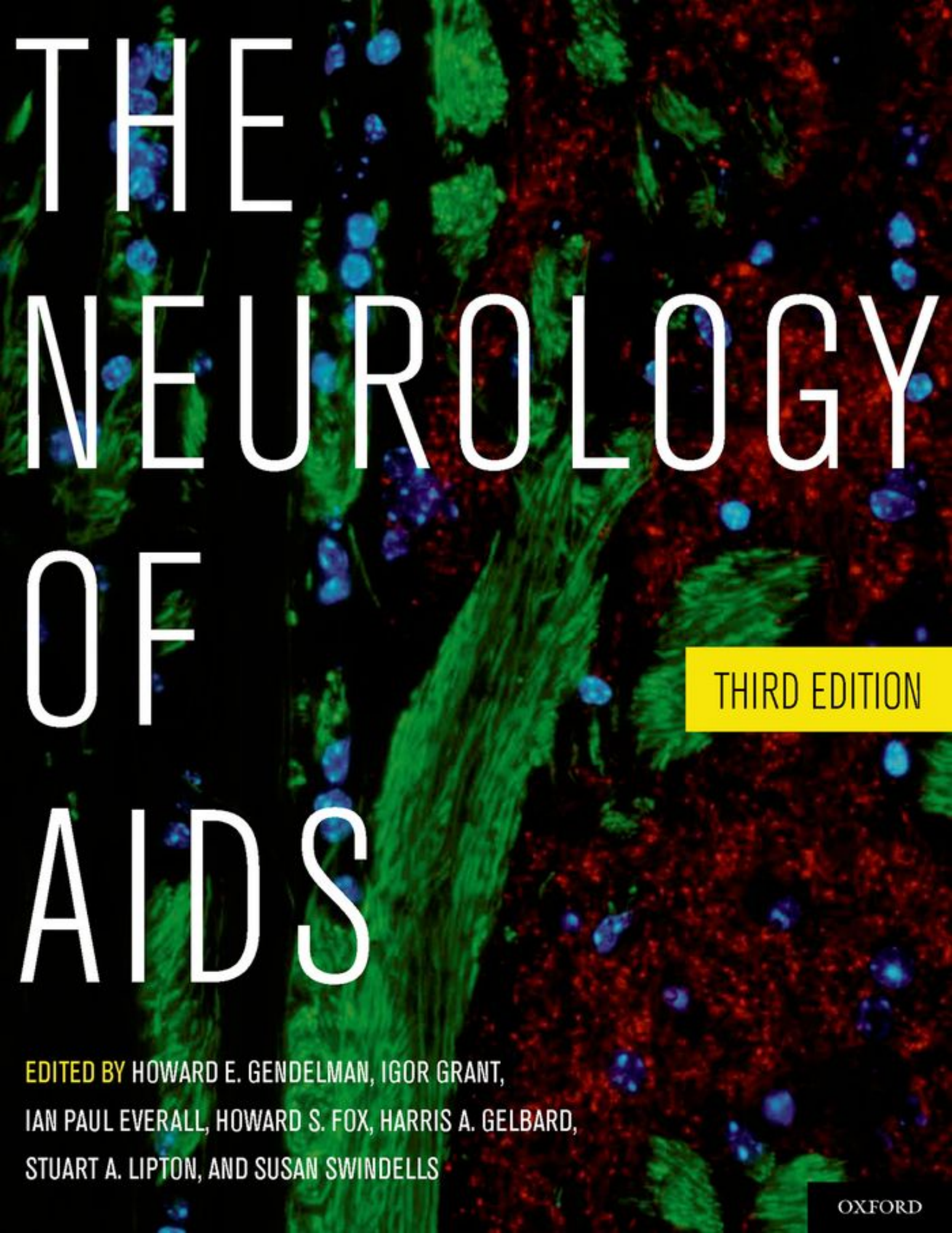
THE
NEUROLOGY
OF
AIDS
THIRD EDITION
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IAN PAUL EVERALL, HOWARD S. FOX, HARRIS A. GELBARD,
STUART A. LIPTON, AND SUSAN SWINDELLS
OXFORD

THE NEUROLOGY OF AIDS

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THE NEUROLOGY OF AIDS

THIRD EDITION

EDITED BY

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To our students, fellows, colleagues and mentors, who teach, guide and inspire us.

To our patients, who provide our research focus and make our efforts meaningful.

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FOREWORD TO THE SECOND EDITION

The Neurology of AIDS is both a well-crafted and timely offering. The book will broadly appeal to the medical community through its thorough evaluation of basic and applied research targeting cognitive, behavioral and mental health issues facing so many HIV-infected people. This book will certainly also be valuable to students, scientists, and health care providers who are involved in research or in the clinical care of people with HIV/AIDS. The contributors are well-respected leaders in AIDS research and clinical care, some of whom have benefitted, in part, from the vision and financial support offered by the American Foundation for AIDS Research (amfAR). We were touched that for this reason and to acknowledge the contributions of amfAR to the fight against AIDS, we were asked to write this dedication.

It is profoundly gratifying to us to see not only the publication of an updated second edition of the encyclopedic text on AIDS neurology reflecting extensive new information collected in the past half-decade, but also to see the contribution we made to it, however modest, so genuinely appreciated.

It is difficult to condense in a few lines how our individual concerns for the people afflicted with HIV/AIDS, and our indignation at the gross prejudices and the many injustices they have had to suffer in addition to their disease, brought the three of us together. It is even more difficult to recount how a myriad of heartbreaks, struggles, disappointments, frustrations and shattered hopes did not destroy our faith in either the power of modern biomedical science or the decency and generosity of spirit of most Americans. In the early 80s, two of us (Mathilde Krim and Elizabeth Taylor) were already deeply concerned by the emergence of a new deadly disease whose epidemic spread appeared so eminently predictable. We were appalled by the failure of our health authorities and elected officials to promptly institute prevention programs in the communities at risk and to provide adequate federal funds for critical research work. We had soon reached the conclusion that the independent nonprofit sector had to step in and start assuming a significant part of these tasks. We proposed that two preexisting organizations (the AIDS Medical Foundation, founded in New York in 1983, and the National AIDS Research Foundation, founded in 1985 in Los Angeles with a substantial gift from the dying Rock Hudson) had to unify their operations and form a single, truly national nonprofit organization. This was done and this larger organization has operated ever since under the name of American Foundation for AIDS Research (amfAR). The third signatory to this dedication (Sharon Stone) became deeply involved somewhat later but with equal determination after the shattering personal experience of losing a close and important friend to the epidemic.

Since its inception, amfAR's mission has been to foster and support biomedical research on the prevention, pathogenesis, and treatment of HIV infection and AIDS, as well as to conduct public education and advocacy efforts on behalf of all people with HIV disease or at high risk for it, so that their civil rights are protected, and their rights to dignity, care, and privacy respected. Much progress was made in these regards through the early and sustained efforts of amfAR.

The support of AIDS-relevant basic biomedical research has always been at the core of amfAR's mission. As do all responsible grant-making agencies, amfAR selects its grantees through stringent peer review. For this, it has enlisted the services of more than 100 volunteer professionals, who are experts in the Foundation's diverse programmatic areas. The senior editor of *The Neurology of AIDS*, Dr. Howard E. Gendelman, is a dedicated member of amfAR's Scientific Advisory Committee (SAC), as are many of this book's contributing authors. For its granting decisions, amfAR relies on its SAC's recommendations. These are based on the originality, promise, and technical excellence of the research proposed in the grant applications it reviews as well as the skills, facilities, and financial needs of the applicants. As an independent foundation, amfAR can respond to funding requests with greater speed and flexibility than government agencies; and it can narrowly focus its funding on HIV/AIDS-relevant proposals. Fields of work to which *The Neurology of AIDS* pays particular attention—innovative treatment strategies, emerging opportunistic infections, disease trends, anti-retroviral drugs, health care delivery, psychosocial aspects of disease, and strategies in vaccine research—all are major concerns of amfAR.

As a research-centered organization, amfAR has helped achieve substantial scientific and medical advances and, in certain instances, it has opened important new avenues to scientific inquiry. This includes the very early studies of Dr. Nancy T. Chang on HIV's protease that spurred the development of protease inhibitors, the drugs that have so dramatically improved the treatment of people with HIV/AIDS in recent years. And, amfAR funded Dr. Ruth M. Ruprecht's preclinical studies in the use of zidovudine for the prevention of vertical retrovirus transmission. Her experiments proved successful and they provided the scientific and ethical rationale for a National Institutes of Health trial in pregnant women that succeeded in "markedly reducing" mother to infant HIV transmission. Another exciting result of amfAR support was the first demonstrations by Dr. Stephen A. Johnston that

"naked DNA" can induce humoral and cellular immunity which opened the novel field of "genetic immunization." In 1992, Dr. Carl Wild, an amfAR Fellow, first synthesized and characterized the anti-HIV activity of the peptide T-20 that the Food and Drug Administration approved for marketing in March 2003. T-20, now called Fuzeon, is the first of a class of drugs known as "entry inhibitors." More recently, in 1996, Dr. Nathaniel R. Landau discovered the CCR5 HIV co-receptor with a grant from amfAR, making it now possible to design drugs to block that receptor to prevent infection.

As early as 1986, amfAR's public education and advocacy efforts had started to impact very significantly on AIDS-related policy decision-making at the federal, state, and municipal levels. This Foundation has, in addition, provided substantial support to community-based clinical research groups. And, after a $30 million amfAR investment over several years in the creation and training of 24 such groups, their nationwide network had become capable of operating independently of amfAR. Its successful completion of a number of amfAR- and industry-sponsored clinical studies has produced important results that have helped improve the medical management of HIV disease. In prevention, innovative, and comprehensive approaches (including syringe exchange programs) were funded and continue to be funded and evaluated.

Our goals remain to contribute significantly to: (1) the control of HIV disease until it is fully medically manageable, and (2) the worldwide control of HIV's epidemic spread, which must include safe and highly effective immunization. To date, amfAR has invested over $220 million in its programs, primarily in grants to more than 2,000 research teams worldwide.

The second edition of *The Neurology of AIDS* provides an important glimpse into how far we have come to a better understanding of how HIV infection can damage the nervous system and, even more importantly, how this damage can be prevented. Fresh perspectives are provided in this second edition on viral infection and opportunistic diseases of the brain, spinal cord, and peripheral nerves, which are among AIDS research's most difficult frontiers.

As did its first edition, this book offers a most comprehensive and well-organized description, in both format and style, of some of the most devastating complications of HIV infection. Topics covered span from a basic science review—the structure and function of HIV genes and gene products—to neurobiology, clinical disease manifestations and diagnosis, pathological outcomes of viral infection, and therapeutics. Recent scientific advances are newly included, such as chemokine receptor expression in brain cells and stem cells; brain immunity in health and disease; common disease mechanisms of neurodegenerative disorders; neuroimaging; and molecular markers. All are discussed in some detail. In addition, this book informatively addresses the psychiatric manifestations of HIV disease and its complications, including its psychological impact on the patients themselves as well as their caregivers and the legal implications of such disease manifestations. Several clinical chapters written by patients themselves—an unusual occurrence in the medical literature—provide unique and very moving insights into the torments of both people living with HIV and their caregivers. Importantly, this book also reports on, and discusses, the extraordinary progress afforded by both highly active antiretroviral and adjunctive therapies that can, in no small measure, reverse the neurological and psychiatric manifestations of nervous system involvement in HIV infection. As HIV disease can be made to change from an acute to a more chronic illness, it is important that state-of-the-art therapeutic approaches be described and discussed in some detail. Altogether, we have here a quite monumental undertaking that offers a view of singular breadth and depth on manifestations of HIV disease that have long appeared mysterious and intractable.

Of course, many unresolved questions remain and research in this important field must continue unabated. But this book can be a solid basis of enlightenment for all those interested in the neurological aspects of HIV/AIDS, and we wholeheartedly congratulate the book's many contributing authors and its editors, for an outstanding achievement.

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PREFACE

The Neurology of AIDS is a comprehensive treatise on the neurological, behavioral, motor, sensory, cognitive, psychiatric, developmental, and basic research of human immunodeficiency virus (HIV) infection. Since the start of the epidemic, neurological disorders emerged rapidly as the most feared and significant complication of progressive HIV infection. Despite considerable advances in combination active antiretroviral therapy (cART), these disorders continue, albeit at a chronic level. The disorders include peripheral neuropathy; myopathy; myelopathy; and, most significantly, HIV-associated neurocognitive disorders (HAND), comprising cognitive, motor, and behavior abnormalities.

The mechanisms by which the virus invades the nervous system and induces neurological disorders are complex. They give rise to novel pathological processes and, when understood correctly, point to therapeutic interventions that are relevant to many neurodegenerative disorders. The neurological disorders are becoming an even more important component of the HIV epidemic, as patients live longer and increasingly productive lives. Accordingly, separate chapters on aging and on linkages between HAND and other neurodegenerative disorders are included in the text. This third edition expresses our collective vision and our collective hope to move research even faster from laboratory bench to patient bedside. With continued new insights emerging for disease causation, considerable emphasis was placed on basic science at the molecular, cellular, animal, and applied human levels. With a focus on genomics, we redoubled our efforts to tie this work to an understanding of host antiretroviral immunity and to include discussions of both the positive potential and the possible pitfalls in vaccine developments. The text goes on to provide thorough and updated reviews of the epidemiological, psychological, and psychiatric aspects of HAND. One unique aspect of the text lies in the section on clinical and personal perspectives. Based on the popularity of this section, the chapters were expanded, and now include a broader disease view from infected patients, who describe their life journey living with HIV/AIDS in their own terms and in their own way. Personal experiences are provided on what it is like living daily with the challenges and difficulties of peripheral neuropathy, sexual dysfunction, substance abuse, anxiety, and depression. These patient perspectives supplement the descriptions written by physicians and scientists. The emerging fields of stem cell biology, proteomics, and adjunctive therapies serve to define how host factors affect active viral replication and neurodegenerative events—and some of the discoveries from these fields are harnessed as biomarkers to improve diagnosis and therapeutic monitoring. The ultimate goal will be to use combined approaches to prevent or reverse disease by positively affecting the processes of neuronal injury and death. Such information, we believe, will serve to define HIV neurology. The advances reflected in this text have already aided in developing new therapeutics for many neurodegenerative disorders, including but not limited to Alzheimer's and Parkinson's disease, amyotrophic lateral sclerosis, and Huntington's disease.

Overall, significant advances in cART have reduced the incidence of HIV-1-associated dementia, while peripheral neuropathy remains common. Neuropsychological tests to monitor cognitive impairments are now more precise, and as patients continue to live longer, more subtle neurological dysfunctions are now the most common disease features. Advances in studies of viral neuropathogenesis, diagnostics, and therapeutics for HAND are provided. A panel discussion by internationally recognized authorities in HIV biology and HAND, including some of the editors, addresses timely questions on neurovirulence, cellular factors influencing viral replication, therapeutic challenges, and the constantly changing epidemiological patterns of disease.

Since the first edition, the book has evolved from 4 to now 12 major sections. The dedication is provided by scientific and public leaders. We acknowledge their contributions, insights, and support for the three decades of the epidemic. Clearly, the merger of efforts between community leaders, scientists, physicians, and policy makers has enabled sustained success in both biomedical research and patient care. Discussion of HAND in the developing world, including links to new research efforts, is provided throughout this volume.

The basic science components of the text were once again revamped considerably from the prior edition. Comprehensive reviews are provided into the molecular and cellular biology, immunology, and neuroscience of HIV infection and into host genomics. A novice reader who picks up the book for the first time can gain a broad understanding of the basic aspects of HIV/AIDS, and its relationships to neurological disease manifestations, without having to refer to other information sources. The next section then focuses on the innate and adaptive immune systems and how they affect the pathogenesis of other neurodegenerative disorders and can be used to develop protective vaccines. A significant feature of the neurological disease complex involves disorders of the blood-brain barrier. Discussions of this barrier's structure and function are provided, as are discussions of mechanisms of viral and cell entry into the brain at both the organ and cellular level. One of the most critical aspects of HIV disease is the disordered regulation

of glial function. The section on this topic was expanded substantively, as deficits in innate glial immunity often underlie the pathogenesis of HIV infection and peripheral neuropathy. Adjunctive medicines that target glial secretory functions, including agents that deactivate microglial responses, can affect both productive viral replication and neurotoxic activities. The means to deliver adjunctive medicines, as well as ART, now have expanded into the field of nanomedicine. Neurotoxicity may manifest through both cellular and viral products and can affect neurogenesis and cellular functions. These neurotoxic products are covered in greater depth, both at the molecular and cellular levels, with viral targets described in relationship to progenitor cells, mononuclear phagocytes, and astrocytes. Animal model systems remain pivotal for studies of viral pathogenesis and developmental therapeutics, and for developing vaccine approaches to prevent disease. As most lentiviral infections show prominent neurological manifestations, a range of infectious disorders is covered, including the simian and feline immunodeficiency viruses, caprine arthritis-encephalitis, and visna-maedi infections of goats and sheep, as well as the more recently developed murine viral systems for HAND, including transgenic and immunodeficient systems. Several chapters discussing the role of drugs of abuse in the disease process are provided. These chapters look at the interrelationships, as observed both in the laboratory and at the patient bedside, among virus, immunity, and drugs of abuse as they pertain to disease progression and protection.

Subsequent sections and chapters provide a "bread and butter" description of the clinical and pathological aspects of disease. These cover features of dementia, neuropathology, drug abuse, spinal cord disease, peripheral neuropathy, myopathy, neoplasms, opportunistic infections including hepatitis C, and psychiatric disorders.

Significant progress has been made in methods for diagnosing and monitoring cognitive function following HIV infection. These methods are now reviewed in chapters on structural brain imaging, magnetic resonance spectroscopy, evoked potentials, neuropsychological assessments, and measures of cellular and viral products as molecular markers of disease and disease progression.

The section on pediatric manifestations of HIV disease was expanded once again to include clinical and neuropathological features along with evaluation of neurodevelopmental deficits and psychosocial aspects. The severity of neurological disease in children is more significant than observed in adults and often devastating. The breakthroughs of ART for children and for prevention of maternal-fetal transmission are also discussed; these breakthroughs have altered markedly the natural history and severity of disease.

A complete section is provided on new treatment paradigms for opportunistic infections. This section should provide the clinician with a comprehensive set of up-to-date management paradigms as well as an understanding of future prospects for adjunctive therapies that target specific disease pathways and mechanisms. The book concludes with a prospectus on changes in HIV epidemiology and the evolution of the disease complex. The clinical and neuropathological aspects of disease in the cART era provide new perspectives for disease and the changing outcomes for neurological impairments that are being developed.

We suspect that the reader, whether a student, researcher, or health care provider, will find this book an important resource and reference on the neurological aspects of HIV infection. Synthesizing in one text contributions from patients, activists, health care providers, scientists, and clinicians proved to be a fascinating undertaking. On reflection, this synthesis provides a unique perspective not only on HIV neurology but on the field of HIV/AIDS as a whole—and on the continued positive prospects for the future.

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The untimely deaths of Al Kerza-Kwiatecki, Anthony Johnson, and Opendra (Bill) Narayan are noted with both sadness and life's celebration. Their lives are a tattooed imprint towards friendship, thoughtfulness, unbridled life determination, inspiration, kindness, and scientific rigor.

To Carol Swarts, a friend, guide, and fellow journeyperson; a thank you for who you are is offered, and also a prayer that you never change.

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To my children and grandchild Lesley, Sierra, Jason, Adam, and Emma, my windows for each today.

Howard E. Gendelman, MD
Editor-in-Chief

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INTERACTIVE TOPICAL DISCUSSION ON AIDS NEUROLOGY

PAST, PRESENT, AND FUTURE

Moderator: Howard E. Gendelman

Participants: Roger J. Bedimo, Linda Chang, Ian Paul Everall, Howard S. Fox, Harris A. Gelbard, Igor Grant, Scott L. Letendre, Stuart A. Lipton, Eliezer Masliah, Susan Swindells, and Babafemi Taiwo

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INTRODUCTION

While the pages of our book offer the reader a comprehensive review of the neurological, behavioral, motor, sensory, cognitive, psychiatric, developmental, and basic research performed on HIV-1 infection of the nervous system, a frank discussion of the field, its future direction, and needs was lacking. To this end, our faculty (the editors, with assistance of other chosen clinician-scientists) used a platform of an interactive conference to discuss what we considered to be the timely questions facing the field. The listed contributors are active investigators and authorities on HIV/AIDS. Particular emphasis is placed within this discussion on current thinking about disease pathogenesis, patient care, and the evolution of these areas for the future. Each question posed to the expert panel is followed by several responses. Each response is preceded by the speaker's last name.

QUESTION 1

"What is the evidence that there are pathobiological and clinical manifestations of human immunodeficiency virus (HIV) infection on the central nervous system (CNS) for the period immediately following the viral seroconversion reaction? What are the mechanisms for such effects and could early intervention attenuate the development of HIV-associated neurocognitive disorders (HAND)?"

Grant: I think that the evidence for early neurocognitive impairment and clinical neurological symptoms in the acute phase of infection remains controversial. There are certainly people who develop early seroconversion illness with neurological symptoms but I think this is not really what we are focusing on here in this discussion. The question is whether the neurocognitive complications, that is, HAND, begin early. There is some evidence that that may be true. For example, Dr. Serena Spudich and colleagues reported on changes in cerebrospinal fluid (CSF) profiles in relationship to systemic infection and antiretroviral treatment early in the course of infections that were also linked to molecular measures of HIV-1 variants (Spudich et al., 2005; Schnell et al., 2009). Recently, our group completed some work that showed that from a neurocognitive standpoint people with acute and early infection scored intermediate between noninfected controls and people with established infection, but the changes were very subtle. So I think it remains an open question. The other point I would make is that there are quite a few data now that suggest that having experienced a very low CD4+ T cell count, that is, a low nadir, predisposes to neurocognitive complications later. And so the question arises whether preventing low CD4 during the acute phase could spare the brain from injury.

Fox: I would like to speak on the mechanism since, as Dr. Grant said, we do not know the cause. What we do know about the early infection period is that there is consistent evidence for early infection of the CNS during this acute period (Chakrabarti et al., 1991; Davis et al., 1992; Lane et al., 1996; Pilcher et al., 2001; Roberts et al., 2004). This evidence is derived from studies on the brain and cerebrospinal fluid in nonhuman primates and people. So certainly, there are early events that affect the brain. This is seen with reactions of both the innate as well as the adaptive immune response in the CNS. Early treatment in the SIV-monkey model revealed a decrease in functional (neurophysiological) CNS abnormalities and a decrease in brain viral load, potentially pointing to a role for early antiviral intervention (Marcondes et al., 2009).

Letendre: I will just add a point that was made about the low nadir CD4 count and what impact that may have on the nervous system. Dr. Ronald Ellis saw a patient in the hospital recently along with our infectious disease team at the University of California-San Diego. The person presented with acute encephalitis and coma and had no infections other than acute HIV infection and had 10 million copies per cubic millimeter of HIV-1 RNA in cerebrospinal fluid (CSF) and with only 1 million copies per cubic millimeter of virus in his

plasma. While this is an extreme case, I think that it is a very clear example that HIV along with the attendant immune response can result in altered sensorium. It may be that there is substantial migration of immune cells in response to new infection and that the damage caused by these cells could account for the cognitive decline that has been described. If this is true, then this damage could potentially be prevented or treated by antiretroviral therapy.

Swindells: The treatment of acute or early or primary HIV infection remains controversial from the clinician perspective. It is unclear really whether we are benefiting people in the long run by starting treatment acutely, and if you do should it then be interrupted? But I think the ongoing debate of when to start antiretroviral therapy is informed to a degree by concern about neurological damage. Concerns exist about virus-induced end organ damage that may be irreversible even if subtle, and there are observational data that support the idea that earlier initiation of therapy may help to prevent neurologic disease from the HIV that develops significantly later on in the course of viral infection (Ellis et al., 2010; CROI abstract 429).

QUESTION 2

What is the importance of combination antiretroviral therapy (cART) for treatment or prevention of HAND? Is the CNS effectiveness of ART important in managing HAND or in preventing it?

Letendre: The data have been accumulating in recent years to support the idea that better-penetrating antiretroviral regimens better reduce HIV in the nervous system. Most—but not all—of the studies that have been performed looking at cognitive performance support the idea that better-penetrating therapy improves impaired performance or maintains normal performance. However, there are no controlled trials. The field has moved from performing cross-sectional analyses to prospective observational uncontrolled studies, but randomized clinical trials have not yet been completed. Dr. Ronald Ellis is leading a randomized controlled clinical trial for treatment of HAND; randomizing people to better-penetrating or worse-penetrating therapy. I am here in Beijing now setting up our new NIH-funded randomized clinical trial of better-penetrating versus worse-penetrating therapy for the prevention of HAND. We need the data from these trials for the field to move forward, particularly since not all the observational data have been supportive. In the last two years there have been a couple of publications that have indicated that antiretroviral therapy could be neurotoxic, at least under certain circumstances. These data highlight the need for randomized clinical trials and tell us that certain drugs with neurotoxic potential may have to be used with caution because they could either cause mild impairment or could limit recovery from impaired neuropsychological performance.

Taiwo: I would just like to add that one of the most important things that we are able to do is to help prevent the neurological consequences of HIV infection that clearly persist despite ART. There is very strong evidence that the longer you wait to start therapy the worse the outcome will likely be. But I think for us to really know how best to intervene we have to understand better the mechanisms of injury to the CNS. As had been said, perhaps it is cryptic viral replication, or maybe persistent immune activation or irreversible neuronal injury from these mechanisms, that plays the dominant role. Immune reconstitution from treatment and direct adverse effects of ART are potential pathways for neurological injury as well. Currently, the field suffers from limited understanding of the relative contribution of these potential mechanisms. So, it is still very difficult to say how best to intervene and in the same vein to define which ART regimen will be most beneficial because there may be subtle differences in their effects. For example, it is unknown if CCR5 inhibition will lead to differential neurological effects. This is one of the important areas for future inquiry.

Lipton: Obviously, with effective ART, the disease has changed. It is a more mild disease, but that said, and this will be brought up in subsequent questions, the prevalence particularly of more mild cases of HAND is still increasing. So, clearly we are not getting to all of the infection or its consequences in the brain. That still needs to be addressed with improved treatments.

Bedimo: Dr. Scott Letendre has done more work on this but I would also like to add that there is not as close a correlation between the viral load in the CNS and the clinical signs of HAND as there is between immune activation and neuronal damage (Kelder, Arthur, Nance-Sproson, McClernon, & Griffin, 1998). In fact, treatment discontinuation in stable patients has recently been shown to be associated with improved cognitive function (Robertson et al., 2010). This suggests that HIV invasion of the CNS might not be sufficient, but other mechanisms might be necessary to cause HAND. So I guess that brings up the next question that you are going to ask about adjunctive therapies. Maybe getting a handle on the mechanisms of HAND will be required in order to evaluate the potential role of adjunctive therapies, and for clinicians to be able to decide whether they're likely to benefit an individual patient.

Swindells: I think from where I sit, the jury is still out on whether clinicians should include CNS antiretroviral penetrance in decision making about choices of antiviral therapy. And it is certainly something we think about and talk about. At the moment, our "go-to" combination for treatment-naïve patients is the simplest: three drugs in one pill—a combination consisting of emtricitabine, tenofovir, and efavirenz—the elements of which do have reasonable penetrance. But there may be patients for whom a different regimen might be preferable for brain-protective effect or for trying to reverse neuronal damage from untreated HIV disease. As Scott mentioned, I think results of randomized controlled clinical trials would be really helpful in informing the field and changing treatment guidelines. So for now, the guidelines don't include this piece and perhaps in the future they will.

Everall: While we still have a long way to go in terms of collecting information regarding efficacy of treating and preventing HAND, as Scott and Susan have just commented on, I think we still have to remember that we have come such a long way since the era when we had no treatment to now having treatment and we have to sort out which is the best

regimen (Everall et al., 2009). I was reminded of just how far we have come a few weeks ago when I saw a patient with the most severe HIV-associated dementia (HAD) that I've seen in 15 years. The patient knew that he was living with HIV for 20 years but refused all treatment and antiretroviral drugs so I saw almost a historical presentation of the HIV dementia complex. So, as I say, we have come a very long way.

QUESTION 3

"What is the place for adjunctive therapies for neuroAIDS? How should it or they be utilized? What is the best developmental scheme?"

Gelbard: I think that after my comments Dr. Stuart Lipton's insights would nicely dovetail for this discussion. I obviously share previously mentioned viewpoints that infection of the CNS happens early with HIV-1, with subsequent events that presumably result in an altered homeostasis between immune effector cells and normal synaptic function. And I believe that normalization of immune indices, particularly those that reflect peripheral immune function, do not adequately reflect the environment that continues to exist in the CNS. I think that even with "perfect" control of viral load, or near-perfect control (currently below 50 copies/mL), we will continue to have CNS disease, and thus we will need adjunctive therapy (Gelbard et al., 2010). The problems with adjunctive therapy in the past have been largely due to limited choice of a single agent with variable pharmocodynamic and pharmocokinetic characteristics that might hinder optimal concentrations in brain regions that are most vulnerable to damage by the virus. The fact remains that many of the same principles that have guided combination chemotherapy for the virus also can and likely should be applied to the adjunctive therapies. By that, I specifically mean because this loss of homoeostasis between the peripheral immune system and cells in the CNS, particularly with respect to normal synaptic function, involves more than one target, it is likely that we need more than one agent at a time to best effect neuroprotection or neurorestoration with adjunctive therapy.

Lipton: I agree with my colleague Dr. Harris Gelbard that there are many cases of HAND even when cART has been optimized. So, clearly, adjunct therapy or better antiretrovirals are needed. I think today, however, the studies with these secondary agents just have not been innovative enough or careful enough. Dr. Gelbard indicated many of the reasons and we need more of an innovative approach to neuroprotection in the brain. We also have to pay more attention to the absorption, distribution, metabolism, excretion, and toxicity (ADME/T) of drugs to insure that they get into the brain adequately, and, unfortunately, many scientists are not remaining aware of these factors in their efforts to make a feasible drug. In the brain a lot of things have to happen for a drug to work and to avoid intolerable side effects. So I think we need a better system of communication between academic institutions and pharmaceutical companies in order to attack this problem in a more coherent manner and produce better neuroprotective agents.

Bedimo: I would like to say as a clinician that the best developmental scheme would be translating currently available information on potential pathogenic mechanisms into clinical trials evaluating therapies that are likely to address those mechanisms. As far as we understand now, potential pathogenic mechanisms leading to HAND include inflammation, excitotoxicity, and oxidative stress. AIDS Clinical Trials Group (ACTG) 5235 is a study that it is focusing on the anti-inflammatory effect of minocycline for the management of HAND and I am not exactly sure of how that study has progressed. Memantine has also been used as a neuroprotective agent to counter the excitotoxicity leading to HAND. To my knowledge, all the adjunctive therapies have yet to yield any positive outcomes.

Lipton: I am involved with the drug memantine as the inventor listed on worldwide patents and I just want to disclose this fact. That said, many of these studies with memantine and other drugs have been ill conceived, and the large variability in the placebo group was belied by the power analysis. At the end of the day, we have found that with several studies either there were not sufficient patients entered or the study was not long enough to allow a real test of efficacy. Then a type II statistical error can be made in which we determine that a drug is not effective, but in fact it might have been had the proper trial be carried out. Having run some of these trials myself, I know that clinical studies are difficult to perform well. Patients are demented, forget to take their medicines, may share medicines between the active group and the placebo group, and I think we need to take better care of exactly how we perform trials. Results can be biased for technical reasons that change the outcome and determine whether the trial was successful or failed for essentially trivial reasons.

Fox: That is a very good point, Dr. Lipton. And let me add, as you and others mentioned, that the clinical presentation is certainly different from severe dementia seen previously. And my question is whether the mechanism is the same, and so, as to the last part of this question, what type of drug should be developed? How much are we using what we have known from the excellent studies on dementia and HIV encephalitis, and how much is due to what we know about the pathogenesis of HAND in its current presentation? I will just leave that as an open question.

Everall: I would like to make one response. I think that the assessment and measurement of neuropsychological outcomes is a critical issue to advising the effectiveness of ARVs and how to treat people with HAND. Let's remember that we still don't have any actual documented guidelines that treatment of HAND with antiretrovirals by themselves improves cognition. I think that maybe we need to have treatment guidelines established that will then help guide us in terms of the use of potential adjunctive therapies as well.

Gendelman: I will add one point. It is clear that HAND in its current form, while mild, remains a persistent problem. It is also clear, despite cART and its therapeutic efficacy in reducing viral load and while significant and confers positive benefits in ameliorating disease severity and comorbid conditions, that adjunctive therapies will likely be needed. The search for new pathways and new means to alter the pathobiological

responses that remain even following effective therapy will be a continued mainstay for the future treatment of HAND (reviewed by Kraft-Terry, Buch, Fox, & Gendelman, 2009; Kraft-Terry, Stothert, Buch, & Gendelman, 2010).

QUESTION 4

"What are the most useful animal model systems for neuroAIDS? How should they be used and do they have a role in studies of disease, pathogenesis, and developmental therapeutics?"

Masliah: Although the simian models and rodent models expressing the HIV genome have demonstrated to be highly useful for studies of pathogenesis and therapeutic development, models over-expressing HIV-1 proteins such as gp120, tat, and vpr have demonstrated to be of interest in terms of uncovering the mechanisms of neurotoxicity. For example, studies in GFAP-gp120 tg mice have shown that selectively neuronal populations including glutaminergic pyramidal neurons are more susceptible via activation of the CDK/cyclin signaling pathway, in contrast, over-expression of Tat under the GFAP promoter has shown activation of other pathways of neurodegeneration such as the GSK3 signaling pathway. This is also of interest because such models could allow us to screen in a rapid manner for compounds with therapeutic potential that selectively target these signaling pathways, such as roscovitine and lithium, respectively.

Fox: One principle advantage of nonhuman primates is that they enable the study of the CNS and immune and other organ systems together, with parameters that are the closest to people. Differences in brain structure and function between rodents and primates are well known, and differences in the immune system are becoming increasingly recognized. Significant attention is now being paid to the relatively poor translatability of rodent studies to humans (Davis, 2008; Schnabel, 2008). While they are more difficult to work with, monkeys have great translatability. One excellent example is the role of the gastrointestinal tract in HIV pathogenesis, discovered in monkeys (Veazey et al., 1998), leading to a wealth of findings in people, including a link between GI microbial translocation and HIV dementia (Ancuta et al., 2008). Other examples are the studies on early viral entry into the CNS (referred to above) and the state of the CNS during stable chronic (non-end stage) infection in the absence or presence of treatment (Roberts et al., 2006; Zink et al., 2010). That said, measuring some of the effects manifest in HAND, in particular the cognitive effects, is difficult. It was much easier in the pretreatment era when people and monkeys got more severe neurologic disease. It is now difficult to measure the finer nuances of cognitive dysfunction in monkeys and correlate those with the current functional findings in people. Still, SIV-infected monkeys are an excellent model for studying a variety of molecular, immune, viral, neuronal, and neurophysiologic properties of the brain due to chronic infection and even with antiretroviral therapy or any adjunctive or other therapy on top of this, with the realization that your group size and number of groups that you can study will be smaller than other lower animal model systems. One has to then balance the translatability with the ability to examine more hypotheses and perform larger studies, uncovering mechanisms that can then be examined in monkeys and people.

Everall: I would also like to say that the primate model has actually provided insights into human disease and especially in the simulation of disease progression. But those experiments take a long time and they obviously cannot be easily repeated due to costs and access. For quicker experiments to generate informative data, you can actually use rodent models. We have been using one of those models and have actually found it very useful in terms in elucidating certain mechanisms of neurotoxicity as well as neuroprotection and they are especially useful in assessing agents and potential agents. A clear experimental path can be to take experiments from tissue cultures through to rodent animal models and then through clinical trial. So while there are limitations with the rodent model, I think this model certainly helped us progress understanding in the field.

Lipton: I would like to say this before giving Dr. Howard Gendelman the floor: I had the great privilege of seeing the data compiled from his new rodent model, which I believe will be revolutionary. It is a new rodent model for neuroAIDS. Many of you know about it but I want to make it clear for our readers; it involved reconstitution of the mouse with a human immune system, allowing HIV infection in the brain, and it is from Dr. Howard Gendelman's laboratory done with several of his colleagues. I really think it is the first time that a mouse model has recapitulated many of the features of HAND, and particularly in its most severe form, HAD. I think this mouse will provide a tremendous advantage to the entire field in elucidating the pathophysiology and in testing drugs before we go into people in clinical trials. With that I would like to hand the floor to Howard so he can tell us a little bit more about this new model.

Gendelman: Thank you, Dr. Stuart Lipton. I would just like to review very briefly the path that led us to these new studies (Dash et al., 2011; Gorantla et al., 2010). There were limited model systems available during the 20 years when we first were involved in developing early systems. Preceding this model were the HIV-1gp120 and viral gene systems, including other transgenic models where HIV subgenomic fragments were put under the control of robust promoters to elicit histopathologic correlates of human disease, along with a broad range of human-cell–mouse-brain reconstitutions and viral chimeras (Toggas et al., 1994; Zou et al., 2007; Potash et al., 2005; Gorantla et al., 2007; Tyor, Power, Gendelman, & Markham, 1993; Persidsky et al., 1996; Avgeropoulos et al., 1998). We have recapitulated human disease components of neuroAIDS, which centered on neuroinflammation as well as the mechanisms of neuronal destruction. These animal model systems have yielded a tremendous amount of data not only on pathobiology but also in many of the developmental adjunctive therapies that have come forward, although none has made it as yet into the drug combination therapies commonly used in patients. These models nonetheless taught us a lot about mechanisms of neuronal destruction and likely built a foundation for the future for new successful therapies that are in the pipeline today. In recent years, our labs and others have begun

to use immunodeficient mice that were reconstituted with HIV-infected cells. In the early stages these were HIV-infected monocyte-derived macrophages, and this model was an encephalitis model where an acute intracranial injection of infected macrophages induces a robust inflammatory process and encephalitis with neuronal dropout and mimics a florid macrophage-induced neuronal and glial process. Following that model system was the reconstitution of human leukocytes of the use of chimeric recombinant viruses that allowed *de novo* infection of murine cells. This model allowed investigators to study and begin to unravel the role of adaptive immune function and that consisted of CD4-positive T cell subsets and CD8 cells in disease, surveillance, and progression. In the last three years, my lab, in collaboration with Drs. Harris Gelbard and Larisa Poluektova, has begun to reconstitute essentially performing bone marrow transplants in immunodeficient animals in generating, with C34-positive human stem cells, a true humanized mouse where the bone marrow progenitor cells are human and the reconstitution of the immune system is human in a homeostatic environment. These are not activated T cells; these are immunocytes that will survive over a year and will respond to a number of antigenic stimuli, including viral infection. These animals can be infected for many months, and about a year and a half ago we began to see results that excited us in the field of neuroAIDS. About 50% of the animals, spontaneously after four, five, or six months of viral infection, would develop a mingle encephalitis, meningitis, or low-level nervous system disease. There was then the characterization of the system demonstrating infiltration and infection of human macrophages in perivascular distribution with low level of innate astrocyte and microglial responses. In 10% of the animals, there was the development of a florid encephalitis. Eight months ago, and in conjunction with other studies,we began to monitor the development of neuronal aberrations, and with support from Dr. Harris Gelbard we began to show changes in neuronal responses. We prospectively monitored these animals by spectroscopy and diffusion tense imaging. This was done by Dr. Michael Boska, who showed very focal changes in N-acetyl aspartate levels in areas that are most affected in humans, so we saw those areas in the cortex and subcortex as well, and they progressed. The animals were sacrificed after several months and these animals showed very significant histopathological correlates of disease, including changes in the neurofilament, which mapped to the same areas of the brain shown to be abnormal by spectroscopy and diffusion tensor imaging techniques. We hope that in conjunction with our own works and works of others that these will be used not only for studies of antiretroviral therapy and combination therapy, but also for adjunctive therapies to combat human disease.

QUESTION 5

"Do drugs of abuse affect the progression of HIV/AIDS and/or neuroAIDS? If so, under what sets of circumstances and which drugs?"

Chang: Regarding the question as to whether drugs of abuse affect the progression of HIV and if so, under what sets of circumstances and which drugs: The published data have shown that additive or interactive effects of neurotoxicity can occur in individuals with HIV who are drug users. For example, neurotoxicity has been shown in HIV patients who use methamphetamine additively (Chang, Ernst, Speck, & Grob, 2005; Alicata, Chang, Cloak, Abe, & Ernst, 2009) or interactively (Jernigan et al., 2005). Our neuroimaging studies that used magnetic resonance spectroscopy to measure N-acetyl-asprate (NAA) and other metabolites showed additional decreased NAA or further elevation of choline or myo-inositol, which is indicative of neurodegeneration and astrogliosis, especially in the subcortical brain regions and notably the basal ganglia (Chang et al., 2005). However, instead of seeing an additive effect, you might actually see an interactive effect, such as in the work done by Dr. Terry Jernigan's group where they saw enlarged subcortical brain structural changes in methamphetamine users but smaller subcortical volumes in HIV-infected people (Jernigan et al., 2005). However, in the HIV subjects who used methamphetamine, they found relatively normal basal ganglia structures (Jernigan et al., 2009). Hence, the different imaging techniques demonstrate different brain changes that are associated with the combined effects of HIV and drugs of abuse. For instance, the interactive effect of HIV and marijuana use on brain glutamate also was documented using MR spectroscopy techniques (Chang, Cloak, Yakupov, & Ernst, 2006). There are other neuroimaging techniques such as PET imaging, which found that HIV patients who used cocaine had additional decreases in the brain dopamine transporters (Chang et al., 2008). The dopamine receptors were also found to be decreased, especially in the HIV+ cocaine users, but the effects on the receptors were primarily related to nicotine use, which is quite prevalent in both the HIV-infected population and amongst drug users. So there are really many examples of human studies as well as animal studies that have actually shown an accelerated disease progression of HIV associated with drug use. For example, both cocaine and methamphetamine can enhance viral replication which can lead to further neurotoxicity.

Everall: This is a very complex issue and the interaction of drugs of abuse with HIV occur at many different levels that could be biological, behavioral, personality, and so on. At the biological level, some studies have shown that drugs such as opiates, through binding at the u-opioid receptor, increase viral replication, which is obviously going to have an effect on progression of HIV disease. With regard to stimulants, such as methamphetamine, I would like to mention the neuropathological findings from the work done by Dr. Eliezer Masliah and myself, that has demonstrated a selective degeneration of calbindin-expressing GABAnergic inter-neurons that results in more severe memory deficits in HIV-infected methamphetmaine users. In addition to the biological mechanisms, there is also the issue of behavior. Drug-using individuals may well be prone to impulsive or sensation-seeking behavior, which puts themselves and others they interact with at risk of infection. It is also known that individuals who regularly take drugs of abuse may well have personality issues and more chaotic lifestyles, which means that they are much less likely to engage and retain in HIV mental health services and be less adherent

to any antiretroviral medication, which again is going to have a negative impact upon their well-being and possibly increase the progression of the HIV disease and/or development of HAND.

Grant: I think Linda and Ian have covered a lot of the issues and I would like to underscore a couple and then add a couple of more. One is that these drugs, some of them like methamphetamine and alcohol and central nervous system depressants, can independently cause neurocognitive impairment. That impairment is probably most likely to be seen in connection with length of abstinence, in other words, in most cases as people refrain from drug abuse their cognitive disturbance relative to drugs decreases. So part of the issue of the interaction between HIV and drugs needs to be seen within a timeframe of detoxification as well. The second point is that not all drugs are alike; so methamphetamine has been mentioned by Linda and Ian as neurotoxic, but, for example, drugs like marijuana are really not known to independently produce substantial neurocognitive changes in the long run in people who are abstinent. And, in fact, some studies suggest that concurrent use of marijuana and meth actually confers a better neuropsychological outcome than using meth alone, perhaps suggesting a neuroprotective action of the cannabinoids. The third point I would make is that substance abusers are also more likely to have dual HIV infection or HCV infections that also can affect neurological function. The final point would be that this has been really a relatively understudied area in the context of large clinical trials such as those being conducted by ACTGs and HPTNs and so forth. Historically, substance users are either excluded or not properly diagnosed in these settings and that is a situation that needs to be corrected if we are really to understand the long-term neurological effects of these drugs in relation to HIV and HIV treatments.

Fox: Dr. Grant nicely raised the issues of different effects of different drugs, and certainly when it comes to people the predominant case is poly drug abuse. It is rare that a drug user uses only one substance, and as pointed out, these may have the opposite effects of different drugs on HIV and the brain. The sum of the data on human drug abusers reveals that there is not a profound effect. People just don't show dramatic changes, and often the apparent effects of drug abuse can be attributed to other factors such as access to health care, medication compliance, and so on. (Ellis et al., 2003; King, Alicata, Cloak, & Chang, 2009). The concept of protection, while seemingly counterintuitive, can arise from the effects drugs have on their receptors not only in the brain but also the immune system. In addition to the human studies with cannabinoids that Dr. Grant refers to, Dr. Robert Donahoe performed a study in SIV-infected monkeys revealing that constant doses of opiates leads to improved survival (Donahoe et al., 2009). But of course human opiate abusers don't have regular supplies of drugs taken on regular schedules. But it serves as an example that, for the true sense of effects of drugs of abuse on infection, they certainly are not dramatic nor always adverse. We did try to mimic human abuse patterns in our studies on the effects of methamphetamine on SIV in monkeys. In the time period that we examined, there was not an effect on progression of disease nor on peripheral viral perimeters; however, we did see increased virus in the brain and effects on macrophages and natural killer cells (Marcondes, Alicata, Cloak, & Chang, 2010). So, I think the results of the drugs may be subtle and require very careful control to get around issues such as poly drug abuse, compliance, co-infections, and other comorbidities. So, I agree with Igor that it is an open question and more studies need to be done but the variables and confounds may be large.

Lipton: Because of drug abuse in humans, the situation is very different from that seen in animal models of HAND, which is much cleaner. Human drug abusers usually bring a whole set of other comorbidities, including head injuries, abuse histories, and so on. Hence, further studies are necessary to look at the effect of interaction of comorbidity, drugs, and HIV.

Everall: I would just like to make an additional comment to Igor's very important point about the potential protection of cannabis. Clinically, my role is a psychiatrist and we know that the more potent forms of cannabis are associated with an increased risk for psychosis. There are a number of active ingredients in cannabis and we need to clarify which ingredients are associated with neuroprotection and which exacerbate psychosis.

QUESTION 6

"How significant is HAND in the cART era? What need clinicians be mindful of?"

Swindells: We have already had some discussion from contributors on the conference call that the overall incidence of what is considered classic AIDS dementia has decreased. Thanks in large part to the availability of effective antiretroviral therapy, this is something we see rarely now in the clinic. However, there are subtle impairments that are still present, and particularly in untreated patients, which are worse with more advanced disease. But, you also have to look for it. Occasionally, it will be obvious, but diagnosis requires careful attention to cognitive impairment and that is difficult in the context of a busy HIV clinic. However, there are some tools that are available that do not require extensive formal neuropsychological testing (Power et al., 1995). There are tools that take a few minutes to apply and we in fact use those in screening and they are extremely helpful for us in terms of assessing severity of impairment and also monitoring progressively to see whether patients make a full or partial recovery neurologically.

Grant: I think this is an important question, as Dr. Swindells mentioned. It is a real challenge in clinics: how to ascertain and monitor these complications. Just to remind readers, we do see at least mild neurocognitive impairment in anywhere from one-third to over one-half of people with HIV disease, most of whom are well treated on cART. So this is a problem that persists. It is important to the patient because it affects their everyday functioning, in some instances including ability to continue with their regular work, may affect driving skills, adherence to complex medical regimens, and other life activities. So, it is not a trivial issue even though the impairments can be considered mild. As to how to monitor this, it is

a bit of a dilemma. As with any complex medical problem, such as for example the diagnosis of mild heart disease, it is not easy to do this at the bedside. It does require specialized evaluations, and so what is then also needed is a high index of suspicion as to the possibility a person may be experiencing neurocognitive decline. Some points to alert the clinician to someone at greater risk would be the patient who has had a low nadir CD4, the patient who has continued viremia despite treatment, and of course patients who complain of memory and other cognitive problems. However, self-report of cognitive difficulties can also be increased when the patient is depressed. Dr. Swindells mentioned that there are brief neurocognitive screens, and they should be employed routinely in the monitoring of persons with HIV, but they do have their limitations, unfortunately. To diagnose mild forms of impairment there is no getting around performing more comprehensive neuropsychological testing whenever the clinician suspects that neurocognitive change may be occurring. Examples of test batteries that are sensitive to neurocognitive complications of HIV can be found in the publication describing the Frascati Criteria for HAND (Antinori, et al., 2007), and in the CHARTER Study report (Heaton et al., 2010).

Chang: In the clinical setting, the scales that are developed to assess HIV-1-associated dementia or HAND are better than the traditional clinical examinations, such as the HIV dementia scale instead of the MMSE (which is more useful for assessing Alzheimer's disease but not too sensitive for detecting HIV-associated dementia or HAND). Another major issue is that it is often difficult to diagnose the comorbid conditions that Igor mentioned and that we discussed earlier, such as, the comorbid effects of substance abuse can additionally impact the cognitive dysfunction. Lastly, in the era of cART, some of the antiretroviral medications themselves may be neurotoxic and may lead to additional brain abnormalities that can be assessed with neuroimaging techniques (Schweinsburg et al., 2005; Chang et al., 2008), and so some of the cognitive deficits you are seeing may not be due to HIV directly, but to the treatment. There are many issues that we don't have complete understanding of and how much of each component contributes to the cognitive dysfunction, and it would be wise to do more research in these areas.

Bedimo: I just wanted to add for the readers one good reference on the incidence and determinants of HAND: the CASCADE cohort (Bhaskaran et al., 2008). They were able to show that compared to the pre-HAART era, the incidence of HAND has decreased about tenfold in the later part of the HAART era (2003–2006). I believe it has also been shown that the prevalence of HAND has not decreased and may in fact be increasing, especially as we are getting better at detecting the more subtle forms of the disorder. A few years ago there was an international HIV dementia scale published that looked at three areas—memory, psychomotor skills, and motor speed (Sacktor et al., 2005). This tool might be more useful at differentiating HIV-1-associated dementia from other forms of dementia in the aging HIV population, given the psychomotor slowing accompanying memory deficits and the accumulation of intraneuronal amyloid beta and alpha synuclein in the former (Achim, Adame, Dumaop, Everall, & Masliah, 2009; Khanlou et al., 2009). And also one thing that I have seen and am not sure what others think about it, it is said that there is significant olfactory deficits in HAND and this might be clinically useful to test (Zucco & Ingegneri, 2004).

Gendelman: Hepatitis C and neuroAIDS in resource-limited settings. Dr. Letendre, if you can respond in order to provide a broad clinical perspective, this would balance the discussion.

Letendre: I want to quickly support what has recently been said, which is that HAND in the clinic has to be interpreted in the context of other complications, which has been termed either multimorbidity or polypathology, depending on which term you prefer. What is clear is that there are several factors that increase with time including age, duration of HIV infection, duration of treatment, the number of comorbidities, and the number of concomitant medications. Those morbidities not only include cardiovascular disease, bone loss, and renal disease, but chronic co-infections, such as the viral hepatitidies. I think it is clear that hepatitis C does affect the brain either by infecting glial cells—and I think there is very strong evidence that supports that—or by other mechanisms such as an indirect effect from liver disease. I think this is going to become increasingly important in the next few years because of the new hepatitis C treatments that are going to be moving into the clinic. With these new treatments, many of the issues that we have dealt with in terms of penetration into the brain of antiretrovirals may now also apply to penetration of these new protease and polymerase inhibitor drugs for hepatitis C. In terms of the international setting, I think the comment that was just made about the international HIV scale is good. Of course, we need geographically and culturally relevant screening instruments to use in the clinic everywhere and the international HIV dementia scale promises to do that, but I am not certain that it always succeeds. We must ensure that we screen for the comorbidities that are relevant in each setting and account for them in our assessments. I just want to quickly add there may also be an impact in differences in host genetics. Gene frequencies of several polymorphisms and copy number variants differ by geographical region. Subtypes of HIV also differ geographically and may also be important to account for since subtype C HIV may be less neurovirulent and subtype D may be more neurovirulent, at least in adults.

Gendelman: We should address survival at this point in time, as people live longer and complications of other neurodegenerative disorders or comorbidity conditions related to Alzheimer's, Parkinson's, or other diseases. What do we think of its importance of comorbid neurodegenerative diseases and in HIV-infected patients? Is this going to be a problem? Is this something we need to be mindful of and what might do we do for the future?

Swindells: The short answer to your question is that it is extremely important and an area of great interest from the affected community and the research institutes and foundations. There are often aging-related complications that appear to occur prematurely in people who are infected with HIV and this is not restricted only to neurological disease, but we see this with cardiovascular disease, bone disease, and overall

frailty, and so this is an area of active ongoing investigation. My prediction is the more that we look for premature brain aging; the more likely we are to find it. There is evidence now from cardiovascular studies, which in some ways are a little easier to implement and interpret, that patients with HIV disease may have a heart of someone ten years older than them. And I think that is a reasonable analogy that also may be applied to brain disease. Lastly, there is some early but concerning signal that other neurodegenerative diseases such as Alzheimer's disease may be becoming more common going forward as the population living with HIV ages.

Lipton: Just to follow up on what has been said by other people, the interplay of HIV infection and aging on neurocognitive effects and structural brain imaging are very complex and merit further study. In part, chronicity of infection, as I believe Dr. Ian Everall mentioned, and the treatment, which itself can be neurotoxic, plus the development of other systemic complications linked to inflammation can all affect cognitive functioning. So, I think that the manifestations of HAND in the elderly may appear somewhat different, both neuropathologically and neurocognitiviely, than HAND in younger people. That is yet to be determined, but something worthy of study. Finally, there may also be an important impact of mild cases of HAND in elderly persons who may already have had some cerebral compromise, such as a stroke or vascular disease, and this could play out with more severe effects on activities of daily living, including driving, and so forth, so this will need to be addressed.

Chang: I just had this thought when we were talking about the neurodegenerative diseases in the aging HIV population. We really don't know what the prevalence is of comorbid Alzheimer's disease, Parkinson's disease, or other diseases that might affect the HIV-infected population as they get older. They should at least have the same prevalence as uninfected individuals. One way to possibly track something like that is to maybe create a registry so that everyone could then log onto to the database and record comorbid conditions, including AD, PD, or other degenerative conditions. This would be similar to how we track other neurological conditions or treatment effects, for example, if we were looking for a new pattern of toxicity associated with a new drug, we would form a registry and everyone would log in and report on that. Perhaps something like that could facilitate and allow us to get a handle on the prevalence of comorbid neurodegenerative conditions. For example, I am especially concerned about Parkinson's disease because HIV-infected patients already have a subcortical type of dementia and they have motor slowing or dysfunction, so perhaps that could hasten the development of Parkinson's disease, and their prematurely aging brains also might lead them to be more vulnerable for Alzheimer's disease; both are diseases of aging.

Lipton: This is often a bit difficult to assess because many young patients with HIV have Parkinson's symptoms because of the involvement of HIV in the striatum. This form of parkinsonism in HIV, as opposed to Parkinson's disease, which is really a progressive neurodegenerative disorder, needs to be discerned.

Chang: That is right, but that is why it is somewhat different because PD is a progressive disease whereas parkinsonism in HIV can be rather static and I think most neurologists can tell the difference. HIV patients come into the clinic with a broad range of brain disorders.

Lipton: Sometimes it can be difficult to tell the difference, particularly early on in the symptomatology.

QUESTION 7

"What is the 'true' neuropathology of HAND in the cART era?"

Masliah: I appreciate the comments of Dr. Lipton crediting our studies of neurodegeneration in the brains of patients with HIV and in animal models. In the cART era, we have noted that HIV encephalitis has gone from a sub-acute neuro-inflammatory disorder to a protected chronic condition accompanied by astrogliosis, microgliosis, and lower levels of HIV, but more widespread degeneration accompanied on several occasions by accumulation of misfolded proteins such as Abeta, Tau, and alpha-synuclein. It appears that in aged HIV-positive individuals, mechanisms of aggregated protein clearance failed and in combination with HIV trigger more widespread neurodegeneration. Other comorbidities that contribute to the neurodegenerative process in patients with HIV include HCV infection, drug abuse, psychiatric conditions, immunological reconstitution syndromes, and toxicity of antiretrovirals.

Everall: Assessment of the "true" neuropathology in HAND is somewhat complicated by the dramatic effectiveness of cART in significantly reducing mortality, thereby limiting the number of brains that we can assess at autopsy. With those brains we do have access to I think that pathologic changes still do exist. We do not see the multinucleated giant cells any longer but we certainly see viral infection in the brain by either immunohistochemistry for viral proteins or by RNA as a measure of brain viral load. We recently published a paper assessing neuropathological changes in a large brain cohort of individuals who were recruited by the National NeuroAIDS Tissue Consortium with advanced HIV disease. We observed high rates of HIV encephalitis and other pathological changes. In addition, there were high rates of clinical cognitive impairment during life in this cohort so it was not possible to assess whether the HIV encephalitis predicted cognitive impairment. In a separate study assessing the brains of individuals with HIV aged 55 years and older, we noted high rates of deposition of a-synuclein and b-catenin, which are markers of Parkinson's disease and Alzheimer's disease, respectively. So, we are now worried that the "true" neuropathology has extended to other neurodegenerative disorders.

Gelbard: I think I would just add that we should emphasize in the future investigation of mechanisms that may underlie impaired homeostasis between immune effector cells and synaptic architecture as opposed to sole focus on viral load because in gaining an equally deep understanding of the biologic substrates for multiple clinical entities that comprise HAND, I think that this body of knowledge will help advance us toward a more informative use of biomarkers and a greater understanding of key molecular targets for the development of new drugs. That may be the next generation of adjunctive therapy.

Chang: A quick comment regarding the value of in vivo neuroimaging studies. In general, when we evaluate neuropathology, we are waiting until the patients have died, and by then many of them might have suffered severe end-stage AIDS or some other kinds of peri-mortem complications. So, being able to assess in vivo living pathology is important; the work we do with neuroimaging is important and can allow us to assess the severity and progression of HIV-associated brain injury, and all the comorbid conditions in the living patients.

Lipton: I wanted to follow up on something that Dr. Gelbard said. I think that Dr. Eliezer Masliah deserves a lot of credit for looking at HAND in terms of a chronic neurodegenerative condition that affects synaptic function. This is why I think we need to look at neurodegenerative diseases in a broader context. I think it is becoming increasingly clear that virtually every neurodegenerative disorder, including cognitive dysfunction in HAND and particularly in HAD, is probably closely correlated with synaptic damage. Protecting the synapse seems to be the real disease modification that is needed, not only in HAD but probably also in Alzheimer's disease and other forms of dementia. The synaptic damage may occur in different areas of the brain for reasons that are still unclear even after all these years, but as Dr. Gelbard said, it is going to be very important to afford neuroprotection at the synapse, and it is going to be critical that our future therapeutic approaches look at this issue very carefully. Certainly, microglial infection leads to the production of toxins, is going to become a dominant way that we need to interfere with the disease in order to affect neuroprotection or prevent neuroinflammation, or both, as Dr. Gelbard stated earlier.

This is why we need to look at neurodegenerative diseases more broadly. It is becoming increasingly clear that nearly that every virtually every neurodegenerative disorders, including cognitive dysfunction in HAND and particularly in HAD, is probably closely correlated with synaptic damage. Protecting the synapse seems to be the real disease modification that is needed, not only in HAD but also in Alzheimer's disease and other forms of dementia. The synaptic damage make occur in different areas of the brain for reasons that are still unclear even after all these years, but as Dr. Gelbard said, it is going to be very important to afford neuroprotection at the synapse, and it is going to be critical that our future therapeutic approaches look at this issue very carefully. Recent papers show that microglia can affect the syanpse. I think that microglia infection, as well as immune stimulation of microglia to produce toxins, are going to become a dominant way that we need to interfere with the disease in order to effect neuroprotection or prevent neuroinflammation, or both, as Dr. Gelbard stated earlier.

Everall: We became interested in looking at whether HIV was also disrupting emerging mechanisms that regulate the translation of mRNA into protein. We assessed specifically the expression of microRNA (miRNA). Micro RNAs are noncoding RNAs that regulate whether the mRNAs become translated into proteins. So we looked at the expression of micro RNAs in the brain in individuals with HIV and compared those with HIV in individuals that had also had a recent documented episode of majoremajor depressive disorder (MDD) (Tatro et al., 2010). We found that there were in fact a two separate signatures of microRNA dysregulation in the brains of individuals who had HIV and HIV with MDD. This may be another mechanisms by which HIV is disrupting the function of the brain during advanced HIV disease.

Letendre: Another broad area that deserves attention is the role of neural progenitor cells in the brains of patients with HAND. I think there is mounting evidence from a number of groups that progenitor cells may be important in the recovery of the brain from injury. Relevant to HIV disease, neural progenitor cells express CXCR4, one of the entry receptors used by virus. Since infection or injury of neural progenitor cells by HIV may limit recovery from nervous system injury, this is a potentially fruitful area for future research.

Gendelman: In closing, let me acknowledge the outstanding support we received from our Oxford colleagues and notably Craig Panner and Kathryn Winder who made this teleconference and other unique aspects of this book possible. Lana Reichardt's organizational and transcriptional skills are appreciated beyond any thank you. The impetus for this teleconference and the book itself rests with our patients and students, who are our singular driver towards excellence in all we do. The neurological complications of HIV-1 infection have continued and will certainly do so until a protective vaccine or a better means to eliminate viral sanctuaries is realized. We are dedicated to seeing the dream of viral eradication, whatever the means, turned into reality. Until this is realized, a thorough means to understand the disease process, the means to best diagnose and stage it, and pathways towards suitable therapeutics as presented herein serves to best address our patients' needs.

REFERENCES

Achim, C. L., Adame, A., Dumaop, W., Everall, I. P., & Masliah, E.; Neurobehavioral Research Center. (2009). Increased accumulation of intraneuronal amyloid beta in HIV-infected patients. *J Neuroimmune Pharmacol*, *4*(2), 190–9.

Alicata, D., Chang, L., Cloak, C., Abe, K., & Ernst, T. (2009). Higher diffusion in striatum and lower fractional anisotropy in white matter of methamphetamine users. *Psychiatry Res*, *174*(1), 1–8.

Ancuta, P., Kamat, A., Kunstman, K. J., Kim, E. Y., Autissier, P., Wurcel, A., et al. (2008). Microbial translocation is associated with increased monocyte activation and dementia in AIDS patients. *PLoS One*, *3*, e2516.

Antinori, A., Arendt, G., Becker, J. T., Brew, B. J., Byrd, D. A., Cherner, M., et al. (2007). Updated research nosology for HIV-associated neurocognitive disorders. *Neurology*, *69*(18), 1789–1799.

Bhaskaran, K., Mussini, C., Antinori, A., Walker, A. S., Dorrucci, M., Sabin, C., et al.; CASCADE Collaboration. (2008). Changes in the incidence and predictors of human immunodeficiency virus-associated dementia in the era of highly active antiretroviral therapy. *Ann Neurol*, *63*(2), 213–21.

Chakrabarti, L., Hurtrel, M., Maire, M. A., Vazeux, R., Dormont, D., Montagnier, L., et al. (1991). Early viral replication in the brain of SIV-infected rhesus monkeys. *Am J Pathol*, *139*, 1273–1280.

Chang, L., Ernst, T., Speck, O., & Grob, C. S. (2005). Additive effects of HIV and chronic methamphetamine use on brain metabolite abnormalities. *American Journal of Psychiatry*, *162*(2), 361–369.

Chang, L., Cloak, C., Yakupov, R., & Ernst, T. (2006). Combined and independent effects of chronic marijuana use and HIV on brain metabolites. *Journal of Neuroimmune Pharmacology*, *1*, 65–76.

Chang, L., Wang, G. J., Volkow, N. D., Ernst, T., Telang, F., Logan, J., et al. (2008). Decreased brain dopamine transporters are related to cognitive deficits in HIV patients with or without cocaine abuse. *Neuroimage*, *42*(2), 869–78.

Chang, L., Yakupov, R., Nakama, H., Stokes, B., & Ernst, T. (2008). Antiretroviral treatment is associated with increased attentional load-dependent brain activation in HIV patients. *J Neuroimmune Pharmacol*, *3*(2), 95–104.

Crews, L., Patrick, C., Achim, C. L., Everall, I. P., & Masliah, E. (2009). Molecular pathology of neuroAIDS (CNS-HIV). *Int J Mol Sci*, *10*(3), 1045–63

Dash, P., Gorantla, S., Gendelman, H. E., Makarov, E., Finke-Dwyer, J., Castanedo, A., et al. (2010). Links between progressive HIV-1 infection of humanized mice and viral neuropathogenesis. *J Neurosci*.

Davis, L. E., Hjelle, B. L., Miller, V. E., Palmer, D. L., Llewellyn, A. L., Merlin, T. L., et al. (1992). Early viral brain invasion in iatrogenic human immunodeficiency virus infection. *Neurology*, *42*, 1736–9.

Davis, M. M. (2008). A prescription for human immunology. *Immunity*, *29*, 835–8.

Donahoe, R. M., O'Neil, S. P., Marsteller, F. A., Novembre, F. J., Anderson, D. C., Lankford-Turner, P., et al. (2009). Probable deceleration of progression of Simian AIDS affected by opiate dependency: Studies with a rhesus macaque/SIVsmm9 model. *J Acquir Immune Defic Syndr*, *50*, 241–9.

Ellis, R. J., Childers, M. E., Cherner, M., Lazzaretto, D., Letendre, S., & Grant, I: (2003). Increased human immunodeficiency virus loads in active methamphetamine users are explained by reduced effectiveness of antiretroviral therapy. *J Infect Dis*, *188*, 1820–1826

Everall, I., Vaida, F., Khanlou, N., Lazzaretto, D., Achim, C., Letendre, S., et al.; National NeuroAIDS Tissue Consortium (NNTC). (2009). Cliniconeuropathologic correlates of human immunodeficiency virus in the era of antiretroviral therapy. *J Neurovirol*, *15*(5–6), 360–70.

Gelbard, H. A., Dewhurst, S., Maggirwar, S. B., Kiebala, M., Polesskaya, O., & Gendelman, H. E. (2010). Rebuilding synaptic architecture in HIV-1 associated neurocognitive disease: A therapeutic strategy based on modulation of mixed lineage kinase. *Neurotherapeutics*, *7*(4), 392–8.

Gorantla, S., Makarov, E., Finke-Dwyer, J., Castanedo, A., Holguin, A., Gebhart, C. L., et al. (2010). Links between progressive HIV-1 infection of humanized mice and viral neuropathogenesis. *Am J Pathol*.

Gorantla, S., Liu, J., Sneller, H., Dou, H., Holguin, A., Smith, L., et al. (2007). Copolymer-1 induces adaptive immune anti-inflammatory glial and neuroprotective responses in a murine model of HIV-1 encephalitis. *J Immunol*, *179*(7), 4345–56.

Heaton, R. K., Clifford, D. B., Franklin, Jr. D. R., Woods, S. P., Ake, C., Vaida, F., et al., the CHARTER Group. (in press). HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy. *Neurology*.

Jernigan, T. L., Gamst, A. C., Archibald, S. L., Fennema-Notestine, C., Mindt, M. R., Marcotte, T. D., et al. (2009). Effects of methamphetamine dependence and HIV infection on cerebral morphology. *J Affect Disord*, *119*(1–3), 84–91.

Jernigan, T. L., Gamst, A. C., Archibald, S. L., Fennema-Notestine, C., Mindt, M. R., Marcotte, T. D., et al. (2005). Effects of methamphetamine dependence and HIV infection on cerebral morphology. *Am J Psychiatry*, *162*(8), 1461–1472.

Kelder, W., McArthur, J. C., Nance-Sproson, T., McClernon, D., & Griffin, D. E. (1998). Beta-chemokines MCP-1 and RANTES are selectively increased in cerebrospinal fluid of patients with human immunodeficiency virus-associated dementia. *Ann Neurol*, *44*(5), 831–5.

Khanlou, N., Moore, D. J., Chana, G., Cherner, M., Lazzaretto, D., Dawes, S., et al.; HNRC Group. (2009). Increased frequency of alpha-synuclein in the substantia nigra in human immunodeficiency virus infection. *J Neurovirol*, *15*(2), 131–8.

King, G., Alicata, D., Cloak, C., & Chang, L. (2010). Neuropsychological deficits in adolescent methamphetamine abusers. *Psychopharmacology (Berl)*, *212*(2), 243–9.

King, W. D., Larkins, S., Hucks-Ortiz, C., Wang, P. C., Gorbach, P. M., Veniegas, R., et al. (2009). Factors associated with HIV viral load in a respondent-driven sample in Los Angeles. *AIDS Behav*, *13*, 145–153.

Kraft-Terry, S. D., Buch, S. J., Fox, H. S., & Gendelman, H. E. (2009). A coat of many colors: Neuroimmune crosstalk in human immunodeficiency virus infection. *Neuron*, *64*(1), 133–45.

Kraft-Terry, S. D., Stothert, A. R., Buch, S., & Gendelman, H. E. (2010). HIV-1 neuroimmunity in the era of antiretroviral therapy. *Neurobiol Dis*, *37*(3), 542–8.

Lane, J. H., Sasseville, V. G., Smith, M. O., Vogel, P., Pauley, D. R., Heyes, M. P., et al. (1996). Neuroinvasion by simian immunodeficiency virus coincides with increased numbers of perivascular macrophages/microglia and intrathecal immune activation. *J Neurovirol*, *2*, 423–432.

Marcondes, M. C., Flynn, C., Huitron-Rezendiz, S., Watry, D. D., Zandonatti, M., & Fox, H. S. (2009). Early antiretroviral treatment prevents the development of central nervous system abnormalities in simian immunodeficiency virus-infected rhesus monkeys. *AIDS*, *23*, 1187–95.

Marcondes, M. C., Flynn, C., Watry, D. D., Zandonatti, M., & Fox, H. S. (2010). Methamphetamine increases brain viral load and activates natural killer cells in simian immunodeficiency virus-infected monkeys. *Am J Pathol*, *177*, 355–61.

Persidsky, Y., Limoges, J., McComb, R., Bock, P., Baldwin, T., Tyor, W., et al. (1996). Human immunodeficiency virus encephalitis in SCID mice. *Am J Pathol*, *149*(3), 1027–53.

Pilcher, C. D., Shugars, D. C., Fiscus, S. A., Miller, W. C., Menezes, P., Giner, J., et al. (2001). HIV in body fluids during primary HIV infection: Implications for pathogenesis, treatment and public health. *AIDS*, *15*, 837–45.

Potash, M. J., Chao, W., Bentsman, G., Paris, N., Saini, M., Nitkiewicz, J., et al. (2005). A mouse model for study of systemic HIV-1 infection, antiviral immune responses, and neuroinvasiveness. *Proc Natl Acad Sci USA*, *102*(10), 3760–5.

Roberts, E. S., Burudi, E. M., Flynn, C., Madden, L. J., Roinick, K. L., Watry, D. D., et al. (2004). Acute SIV infection of the brain leads to upregulation of IL6 and interferon-regulated genes: Expression patterns throughout disease progression and impact on neuroAIDS. *J Neuroimmunol*, *157*, 81–92.

Roberts, E. S., Huitron-Resendiz, S., Taffe, M. A., Marcondes, M. C., Flynn, C. T., Lanigan, C. M., et al. (2006). Host response and dysfunction in the CNS during chronic simian immunodeficiency virus infection. *J Neurosci*, *26*, 4577–85.

Robertson, K. R., Su, Z., Margolis, D. M., Krambrink, A., Havlir, D. V., et al.; A5170 Study Team. (2010). Neurocognitive effects of treatment interruption in stable HIV-positive patients in an observational cohort. *Neurology*, *74*(16), 1260–6.

Sacktor, N. C., Wong, M., Nakasujja, N., Skolasky, R. L., Selnes, O. A., Musisi, S., et al. (2005). The International HIV Dementia Scale: A new rapid screening test for HIV dementia. *AIDS*, *19*(13), 1367–74

Schnabel, J. (2008). Neuroscience: Standard model. *Nature*, *454*, 682–5.

Schnell, G., Price, R. W., Swanstrom, R., & Spudich, S. (2010). Compartmentalization and clonal amplification of HIV-1 variants in t he cerebrospinal fluid during primary infection. *J Virol*, *84*(5), 2395–407.

Schweinsburg, B. C., Taylor, M. J., Alhassoon, O. M., Gonzalez, R., Brown, G. G., et al.; HNRC Group. (2005). Brain mitochondrial injury in human immunodeficiency virus-seropositive (HIV+) individuals taking nucleoside reverse transcriptase inhibitors. *J Neurovirology*, *11*(4), 356–64.

Spudich, S. S., Nilsson, A. C., Lollo, N. D., Liegler, T. J., Petropoulos, C. J., Deeks, S. G., et al. (2005). Cerebrospinal fluid HIV infection and pleocytosis: Relation to systemic infection and antiretroviral treatment. *BMC Infect Dis*.

Tatro, E. T., Scott, E. R., Nguyen, T. B., Salaria, S., Banerjee, S., Moore, D/J., et al. (2010). Evidence for alteration of gene regulatory networks through microRNAs of the HIV-infected brain: Novel analysis of retrospective cases. *PLoS One*, *5*(4), e10337.

Veazey, R. S., DeMaria, M., Chalifoux, L. V., Shvetz, D. E., Pauley, D. R., Knight, H. L., et al. (1998). Gastrointestinal tract as a major site of CD4+ T cell depletion and viral replication in SIV infection. *Science*, *280*, 427–31.

Toggas, S. M., Masliah, E., Rockenstein, E. M., Rall, G. F., Abraham, C. R., & Mucke, L. (1994). Central nervous system damage produced by expression of the HIV-1 coat protein gp120 in transgenic mice. *Nature*, *367*(6459), 188–93.

Tyor, W. R., Power, C., Gendelman, H. E., & Markham, R. B. (1993). A model of human immunodeficiency virus encephalitis in SCID mice. *Proc Natl Acad Sci USA*, *90*(18), 8658–62.

Zink, M. C., Brice, A. K., Kelly, K. M., Queen, S. E., Gama, L., Li, M., et al. (2010). Simian immunodeficiency virus-infected macaques treated with highly active antiretroviral therapy have reduced central nervous system viral replication and inflammation but persistence of viral DNA. *J Infect Dis*, *202*, 161–70.

Zou, W., Kim, B. O., Zhou, B. Y., Liu, Y., Messing, A., & He, J. J. (2007). Protection against human immunodeficiency virus type 1 Tat neurotoxicity by Ginkgo biloba extract EGb 761 involving glial fibrillary acidic protein. *Am J Pathol*, *171*(6), 1923–35.

Zucco, G. M. & Ingegneri, G. (2004). Olfactory deficits in HIV-infected patients with and without AIDS dementia complex. *Physiol Behav*, *80*, 669–674.

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SECTION 1

HIV-1 BIOLOGY AND IMMUNOLOGY

Harris A. Gelbard

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1.1

HIV-1 BIOLOGY

Sergey Iordanskiy and Michael Bukrinsky

Viral determinants necessary for efficient human immunodeficiency virus (HIV) infection of cells of the central nervous system (CNS) parallel those of the immune system. Factors that regulate viral infection of its host and instigate specific pathogenic outcomes are often a combination of host and virus-specific products. Any understanding of how HIV affects the CNS must first start with a thorough analysis of functional and molecular properties of the virus. This chapter serves this stated purpose.

OVERVIEW

Many patients infected with human immunodeficiency virus type 1 (HIV-1) develop a syndrome of neurologic deterioration known as HIV-1-associated neurocognitive disorders (HAND); the most severe form is HIV-associated dementia (HAD). Well-accepted neuropathologic correlates of HAD include encephalitis, increased numbers of macrophages in the brain, and the presence of HIV antigen-positive microglial cells (for a recent review see Kaul, 2009). Neurons are not productively infected by HIV-1; thus, the mechanism of HIV-induced neuronal injury is most likely indirect. It is generally accepted that the primary host cells for productive HIV infection within the brain are cells of the macrophage lineage, such as perivascular macrophages and microglial cells, pointing to a pivotal role for the macrophage in the development of HAND. The most likely mechanisms for HIV-induced central nervous system (CNS) injuries is neuronal apoptosis. Microglial and glial activation, directly or indirectly related to HIV infection, plays a major role in neuronal death, possibly through the mediation of oxidative stress (Turchan et al., 2003; Gray et al., 2001; Louboutin, Agrawal, Reyes, Van Bockstaele, & Strayer, 2007).

HIV neurotropism does not always correlate with neurovirulence, as some patients do not show signs of neuronal damage despite the presence of replicating HIV-1 in the brain (Gorry et al., 2001). Prototype HIV-1 isolates from the CNS are macrophage (M)-tropic, non-syncytia-inducing (NSI), and use CCR5 for entry (R5 strains). However, in vitro studies have shown that X4 viruses induce neuronal apoptosis more frequently than R5 viruses (Ohagen et al., 1999; Zheng et al., 1999), and highly neurovirulent X4 strains can be isolated from the CNS (Yi et al., 2003; Zheng et al., 1999). Interestingly, despite X4 tropism, these viruses effectively replicate in macrophages. Similarly, X4 viruses are generally more cytopathic than R5 viruses for cells of the immune system.

In this chapter, which will overview the molecular biology of HIV, we will focus on those aspects of HIV biology that are important for replication of this virus within cells of the CNS.

HIV-1 GENOMIC ELEMENTS AND PROTEINS

HIV-1 belongs to the group of complex retroviruses whose replication is controlled by several regulatory and accessory proteins that are expressed in addition to classical retroviral structural proteins and enzymes encoded by *gag*, *pol*, and *env* genes. The nucleotide sequencing of the original HIV-1 isolates revealed multiple overlapping open reading frames (ORFs) in addition to these archetypal retroviral genes (Figure 1.1.1).

HIV-1 LONG TERMINAL REPEAT (LTR)

The LTRs are two identical regions at the ends of viral DNA that are generated during the process of reverse transcription. In the integrated proviral DNA, the LTRs serve as the main function of regulating viral RNA synthesis. The highly complex structure of the LTR contains important regulatory regions for transcription initiation and polyadenylation. The 630 bp-long HIV-1 LTR is functionally divided into three regions. The R (repeat) region of the LTR corresponds to a 92 nucleotide repeat located at both termini of the HIV-1 genomic RNA. The U5 region is an 84 nucleotide sequence located at the 5' end of the viral genome; it is positioned immediately downstream of the R region in the LTR. The U3 segment (454 nucleotides) corresponding to the sequence at the 3' end of the viral genome is located 5' to the R region in the LTR. Thus, the overall arrangement in the LTR is 5'-U3-R-U5–3'. The U3 region of the LTR also harbors part of the *nef* ORF, which contributes to the larger size of HIV-1 U3 as compared to other retroviruses.

During replication, LTR sequences serve additional functions (beyond regulation of transcription), both at the DNA and RNA levels. DNA and RNA sequences in the R region of LTR participate in the formation of DNA–RNA hybrids in an early step of reverse transcription (first template switch). During integration, LTR sequences (*att*) at the

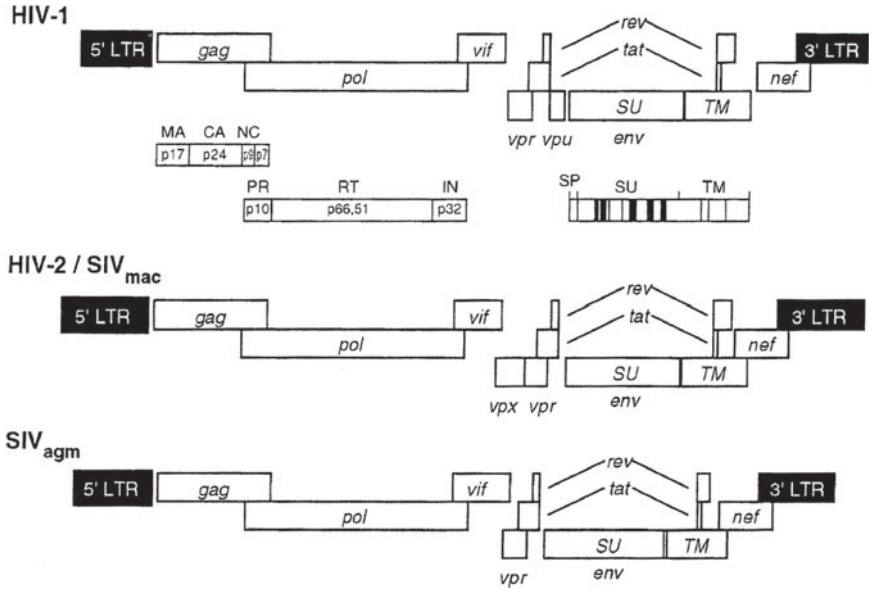

Figure 1.1.1 Genomic maps of HIV-1, HIV-2, and SIV. The provirus genomic organization is shown.

termini of the full-length linear DNA mediate insertion into the host cell genome (Masuda, Kuroda, & Harada, 1998). Sequences in the U5 RNA are involved in the packaging of the progeny HIV-1 RNA genome during viral assembly.

Since the main function of the LTR is to serve as a promoter region, it contains several elements that facilitate Pol II activity on an HIV-1 template. These include a variety of general transcription factors, such as TFIID, that bind to the TATA elements upstream of the transcription initiation site (Baltimore, 1970), thus setting the stage for the assembly of the transcription complex. Other transcription factors that bind to HIV-1 LTR include Sp1, NF-κB, NRE, Ap-3-like, DBF-1 (Pereira, Bentley, Peeters, Churchill, & Deacon, 2000). The HIV-1 genome codes for three factors that can modulate 5'-LTR transcription - Nef, Vpr, and Tat (see below). In addition, chromatin structure associated with the LTR contributes to the activation and repression of the LTR in response to external stimuli (He, Ylisastigui, & Margolis, 2002; Blazkova et al., 2009; Holmes, Knudsen, Key-Cushman, & Su, 2007). Regulation of the HIV-1 genome expression plays a pivotal role in the replication and pathogenesis of this virus and the LTR is central to these events.

TAT RESPONSIVE ELEMENT (TAR)

TAR, or the target sequence for viral transactivation, is the binding site for the viral transactivating protein, Tat, and its partner cellular proteins involved in HIV-1 transcription. TAR RNA forms a hairpin stem-loop structure 59 nucleotides long in HIV-1 and 100 nucleotides long in HIV-2 and simian immunodeficiency virus [SIV]) with a side bulge. The bulge is necessary for Tat binding and function. The minimal TAR element maps between bases +19 and +43 and contains three important components: a base-paired stem, a trinucleotide bulge that contains UCU at positions +23 to +25 and a hexanucleotide G-rich loop (Greenbaum, 1996). TAR functions as an RNA element that recruits cellular RNA polymerase machinery to the HIV-1 promoter, thus stimulating viral transcription.

REV RESPONSIVE ELEMENT (RRE)

RRE is a cis-acting RNA element present in all unspliced and partially spliced viral mRNAs. It consists of approximately 200 nucleotides (positions 7327 to 7530 from the transcription start in HIV-1, spanning the border of gp120 and gp41). It has a complex secondary structure containing several stem-loop arrangements branching from a large central bubble (Grate & Wilson, 1997). The RRE is a binding site for Rev, a viral protein regulating nuclear export of viral intron-containing RNAs (e.g., Gag, Env, and genomic RNAs). Other lentiviruses (HIV-2, SIV, visna) have similar RRE elements in similar locations within *env*.

STRUCTURAL PROTEINS (GAG, POL, ENV)

Gag

Gag is the genomic region encoding group-specific antigens. Gag proteins are necessary and sufficient for the formation of noninfectious, virus-like particles. Retroviral Gag proteins are synthesized as a polyprotein precursor (Figure 1.1.2) containing the p55 myristylated protein (Pr55 Gag), which is processed to p17 (MAtrix), p24 (CApsid), p7 (NucleoCapsid), and p6 proteins by the viral protease during virus maturation, which occurs after budding of the nascent virions from the infected cell. Gag associates with the cholesterol- and sphingomyelin-enriched lipid raft regions of the plasma membrane, where virus assembly takes place. This association

is determined by membrane-targeting signals within the MA portion of the Pr55 Gag (see below).

Matrix Antigen (MA)

The MA domain is located at the N-terminal end of the Gag precursor and plays several important roles during viral life cycle. During the assembly step, it directs Pr55 Gag to the plasma membrane via membrane-binding signals. The affinity towards membrane is provided by the myristoylation of the N-terminal glycine residue of p17 (Morikawa, Hockley, Nermut, & Jones, 2000). Another MA feature responsible for interaction with the membrane is a patch of basic amino acid residues that can interact with the negatively charged phospholipids on the inner surface of the plasma membrane (Ono & Freed, 1999). Specific targeting of Pr55 to lipid rafts is determined by phosphatidylinositol-(4,5)-bisphosphate, a phospholipid that is enriched in lipid rafts and can specifically interact with the MA globular domain (Ono, Ablan, Lockett, Nagashima, & Freed, 2004; Saad, Miller, Tai, Kim, Ghanam, & Summers, 2006; Chukkapalli, Hogue, Boyko, Hu, & Ono, 2008). Thus, matrix plays an important role in the trafficking of Gag to the plasma membrane and participates in assembly of the viral particles.

It has been suggested that matrix participates in early events following fusion between the viral and cellular membranes. Mutations in the matrix protein have been shown to impair viral reverse transcription (Kiernan, Ono, Englund, & Freed, 1998). This effect is supposedly due to an inability of the mutant matrix to disengage from the lipid bilayer resulting in an uncoating defect and failure to form a functional reverse transcription complex (Koh et al., 2000).

One of the properties of matrix that is of special interest to this chapter is its activity in the HIV-1 infection of nondividing cells, in particular, macrophages. The basic domains located in the N-terminal part of matrix resemble nuclear localization signals of cellular nuclear proteins and were proposed to play a role in translocating the viral preintegration complex to the nucleus (Bukrinsky et al., 1993; Haffar et al., 2000). This interesting step of the HIV-1 life cycle is discussed later in this chapter.

Capsid Antigen (CA)

The CA protein also functions in the early and the late stages of the viral replication. It contains two structural and functional domains. The N-terminal portion forms the core domain and the C-terminal is the dimerization domain (Freed, 2001). The core domain is a helical structure and has the cyclophylin A (CypA)–binding loop (Luban, Bossolt, Franke, Kalpana, & Goff, 1993). Incorporated CypA is required during an early post-entry step of de novo infection (Braaten & Luban, 2001), likely by protecting the incoming HIV from a host restriction factor (Sokolskaja & Luban, 2006), but exact mechanisms of this activity are still debated.

Nucleocapsid (NC)

The NC is involved in the specific encapsidation of full-length, unspliced genomic RNA into virions. HIV-1 NC

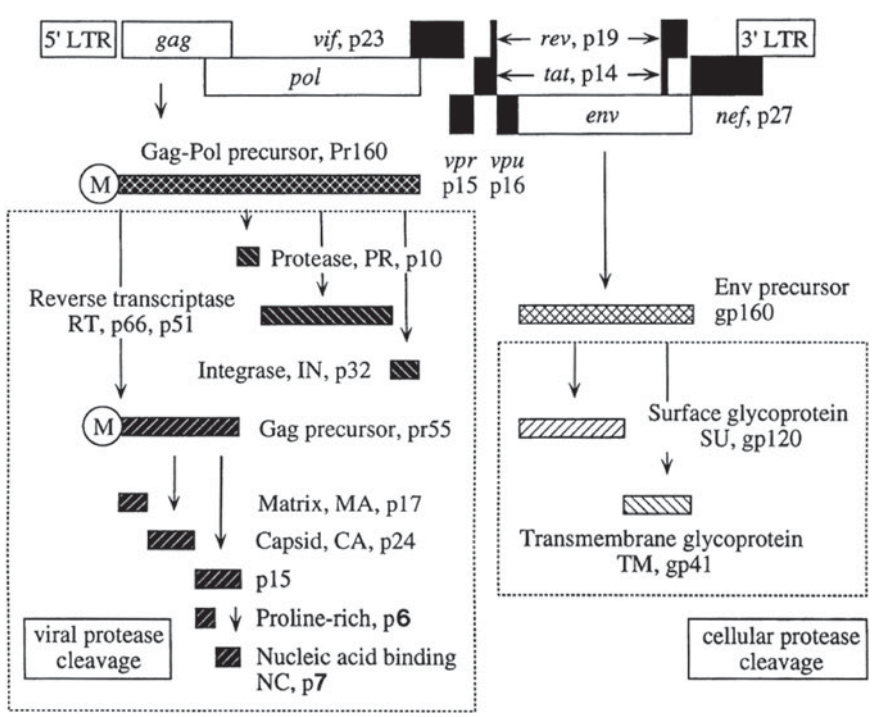

Figure 1.1.2 HIV-1 protein processing. The Gag-Pol precursor of 160 kDa (Pr160) is processed by the viral (aspartyl) protease into seven proteins, which include four Gag proteins (MA, p17; CA, p24; proline—rich, p6; NC, p9), protease (PR, p10), reverse transcriptase (RT, p66/p51) and integrase (IN, p32). The Env precursor (gp160) is processed by a cellular protease into the surface glycoprotein (SU, gp120) and the transmembrane glycoprotein (TM, gp41). Viral regulatory and accessory proteins, which include Tat (p14), Rev (p19), Nef (p27), Vif (p23), Vpr (p15), and Vpu (p16), are not processed. M, myristyolated (Figure courtesy of J. Levy).

protein contains two zinc-finger motifs, which can bind zinc strongly in virions and in vitro (Feng et al., 1996). Mutations or drugs that eliminate zinc-binding capacity of NC greatly diminish viral infectivity (McDonnell, De Guzman, Rice, Turpin, & Summers, 1997). The specificity of HIV genome encapsidation is based on the interaction between NC and a 120-nucleotide sequence located between the 5'-LTR and the Gag initiation codon. Thus, this sequence is called packaging signal, encapsidation element, or ψ-site (Lawrence, Stover, Noznitsky, Wu, & Summers, 2003).

NC is also required for efficient reverse transcription and initial integration processes in a target cell (Buckman, Bosche, & Gorelick, 2003). This NC activity also involves the zinc fingers and appears to be mediated by a protective effect of NC on the full-length viral DNA.

p6 Protein

Retroviral *gag* genes encode a variety of additional ORFs that are generally unique to particular genera of retroviruses. HIV-1 has a proline-rich 6 kD protein, called p6, at the C-terminus of the Pr55 Gag. The p6 region of p55 is responsible for incorporation into virions of another viral protein, Vpr (Checroune, Yao, Gottlinger, Bergeron, & Cohen, 1995). P6 also contains specific sequences, YPLTSL and PTAP, called the late domains, which bind cellular proteins normally functioning as part of the endosomal sorting complexes required for transport (ESCRT) machinery (Garrus et al., 2001). The ESCRT machinery normally mediates the budding of vesicles into the late endosomal lumen, and by recruiting this machinery HIV achieves separation of the budding virions from the membrane of a producer cell (Bieniasz, 2006; Morita & Sundquist, 2004). Thus, PTAP binds Tsg101 (a component of ESCRT-I) and YPLTSL binds ALIX (an ESCRT-I- and ESCRT-III-binding protein). Mutations in late domain sequences lead to inefficient release of the virions, which accumulate at the plasma membrane.

Pol

The *pol* gene represents the genomic region encoding the viral enzymes protease (PR), reverse transcriptase (RT) and integrase (IN). These enzymes are produced as a Gag-Pol precursor polyprotein, Gag-Pol Pr160, which is processed by the viral protease. The Gag-Pol precursor is produced by ribosome frameshifting at the C-terminus of the *gag* gene, thus allowing the ribosomes to avoid the Gag termination codon and fusing the Gag and Pol reading frames (Figure 1.1.2). The *pol* gene is the most conserved region of HIV-1. The *pol*-encoded viral enzymes are targets for currently used anti-HIV drugs.

Protease

The HIV-1 protease plays a critical role in late stages of the viral life cycle. It mediates the production of mature, infectious virions by cleaving the Gag Pr55 and Gag-Pol Pr160. This proteolytic digestion is mandatory for viral infectivity. Retroviral proteases are related to pepsin and similar "aspartic" proteases (Dunn, Goodenow, Gustchina, & Wlodawer, 2002). Interestingly, while the primary sequences of retroviral proteases are highly divergent, their tertiary structures are remarkably similar. The HIV-1 protease functions as a dimer, which carries flexible overhangs that cover the binding site (Dunn et al., 2002). Introduction of protease inhibitors into anti-retroviral drug cocktails has revolutionized the efficacy of anti-HIV therapy.

Reverse Transcriptase (RT)

The retrovirus nomenclature is consistent with the properties of this enzyme. By definition, the replication cycle of all retroviruses includes a step of reverse transcriptase (RNA-dependent DNA polymerase) mediated conversion of the RNA genome into a DNA copy. RT directs synthesis of both minus and plus strands of viral DNA and also degrades the tRNA primer and genomic RNA present in the RNA-DNA hybrid intermediates with its RNAse H activity. Reverse transcription of the HIV-1 RNA genome is a complex process completed via multiple steps (Jonckheere, Anne, & De Clercq, 2000). A plethora of RT inhibitors have been developed and are currently used in highly active antiretroviral therapy (HAART).

Integrase (IN)

Retroviruses insert their full-length genome into the host cell genome via activity of the integrase. Integrase is a 32 kDa protein generated by the viral protease-mediated cleavage of the C-terminal portion of the HIV-1 Gag-Pol polyprotein precursor. Integrase inhibitors potently suppress HIV replication and represent a recent advance in anti-HIV therapy (Marchand, Maddali, Metifiot, & Pommier, 2009).

Envelope (Env)

Viral envelope glycoproteins are produced as a heavily glycosylated precursor protein (gp160). At a late stage of synthesis, most probably in the trans-Golgi network, gp160 is cleaved by furin or other related subtilisin-like proteases into the surface (SU; gp120) and transmembrane (TM; gp41) subunits (Moulard & Decroly, 2000). The gp120 and gp41 proteins then remain noncovalently associated, forming the functional, native trimeric gp120-gp41 complex (Figure 1.1.3), which is delivered to the plasma membrane via the endoplasmic reticulum network (Chen, Lee, & Wang, 2001; Ou & Silver, 2005; Bultmann, Muranyi, Seed, & Haas, 2001; Leung et al., 2008). Since the noncovalent interaction between gp120 and gp41 is weak, a substantial amount of gp120 can be released in the medium. The gp120 is the major antigenic determinant of HIV-1 that triggers a potent antibody response in infected individuals. The viral Env glycoproteins play a major role in viral entry into target cells. Gp120 contains the binding sites for the CD4 receptor and the chemokine co-receptor that serve as attachment sites for HIV-1, whereas gp41 mediates fusion between the viral and target cell membranes (Melikyan, 2008). Interestingly, virus-cell fusion occurs not at the cell surface, as was surmised for a long time, but inside the cell, after endocytosis of the virus-receptor complex (Miyauchi, Kim, Latinovic, Morozov, & Melikyan, 2009).

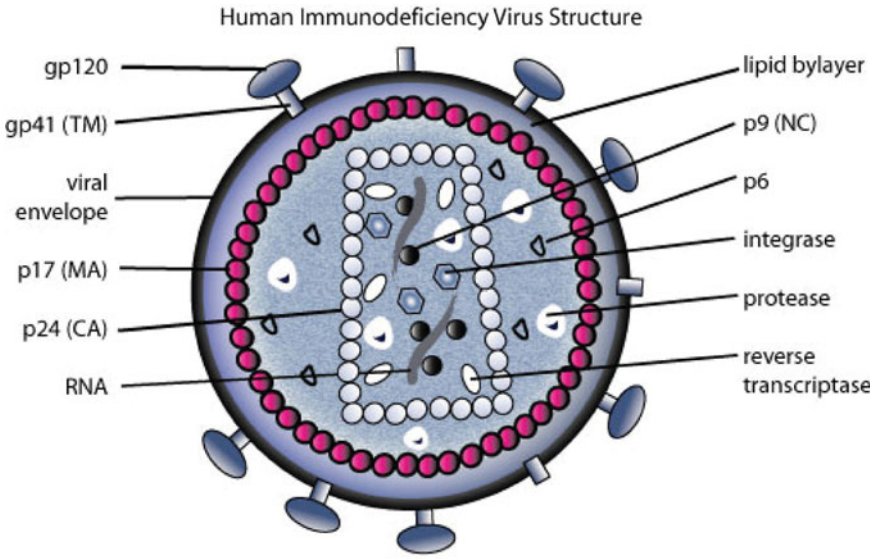

Figure 1.1.3 Schematic representation of the mature virion. This schematic representation of the virus shows the relative locations of the structures and proteins. The virion proteins that make up the envelope (each knob represents trimeric gp120 non-covalently attached to trimeric gp41) and nucleocapsid (p24, p17, p9, p7) are noted. The genome consists of diploid RNA associated with reverse transcriptase and integrase.

Regulatory Proteins (Tat, Rev)

HIV-1 encodes two essential regulatory proteins, Tat and Rev. The Tat protein interacts with the regulatory element TAR and Rev interacts with RRE. They both modulate transcriptional (Tat) and posttranscriptional (Rev) steps of viral gene expression.

TransActivator of Transcription (Tat)

The HIV Tat increases the steady-state levels of viral RNA several hundred fold (Marcello, Zoppe, & Giacca, 2001). HIV-1 Tat is a nuclear protein containing 101 amino acids encoded by two exons. It acts by binding to the TAR RNA element and activates transcription elongation by recruiting to the HIV-1 transcription unit the pTEFb factor, which phosphorylates the C-terminal domain of RNA polymerase II, thus increasing its processivity (Liang & Wainberg, 2002). It is the first eukaryotic transcription factor known to interact with RNA rather than DNA and may have similarities with prokaryotic anti-termination factors. Tat is also found in the extracellular milieu and can be taken up by cells in culture to activate LTR-driven transcription.

Regulator for Expression of Viral Proteins (Rev)

For HIV progeny to be produced, unspliced HIV-1 Gag-Pol encoding RNA and partially spliced Env-encoding RNA need to exit the nucleus to associate with ribosomes for translation. However, cellular nuclear export machinery does not support export of unspliced or incompletely spliced RNAs. To circumvent this limitation, HIV-1 relies on Rev. Rev is a 19 kDa phosphoprotein localized primarily in the nucleolus/nucleus. It binds to the RRE sequence present in viral Gag, Gag-Pol, and Env mRNAs and promotes their nuclear export by connecting them to the cellular exportin protein, CRM1, which is responsible for nuclear export of cellular messenger RNAs (Cullen, 1998). In addition, Rev regulates HIV splicing by engaging a number of cellular cofactors, such as eIF5a, hRIP, Sam68 and RNA helicases (Suhasini & Reddy, 2009).

Accessory/Auxiliary Proteins (Vif, Vpr, Vpu, Nef)

HIV-1 encodes four additional virion- and non-virion-associated proteins, Vif, Vpr, Vpu and Nef. They are referred to as accessory or auxiliary proteins as early evidence suggested that their presence was not essential for HIV-1 replication. However, later studies demonstrated that these proteins are highly conserved in viral isolates and play important roles in viral replication in vivo. The mechanisms of their activity are actively investigated, and current evidence suggests that the role of these HIV proteins is to either counteract intrinsic cellular anti-viral activities termed restriction factors (Bieniasz, 2004) or down-regulate cellular proteins that inadvertently interfere with HIV replication.

Viral Infectivity Factor (Vif)

Vif is a basic 192 amino acid protein with the molecular mass of 23 kDa, which promotes the infectivity of HIV-1 without affecting production of viral particles. Vif is essential for viral replication in HIV natural targets, primary CD4+ T cells and macrophages. Certain cell lines support replication of *vif*-deficient viruses, and cell fusion experiments indicated that such cells lack expression of inhibitory factor(s) that naturally block viral replication when Vif is absent (reviewed in Malim & Emerman, 2008). Analysis of mRNA expression profiles in cells restrictive for replication of Vif-deficient HIV-1 identified *APOBEC3G* (*A3G*) as a cellular anti-HIV inhibitory factor targeted by Vif (Sheehy, Gaddis, Choi, & Malim, 2002). This protein is a member of the APOBEC family of editing deaminases, and its activity leads to

deamination of the cytidine (C) to uridine (U) in the first newly synthesized DNA strand of HIV-1 cDNA, resulting in massive guanosine (G)-to-adenosine (A) transitions in the complementary strand (Zennou, Perez-Caballero, Gottlinger, & Bieniasz, 2004). In the absence of Vif, host cellular A3G is packaged into budding viral particles through interactions with viral RNA and Gag (Bogerd & Cullen, 2008; Soros, Yonemoto, & Greene, 2007) and consequently carried forward to newly infected cells where it impairs viral reverse transcription (Harris & Liddament, 2004). About 10% of G residues can be mutated to A as a result of this process, which is sufficient to stop further viral spread through the gross loss of genetic integrity (Malim & Emerman, 2008).

Vif effectively antagonizes the antiviral effects of A3G through induction of polyubiquitylation and subsequent degradation of A3G (Conticello, Harris, & Neuberger, 2003; Mehle et al., 2004). By eliminating A3G from virus-producing cells, Vif allows progeny viral particles to be free of A3G. It should be noted here that *A3G* is one of a family of seven cytidine deaminase genes encoded in human genome (Holmes, Malim, & Bishop, 2007b); another member of this family, A3F, is also active in restricting replication of Vif-deficient HIV-1, and Vif induces its polyubiquitylation and degradation (Liu et al., 2005).

Viral Protein R (Vpr)

Vpr is a 96-amino acid (14 kDa) accessory protein, highly conserved among primate lentiviruses (Tristem, Purvis, & Quicke, 1998). Vpr is incorporated into the virion due to interaction with the NC p7 and p6 portions of Pr55 Gag precursor (Lavallee et al., 1994; de Rocquigny et al., 1997). Proposed activities of Vpr include the nuclear import of the viral pre-integration complexes, growth arrest of target cells in the G2 phase, induction of apoptosis in infected cells, and transactivation of viral genes (Bukrinsky & Adzhubei, 1999; Planelles & Benichou, 2010). The ability to cause cell cycle blockade is conserved among Vpr proteins from different isolates and thus is important for HIV-1 infection. A likely reason for this is that G_2 arrest may promote optimal transcription from the LTR and an increase in viral output (Goh et al., 1998; Hrimech, Yao, Bachand, Rougeau, & Cohen, 1999; Andersen, Le, & Planelles, 2008). Data about pro-apoptotic activity of Vpr and association of the Vpr-induced apoptosis with cell-cycle arrest are still controversial. Some studies suggest that Vpr-induced apoptosis of infected cells is linked to the pathway leading to G2 arrest (Andersen et al., 2006). However, other evidence suggests that cell cycle blockade and cytopathicity are independent functions, at least in CD4+ T (Chen et al., 1999; Nishizawa, Kamata, Mojin, Nakai, & Aida, 2000; Bolton & Lenardo, 2007). Moreover, some reports indicate that the cytostatic effect of Vpr serves to prevent cell death and, by some accounts, protects against apoptotic stimuli ((Bartz, Rogel, & Emerman, 1996; Conti et al., 1998). The reason for this controversy is likely due to the different experimental conditions used in different studies, and in particular the level of Vpr expression. It is possible that low levels of Vpr expression protect cells from apoptosis, whereas high expression promotes cell death. Notably, some of these activities, and in particular the G2 arrest, depend on association of Vpr with DCAF1/VprBP (Le et al., 2007). DCAF1 bridges Vpr to DDB1, a core subunit of Cul4 E3 ubiquitin ligases, which are involved in regulation of protein degradation by proteasomes.

Intriguingly, Vpr activity is much more pronounced in macrophages than in CD4+ T cells (Subbramanian et al., 1998). Initially, this finding was explained by the role of Vpr in HIV nuclear import, which was considered to be required for viral replication in non-dividing, but not in proliferating cells. However, later studies demonstrated that travel through the nuclear pore is a necessary step in HIV replication in both dividing and non-dividing cells (Bukrinsky, 2004; Yamashita & Emerman, 2006; Riviere, Darlix, & Cimarelli, 2010). Recent evidence suggests that a close relative of Vpr, the Vpx protein found in SIV and HIV-2, functions to counteract an as yet unidentified cellular restriction factor that limits viral replication in macrophages (Sharova et al., 2008; Srivastava et al., 2008). This activity of Vpx depends on interaction with DDB1-Cul4 E3, the same ubiquitin ligase that interacts with Vpr (Bergamaschi et al., 2009; Gramberg et al., 2010; Sharova et al., 2008; Srivastava et al., 2008). Nevertheless, Vpr appears not to target this hypothetical restriction factor, as Vpx potently stimulates replication in macrophages of a Vpr-positive HIV-1 (Sharova et al., 2008). Therefore, either Vpr is a weaker inhibitor of a Vpx-targeted restriction factor, or Vpr's target is different from that of Vpx. Vpr and Vpx may both work in concert with an ubiquitin-proteasome system to limit cellular factor(s) restricting HIV replication in macrophages, and additional factors determine the target of this activity.

Viral Protein U (Vpu)

Vpu is unique to HIV-1 and SIVcpz, a close relative of HIV-1. There is no similar gene in HIV-2 or other SIVs. Vpu is a 16-kDa (81-amino acid) type I integral membrane protein with at least two biological activities: (a) degradation of CD4 in the endoplasmic reticulum, and (b) virion release from the plasma membrane. Vpu is not incorporated into virions, and its function is essential in virus-producing cells. The effect of Vpu on CD4 disrupts the complex between nascent gp120 and CD4 formed in the endoplasmic reticulum and allows delivery of Env proteins to the plasma membrane. The effect on virus release is due to ability of Vpu to counteract activity of a cellular interferon-induced restriction factor called B cell stromal factor 2 (BST-2), also known as CD317 or tetherin. This factor prevents release of enveloped viruses, including retroviruses, from the plasma membrane (Neil, Zang, & Bieniasz, 2008; Neil, Sandrin, Sundquist, & Bieniasz, 2007; Van Damme et al., 2008). Since HIV-1, as many other enveloped viruses, is known to accumulate and bud from lipid raft regions of the plasma membrane, tetherin may form connections between lipid rafts on plasma and viral membranes and thereby physically tether virions to cells (Perez-Caballero et al., 2009). As a result, viral particles made in the absence of Vpu in tetherin-expressing cells fail to detach from the plasma membrane and are transported to endosomes and degraded (Malim & Emerman, 2008). It is still not clear how Vpu counteracts tetherin activity. It may directly target tetherin for

proteasomal degradation (Neil et al., 2008) or reduce the surface expression of tetherin (Van Damme et al., 2008). Interestingly, macrophages express higher levels of tetherin than CD4+ T cells, explaining ability of viruses carrying certain Vpu mutations to replicate in T cells but not in macrophages (Schindler et al., 2010).

Negative Factor (Nef)

Nef is a multifunctional 27-kDa myristoylated protein produced by an open reading frame located at the 3'-end of the HIV-1 or SIV genome. It is an early protein abundantly expressed at all stages of infection. Nef is predominantly distributed in cytoplasm but can associate with the plasma membrane via the myristyl residue linked to the conserved second amino acid (Gly). One of the first HIV proteins to be produced in infected cells, Nef is the most immunogenic of the accessory proteins. Despite its original misleading name—the "negative factor," attributed to dispensability of Nef for in vitro infection—Nef in HIV and SIV is essential for efficient viral spread and disease progression in natural infections of different species of primates (Schindler et al., 2006). Viruses with defective Nef have been found in some HIV-1-infected long-term non-progressors (Dyer et al., 1997). Nef downregulates CD4 and MHC class I molecules, and these functions map to different parts of the protein (Geyer, Fackler, & Peterlin, 2001).

Interaction of Nef with the cytoplasmic tail of CD4 accelerates endocytosis of CD4 from the surface of infected cells with subsequent internalization through clathrin-coated pits, with transport to endosomes, and then lysosomes for degradation (Chaudhuri, Lindwasser, Smith, Hurley, & Bonifacino, 2007). Nef performs this function synergistically with another accessory protein, Vpu (described in the previous section). Downregulation of MHC I by Nef proceeds through a different, endocytosis-independent mechanism. Instead, Nef-induced MHC-I downregulation depends on PACS-1 (phosphofurin acidic cluster sorting protein-1) to sort and sequester MHC-I to the trans-Golgi network (Piguet et al., 2000; Crump et al., 2001). Suppression of MHC class I function blunts cytotoxic T cell (CTL) recognition of infected cells and provides a selective advantage to the virus (Malim & Emerman, 2008). Nef also downregulates CD28, a major co-stimulatory receptor that mediates effective T-cell activation (Swigut et al., 2001). In addition, Nef interacts with components of host cell signal transduction and clathrin-dependent protein sorting pathways (Fackler & Baur, 2002). Nef also is a major regulator of viral cholesterol, as it targets the cellular cholesterol transporter ABCA1, which reduces the amount of cholesterol delivered to assembling virions (Mujawar et al., 2006). Nef is incorporated into HIV virions and stimulates early post-entry steps of HIV replication through the interaction with proteins involved in the rearrangements of actin filaments to penetrate the cortical actin network, a known barrier for intracellular parasitic organisms (Campbell, Nunez, & Hope, 2004; Pizzato et al., 2007). In summary, most Nef activities are associated with modulation of cellular proteins in the infected cell to provide optimal conditions for viral propagation.

HIV-1 REPLICATION IN CELLS OF THE MONOCYTE/MACROPHAGE LINEAGE

It is generally accepted that the primary host cells for productive HIV infection within the brain are cells of the macrophage lineage, such as perivascular macrophages and microglial cells. Therefore, in this section we will focus on peculiarities of HIV-1 infection of macrophages as compared to infection of another susceptible cell type, $CD4^+$ T lymphocyte. The differences in HIV-1 replication between these two types of susceptible cells provide an understanding of what viral features define the neurotropism of HIV-1.

HIV-1 ENTRY/TROPISM

Since identification of CD4 as an essential component of HIV-1 receptor, it has been appreciated that additional molecules are required for the entry of HIV-1 into target cells. This idea came from experiments with several nonhuman cell lines, which remain resistant to HIV infection even when engineered to express human CD4. The nature of additional co-receptors remained elusive for many years, until the breakthrough report from Berger's group (reviewed in Berger, 1998). Using expression cloning, these authors identified an orphan 7-transmembrane domain G protein-coupled receptor (which they named fusin) as a co-receptor for T cell line-adapted HIV-1 strains. This co-receptor was later identified as a receptor for chemokine SDF-1α and renamed CXCR4. Following this report, another chemokine receptor, CCR5, was described by several groups as the main co-receptor for primary, macrophage-tropic HIV-1 isolates (Alkhatib et al., 1996; Deng et al., 1996; Dragic et al., 1996). The importance of CCR5 for HIV-1 transmission is underscored by the finding that individuals homozygous for a defective CCR5 allele (a 32 base pair deletion in the gene encoding CCR5 resulting in truncation of the receptor and its retention in the endoplasmic reticulum) are almost completely protected from HIV-1 infection (Liu et al., 1996). Transmitted HIV-1 strains are almost uniformly CCR5-tropic, but later in disease X4 strains may evolve. Emergence of X4 viruses is usually associated with rapid development of immunodeficiency and poor clinical prognosis (Casper et al., 2002).

In addition to promoting virus entry, interaction with chemokine co-receptor also contributes to post-entry steps of HIV replication by initiating signal transduction (Davis et al., 1997; Yoder et al., 2008). The signaling induced by gp120-CCR5 or CXCR4 interaction might facilitate various stages of the HIV replicative cycle, such as trafficking of the RTC through the cytoplasm, reverse transcription, and integration (Yoder et al., 2008; Lin et al., 2002). A correlation between the capacity of R5 HIV-1 strains to signal through CCR5 and to replicate in macrophages has been reported (Arthos et al., 2000). These data are consistent with a model of M-tropism in which early events in HIV replication in macrophages, in particular reverse transcription, are facilitated by signaling initiated by envelope-CCR5 interaction. Intriguingly, replication in macrophages of some primary X4 isolates could be enhanced by addition of CC chemokines (Arthos et al., 2000).

This observation indicates that signaling through CC chemokine receptors may promote the replication in macrophages of some primary CXCR4-utilizing isolates.

The mechanism by which envelope or CC chemokine signaling promotes HIV-1 replication in MDMs may involve changes in the cytoskeleton. Bukrinskaya et al. demonstrated that after viral entry, reverse transcription complexes rapidly localize to the cytoskeletal compartment (Bukrinskaya et al., 1998). In addition, they showed that actin polymerization is a prerequisite for efficient reverse transcription. CCR5 stimulation by CC chemokines promotes actin polymerization (Premack & Schall, 1996). Because M-tropic envelopes share with CC chemokines the ability to transduce signals through CCR5, it appears likely that CCR5-utilizing envelopes may also induce actin polymerization. This hypothesis is supported by our observation that heterologous desensitization of CCR5 by the B-oligomer of the pertussis toxin blocks gp120-induced capping of CD4 and CCR5, a process dependent on actin polymerization (Alfano, Schmidtmayerova, Amella, Pushkarsky, & Bukrinsky, 1999). A recent report indicated that signaling through CXCR4 activates a cellular actin depolymerization factor, cofilin, to promote the cortical actin dynamics that are critical for viral intracellular migration across the cortical actin barrier (Yoder et al., 2008). While this effect has been described for infection of T cells (Wu et al., 2008), it remains to be determined whether similar activities can be found in macrophages.

Molecular studies of viral sequences found in the brain underscore the critical role of macrophages and macrophage-tropic viruses in CNS disease. While an early report suggested that CCR3 (a receptor for chemokine eotaxin [CCL11]) may function as an HIV-1 co-receptor for infection of microglial cells (He et al., 1997), later studies demonstrated that CCR5 is the major co-receptor for HIV-1 infection of macrophages and microglia (Gabuzda & Wang, 1999). Furthermore, CCR5 is the principal co-receptor used by HIV-1 viruses isolated from the brain (Smit, et al., 2001; Li et al., 1999). However, CCR5 usage by primary brain-derived HIV-1 isolates is neither necessary nor sufficient for neurotropism (defined as the ability of viruses to replicate in microglia), as infection of microglial cells by some isolates occurs via the CXCR4 co-receptor (Gorry et al., 2001; Yi et al., 2003). Importantly, some R5 isolates described in the Gorry et al. study did not replicate in macrophages due to a block to virus entry, indicating that additional factors, beside co-receptors, are involved in regulating macrophage- and neuro-tropism.

One such factor may be CD4 density on the surface of macrophages. Studies in rhesus macaques demonstrated that low levels of CD4 on macrophages account for the lack of infection by M-tropic HIV-1 or T-tropic SIV strains (Bannert, Schenten, Craig, & Sodroski, 2000; Mori et al., 2000). Indeed, CD4 expression levels on macrophages are lower than on T lymphocytes, and this difference might explain in part low efficiency of entry into macrophages of X4 strains (Dimitrov et al., 1999). It may also restrict entry of some R5 viruses (Platt, Wehrly, Kuhmann, Chesebro, & Kabat, 1998). Other possible factors affecting macrophage tropism are affinity of the interaction between gp120 and the co-receptor (CCR5 or CXCR4) and the exposure of the co-receptor binding site on gp120 (Doms, 2000).

Overall, macrophage (M)-tropism appears to fully determine the capacity of an HIV or SIV isolate to replicate in the CNS. Macrophage tropism is necessary for the virus to remain in the CNS and for the development of encephalitis.

REVERSE TRANSCRIPTION

Following HIV-1 entry into a target cell, viral RNA genome is converted to a DNA form by the viral RNA-dependent DNA polymerase, reverse transcriptase. While reverse transcription is completed intracellularly, it may be initiated within virions outside the cell ("endogenous" reverse transcription); and this capacity may contribute to efficiency of HIV infection process (Zhang, Dornadula, & Pomerantz, 1998; 1996). Intracellular reverse transcription of HIV-1 entirely depends on the cellular dNTP pool. Treatment with hydroxyurea, which blocks de novo dNTP synthesis, has been shown to inhibit HIV-1 replication in acutely infected peripheral blood mononuclear cells (PBMC) and primary macrophages (Lori et al., 1994). The observed low dNTP pools in quiescent T cells and macrophages might explain the slow rate of reverse transcription in these cells as compared to activated T lymphocytes (Meyerhans et al., 1994; O'Brien et al., 1994; Collin & Gordon, 1994; Zack et al., 1990). Indeed, activated T cells reverse transcription is completed within 6 hours, while in macrophages it can take as long as 36–48 hours (O'Brien et al., 1994). However, in contrast to quiescent T lymphocytes, where inefficient reverse transcription is one of the main blocks to HIV replication (Zack et al., 1990), substrate limitations in mononuclear phagocytes slow but do not arrest HIV-1 reverse transcription. As a result, virus replicates efficiently in macrophages, albeit with slower kinetics.

Interestingly, the effects of cell activation on the fate of the virus are opposite in T cells and macrophages. While activated T cells are highly susceptible to HIV-1 infection, in part due to high efficiency of viral reverse transcription in such cells, activated macrophages are resistant to infection by HIV-1 (Zybarth et al., 1999; Pushkarsky et al., 2001). This resistance probably stems from increased degradation of the viral RNA, which is not compensated by an increased efficiency of reverse transcription in activated macrophages.

One characteristic feature of the genome of HIV-1 and other lentiviruses is the presence of two additional *cis*-acting sequences, the central polypurine tract (cPPT) and the central termination sequence (CTS). The effect of these sequences on reverse transcription is formation of a three-stranded DNA structure, the central DNA flap, which is essential for viral replication (Charneau, Alizon, & Clavel, 1992; Charneau et al., 1994; Arhel et al., 2006). This central DNA flap contributes to PIC (pre-integration complex) nuclear import (Zennou et al., 2000), likely by promoting uncoating (loss of CA p24) of the RTC at the nuclear membrane, thus preparing the RTC for translocation through the nuclear pore (Arhel et al., 2007).

NUCLEAR IMPORT

A crucial factor in HIV-1 replication in macrophages is the capacity of this virus to transport its reverse transcribed genome through the intact nuclear membrane. Although one report suggested that macrophage infection occurs during rare cell division events, possibly during monocyte-to-macrophage maturation process (Schuitemaker et al., 1994), an overwhelming evidence supports the notion that HIV-1 can infect terminally differentiated, non-dividing macrophages (Weinberg, Matthews, Cullen, & Malim, 1991; Schmidtmayerova et al., 1997; and reviewed in Stevenson & Gendelman, 1994). To be able to replicate in such cells, a mechanism for nuclear importation through intact nuclear membrane of the viral pre-integration complex (PIC) carrying HIV-1 genome is required. Inability to cross the nuclear membrane of the interphase cell is the main barrier that restricts replication of simple retroviruses to proliferating cells (Roe, Reynolds, Yu, & Brown, 1993).

The journey of the HIV-1 PIC to the cell's nucleus is comprised of several distinct steps. To deliver its PIC through the cytoplasm and into the nucleus, HIV-1 relies on the cellular machinery. Following the fusion of the viral and cell membranes, the HIV-1 nucleocapsid is released into the cytoplasm. This process is accompanied by partial dissociation of the capsid shell composed of the CA p24 (uncoating) and the formation of a reverse transcription complex (RTC) (step 1 in Fig. 1.1.4).

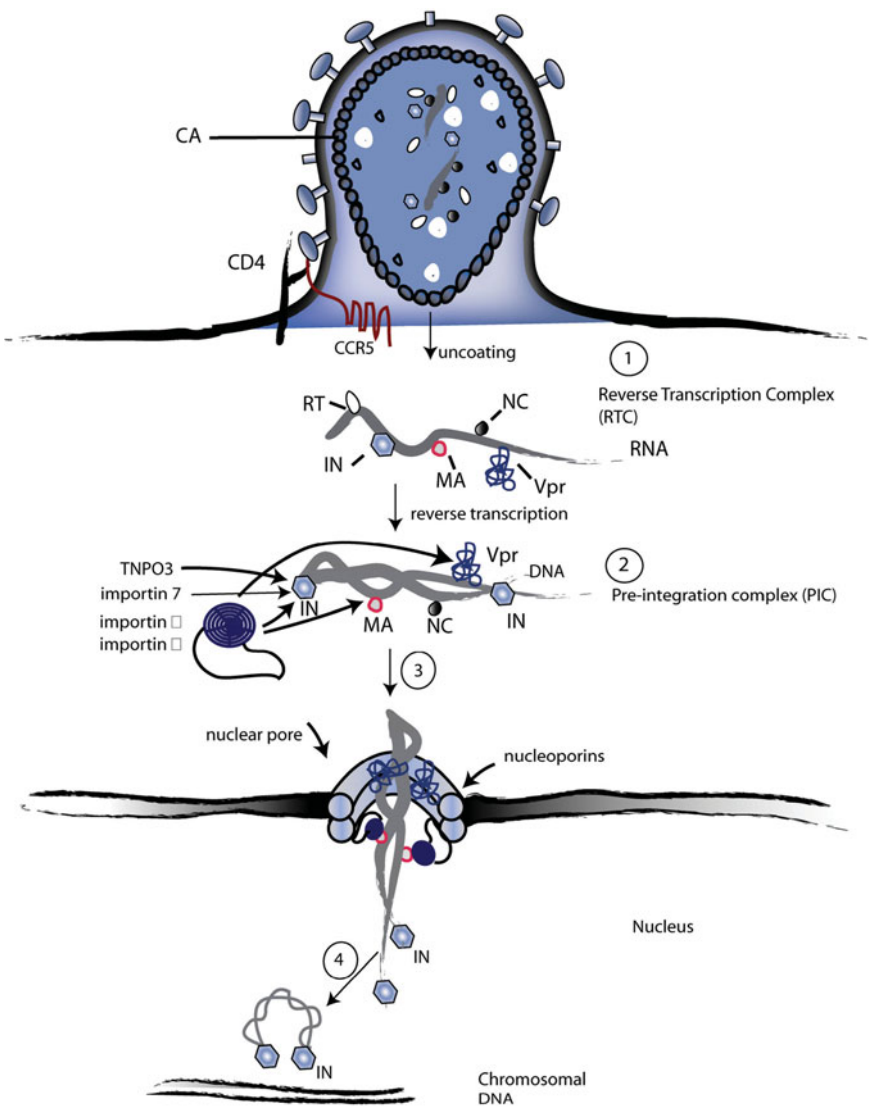

Figure 1.1.4 A model of HIV-1 nuclear translocation. Following binding to cell-surface receptors (CD4 and CCR5 or CXCR4), HIV-1 fuses with the cellular membrane resulting in the entry of the viral core into the cytosol. Entry is accompanied by initiation of a poorly defined step termed *uncoating*, the most characteristic feature of which is dissociation of the capsid shell made of capsid antigen (CA) and the formation of a reverse transcription complex (RTC) (step 1). Of note, uncoating continues along the translocation of the RTC through the cytoplasm and is completed at the nuclear membrane. Upon reverse transcription of the viral genomic RNA, the complex becomes competent for integration and is termed pre-integration complex (PIC) (step 2). Several cellular nuclear transport proteins, including importins α and β, bind to karyophilic proteins within the PIC, such as Vpr, MA, and IN, and target the complex to the nuclear pore (step 3). Importin β and Vpr mediate the interaction between the PIC and the nuclear pore proteins (nucleoporins). Transfer of the PIC through the nuclear pore is likely accompanied by dissociation of MA, Vpr, and NC proteins (step 4), leaving IN, which mediates integration of the viral genome into the host cell DNA.

Uncoating is likely a gradual process which proceeds in parallel to reverse transcription and the movement of the RTC through the cytoplasm, with final steps occurring at the nuclear membrane (Arhel et al., 2007). Because of the high viscosity of the cytoplasm, movement of RTCs by diffusion is likely to be very limited, especially considering the size of the HIV-1 RTC, which has been estimated to be at least 50 nm in diameter (Miller, Farnet, & Bushman, 1997). To overcome this obstacle, HIV-1 utilizes the cellular cytoskeleton. Initial movements of the virus within the peripheral regions of the cell cytoplasm occur in association with the actin cytoskeleton (Bukrinskaya et al., 1998). However, subsequent translocation of the HIV-1 pre-integration complex towards the nucleus takes place along the microtubule network (McDonald et al., 2002). Transfer from the actin to the microtubule network is consistent with the evidence that actin can be used to gain access to tubulin (Taunton, 2001). The structural basis for interaction of the RTC and microtubules remains to be determined. This interaction likely engages a cellular dynein-dependent motor complex, which has both minus end (towards the microtubule-organizing center located close to the nucleus)- and plus end (towards cell periphery)–directed motor activities. A similar mechanism is used by herpes simplex virus (HSV)-1 (Sodeik, Ebersold, & Helenius, 1997) and adenovirus (Suomalainen et al., 1999) for intracellular transport.

Reverse transcription occurs during this translocation converting the HIV-1 RTC to an integration competent pre-integration complex (PIC) (step 2 in Fig. 1.1.4). Once near the nuclear membrane, HIV-1 most likely relies on the cellular nuclear import proteins to pass through the nuclear pore (step 3 in Figure 1.1.4), although other mechanisms, such as entry through reversible ruptures of the nuclear membrane made by the Vpr protein (de Noronha et al., 2001), might contribute to this process.

Cellular Nuclear Import Machinery

Nuclear transport of macromolecules occurs through the nuclear pore complexes and is controlled by nuclear localization signals (NLSs). The most common type of NLS is a short stretch of basic amino acids introducing an overall net positive charge crucial for the nuclear targeting properties of these sequences (reviewed in Dingwall & Laskey, 1991). Import of NLS-containing proteins across the nuclear pore complex is mediated by karyopherin α/β heterodimers (also termed NLS receptor/importin), which bind NLS-containing proteins in the cytosol and target them to the nucleus (Mattaj & Englmeier, 1998). Karyopherin α affinity for the NLS is enhanced by karyopherin β. Karyopherin β also mediates docking of karyopherin-NLS protein complexes to nucleoporins (a collective term for nuclear pore complex proteins) containing FG peptide repeats (Terry & Wente, 2009; Moroianu et al., 1995; Rexach & Blobel, 1995). Some NLSs interact with karyopherin β directly, without engaging the adapter protein karyopherin α (Barry & Wente, 2000). The small guanosine 5'-triphosphate (GTP) binding protein, Ran (Moore & Blobel, 1993; Melchior, Paschal, Evans, & Gerace, 1993), is a key regulator of the import process. Ran switches between the guanosine 5'-diphosphate (GDP) and GTP-bound states by nucleotide exchange and GTP hydrolysis. The concentration of Ran-GTP is high in the nucleus and low in the cytoplasm. It is believed that this gradient is used to provide direction to the nucleo-cytoplasmic exchange. In particular, by directly binding to karyopherin β: in the nucleoplasm, Ran-GTP disassembles the import complex and thus terminates the import process (Gorlich et al., 1996). It also stimulates assembly of the karyopherin α complex with cellular apoptosis susceptibility (CAS) factor, a protein originally implicated in apoptosis and cell proliferation but then rediscovered as an export factor (Kutay, Bischoff, Kostka, Kraft, & Gorlich, 1997). CAS promotes re-export of karyopherin α into the cytoplasm for recycling.

Nuclear Entry of the HIV-1 PIC

The model favored by investigators working in the area of HIV nuclear import is that the PIC itself is nucleophilic. This implies that a component or components of the complex contain targeting signals that direct the PIC to the nucleus. The most likely candidates for this role are viral proteins associated with the PIC (see below), although they can do it indirectly by binding to karyophilic cellular proteins (Gupta, Ott, Hope, Siliciano, & Boeke, 2000). Nuclear import of HIV PIC requires timely uncoating to expose the complex to cellular nuclear targeting machinery, so it is not surprising that mutations that accelerate or delay uncoating impair nuclear translocation and replication of HIV (Yamashita, Perez, Hope, & Emerman, 2007; Yamashita & Emerman, 2006). Several karyopherins/importins, including importins α and β (Hearps & Jans, 2006; Nitahara-Kasahara et al., 2007), importin 7 (Fassati, Gorlich, Harrison, Zaytseva, & Mingot, 2003), and TNPO3/transportin-SR2 (Christ et al., 2008) have been implicated in nuclear targeting of the HIV-1 PIC. TNPO3 was also identified in genome-wide screens for factors essential for HIV-1 replication, which used proliferating cells to monitor HIV replication, indicating that this importin, and by extension the process of nuclear import, is critical for HIV replication regardless of cellular proliferation status (Goff, 2008; Riviere, Darlix, & Cimarelli, 2010).

Role of MA, IN, and Vpr in HIV Nuclear Import

MA was the first protein implied in HIV-1 nuclear import (Bukrinsky et al., 1993). However, later studies questioned this role of MA (Fouchier et al., 1997; Freed, Englund, & Martin, 1995). It appears that HIV-1 MA carries functional, yet rather weak, NLSs whose activity may be enhanced by Vpr (Haffar et al., 2000). While MA is clearly required for efficient nuclear import of the HIV-1 PIC, its role is most likely nonessential (Reil, Bukovsky, Gelderblom, & Gottlinger, 1998).

Gallay and coworkers have proposed a role for IN in HIV-1 nuclear import (Gallay, Hope, Chin, & Trono et al., 1997). They demonstrated that IN associates with karyopherin α and can target a fusion GST-IN protein into the nucleus of microinjected cells. A report from Malim's group (Bouyac-Bertoia et al., 2001) extended this observation. This

group identified an unusual NLS spanning residues 161–173 within the central core domain of IN and demonstrated that certain mutations within this NLS, such as V165A or R166A, disrupt nuclear import of the viral PIC but do not affect IN catalytic activity. However, these results could not be reproduced in later studies demonstrating that the mutations described by Bouyac-Bertoia and colleagues primarily affected integration and not the PIC nuclear import (Bouyac-Bertoia et al., 2001; Limon, Nakajima, Lu, Ghory, & Engelman, 2002). IN was also reported to interact with lens epithelium-derived growth factor/p75 (LEDGF/p75), which is a karyophilic protein that may facilitate nuclear localization of IN and PIC (Maertens, Cherepanov, Debyser, Engelborghs, & Engelman, 2004). HIV-1 IN is likely an important factor in HIV-1 nuclear import (Woodward, Prakobwanakit, Mosessian, & Chow, 2009; Levin et al., 2009).

Vpr is another important contributor to HIV-1 nuclear import (Nie et al., 1998; Popov, Rexach, Ratner, Blobel, & Bukrinsky, 1998a; Popov et al., 1998b; Vodicka, Koepp, Silver, & Emerman, 1998). Three hypotheses (not necessarily mutually exclusive) for the mode of action of Vpr have been proposed: i) Vpr targets the HIV-1 PIC to the nucleus via a distinct, karyopherin α/β-independent pathway (Gallay, Stitt, Mundy, Oettinger, & Trono, 1996; de Noronha et al., 2001; Jenkins, McEntee, Weis, & Greene, 1998); ii) Vpr-mediated import requires karyopherin α, but not β (Vodicka et al., 1998); and iii) Vpr modifies cellular karyopherin α/β-dependent import machinery (Popov et al., 1998a; Popov et al., 1998b). The first model is based on the observation that in an in vitro nuclear import assay Vpr can enter nuclei in the absence of soluble import factors (Jenkins et al., 1998). Later, the same group reported that Vpr induces dynamic disruptions in nuclear envelope integrity, which may serve as entry points for large complexes such as HIV-1 PIC (de Noronha et al., 2001; Segura-Totten & Wilson, 2001). However, this latter assumption has not been tested experimentally. In addition, the effect of Vpr on the nuclear envelope was demonstrated in HeLa cells, where HIV-1 replication is Vpr-independent (Gallay et al., 1997). The second hypothesis relies on the ability of Vpr to bind nucleoporins (Fouchier et al., 1998; Popov et al., 1998a) and proposes that nuclear translocation of the HIV-1 PIC is mediated by direct binding of Vpr to the nuclear pore. The third model is based on findings that Vpr binds to karyopherin α (Popov et al., 1998b; Vodicka et al., 1998) and changes its affinity for the nuclear localization signal (NLS) (Popov et al., 1998b), and that nuclear import and docking of the HIV-1 pre-integration complex to nucleoporins are inhibited by antibodies to karyopherin β (Popov et al., 1998a). Most of the experimental data supporting these models have been obtained using individually expressed Vpr. The actual mechanism for Vpr activity in the nuclear importation of the HIV-1 PIC remains unresolved. In any case, Vpr is definitely involved in the process of HIV-1 nuclear import in macrophages. However, its role is not strictly essential, as viruses lacking Vpr can still replicate in macrophages, albeit with reduced efficiency (Heinzinger et al., 1994).

How could all these seemingly conflicting results be assembled into a unified model? One likely possibility is that all the mentioned constituents contribute to HIV-1 nuclear import in a redundant manner. Such redundancy would ensure the efficiency of this extremely important step for the virus. It is also possible that HIV-1 uses different pathways for the nuclear import, depending on the cell type and the activation state of the target cell (Lee et al., 2010; discussed in Levin, Loyter, & Bukrinsky, 2010). Such flexible use of the nuclear import machinery would employ different viral proteins under different conditions. Based on studies with HIV and other viruses replicating in the nucleus, it appears safe to assume that the HIV-1 PIC is delivered to the nuclear envelope along the microtubule network and enters the nucleoplasm through the nuclear pore. The simplified model depicting the main steps in the process of nuclear translocation of the HIV genome is shown in Figure 1.1.4. During the passage through the nuclear pore or shortly after entering the nucleus, the PIC likely undergoes additional disassembly, getting rid of the factors unnecessary for integration, such as MA and Vpr (step 4 in Fig. 1.1.4). The detailed mechanisms responsible for the steps shown in Figure 1.1.4 await their resolution.

INTEGRATION

Integration of the HIV-1 DNA into the host-cell genome, catalyzed by the integrase (IN) protein, is a prerequisite for viral replication in both T cells and macrophages. Integrase is a recombinase responsible for the cutting-and-joining reaction that leads to the covalent ligation of the viral genome with the cellular DNA and the formation of the provirus. Integration usually proceeds in multiple steps (Pluymers, De Clercq, & Debyser, 2001). First, two to three nucleotides from the initially blunt 3' ends of the viral DNA are removed to form a preintegration substrate. This step presumably occurs in the cytoplasm (Kulkosky & Skalka, 1994). Second, in the nucleus, integrase catalyzes a staggered cleavage of the cellular target DNA. Third, the cellular DNA repair machinery repairs the gaps and ligates the 3' recessed ends to the 5' overhangs. The integrated viral DNA is called provirus and is retained in the host genome throughout the life of the infected cell. Some low-level replication in macrophages of an integrase-defective HIV-1 mutant has been described (Cara, Guarnaccia, Reitz, Gallo, & Lori, 1995). However, replication of this mutant virus was inefficient and self-limiting. While extra-chromosomal circular forms of viral DNA are produced in both peripheral blood lymphocytes (PBLs) and macrophages, they cannot sustain viral replication, despite their reported stability, at least in T cells (Pierson et al., 2002).

Studies using integration in vitro have clarified factors influencing integration site selection in simplified models. Access of integration complexes to target DNA can be obstructed by DNA binding proteins bound at or near the integration sites (Pryciak & Varmus, 1992). In contrast, proteins that induce DNA bending, such as nucleosomal histones, can actually promote integration (Pryciak & Varmus, 1992; Pruss, Reeves, Bushman, & Wolffe, 1994b; Pruss, Bushman, & Wolffe, 1994a). In vivo, nucleosomal DNA is assembled into

higher-order chromatin. A recent analysis of HIV-1 integration sites in vivo using genomics approaches revealed that genes were clearly preferential integration targets in vivo but not in vitro (Schroder et al., 2002). Transcriptional profiling revealed a strong correlation between gene activity and integration targeting. In addition, hotspots for integration were detected, for example, a 2.5 kb region that contained 1% of all integration events (Schroder et al., 2002). Therefore, there is a certain degree of specificity in the integration targeting by HIV.

How can this specificity be accomplished? Obviously, cellular proteins must be involved, as integration in vitro is random. Search for cellular proteins interacting with HIV-1 integrase produced a number of candidates, including LEDGF/p75, transportin-SR2 (TNPO3), von Hippel-Lindau binding protein 1 (VBP1), and sucrose non-fermenting 5 (SNF5) (Rain, Cribier, Gerard, Emiliani, & Benarous, 2009). Further studies are required to determine the role of these factors in HIV-1 integration.

Efficiency of HIV-1 integration in $CD4^+$ T cells and macrophages appears to be influenced by the activation state of the target cell. HIV gp120 induces multiple cellular signaling pathways, including the phosphatidylinositol 3-kinase (PI3-kinase) pathway. The PI3-kinase inhibitor LY294002 inhibited infection of $CD4^+$ T cells and macrophages with X4 and R5 HIV-1 strains (Francois & Klotman, 2003). The inhibition of the PI3-kinase signaling pathway suppressed virus infection post-entry and post-reverse transcription but prior to HIV gene expression, suggesting an effect at the step of integration. So far, there is no indication in the literature that differences exist between T cells and macrophages with regard to the mechanism of HIV-1 integration.

TRANSCRIPTION

Despite some evidence that unintegrated HIV-1 DNA can be transcribed (Poon & Chen, 2003; Wu & Marsh, 2003), the vast majority of HIV-1 transcription occurs from integrated proviral DNA. Main elements of HIV transcriptional control are located in the 5'-LTR. Transcriptional regulation depends on the recognition of *cis* regulatory regions in the 5'-LTR by a set of transcription factors which interact with the basal transcriptional complex (reviewed in Cullen, 1991). A TATA box, which recruits host cell RNA polymerase II, three Sp1 sites, and a strong enhancer composed of two NF-κB sites are located in the U3 region (Nabel & Baltimore, 1987). Additional binding sites for transcriptional regulatory proteins have been identified 5' to the NF-κB enhancer, in the so-called NRE of the HIV-1 LTR. The NRE includes binding sites for USF, AP1, LEF, NF-AT, and ETS transcription factors (Naghavi et al., 2001, and references therein). Inducible activation of the viral LTR appears to depend principally on the generation of a functional NF-κB complex and requires a trans-activating protein, Tat, to interact with a trans-activating responsive element, TAR. NF-κB defines a family of transcription factors composed by members of the NF-κB/Rel family, namely NF-κB1 (p50), NF-κB2 (p52), RelA (p65), RelB, v-Rel, and c-Rel, all sharing a sequence homology over a 300 amino acids ("rel homology domain") (Siebenlist, Franzoso, & Brown, 1994). NF-κB proteins form homo- or heterodimers that bind with different affinities to the NF-κB enhancer of HIV-1.

Transcription and splicing of HIV mRNAs is a complex process, in which the viral regulatory proteins Tat and Rev play essential roles. HIV-1 genes are expressed through the complex splicing of a single mRNA precursor, leading to three major classes of transcripts: unspliced (US), singly spliced (SS), and multiply spliced (MS) mRNAs. In vitro, reactivation of latent HIV-1 infection is characterized by an early increase in MS transcripts, followed by a rise in SS and US viral mRNA (Cullen & Greene, 1989). Thus, early in the infection only MS transcripts are produced, which encode the regulatory proteins Tat, Rev, and Nef. Accumulation of Tat and Rev has profound effects on viral transcription. Tat greatly enhances the processivity of transcription from the HIV LTR, while Rev is essential for the export of SS and US viral transcripts from the nucleus into the cytoplasm. Together, these regulatory proteins ensure accumulation of the viral structural proteins required for efficient production of new viral particles. Interestingly, HIV infection of macrophages in vitro is characterized by a dramatic reduction of Tat expression following the peak of productive infection, perhaps accounting for the slower non-cytopathic infection of cells of the macrophage lineage (Sonza et al., 2002).

VIRUS PARTICLE ASSEMBLY

The final steps of the viral life cycle include assembly of an immature viral particle on the cytoplasmic face of a cell membrane, encapsidation of the viral RNA, viral budding and release of the viral particles from the infected cell, and proteolytic processing of Gag and Gag-Pol polyprotein-precursors resulting in formation of infectious virions. These steps are controlled by Gag polyproteins, Pr55 Gag and Pr160 GagPol synthesized from unspliced genomic RNA. Gag itself is enough for a formation and budding of virus-like particles (VLP) independently of an active viral encoded protease (PR) (Klein, Reed, & Lingappa, 2007). The assembly process is driven primarily by elements within Gag, such as the N-terminal membrane-targeting domain (M), central Gag-Gag interaction domain (I), the NC (nucleocapsid) domain which binds RNA enabling packaging of the viral genome, and a C-terminal proline-rich late assembly (L) domain located in the p6 region of Gag, which is required for separation of the virion from the host cell membrane (Pincetic & Leis, 2009; Bieniasz, 2009).

HIV assembly begins with Gag precursor molecules coming into close contact with each other to form a Gag protein sphere within a lipid bilayer envelope (Hermida-Matsumoto & Resh, 2000). Gag localization in the cell membrane is not uniform and seems to be concentrated in lipid rafts (the membrane areas highly enriched in cholesterol, sphingolipids, and glycosylphosphatidylinositol-linked proteins) (Ono & Freed, 2001) where the Env complexes are also preferentially accumulated. Some data provide evidence that HIV-1 Gag monomers might multimerize and associate with

viral RNA in the cytoplasm prior to arriving to the membrane and then diffuse through the cytoplasm to sites of assembly on cell membranes (Yuan, Yu, Lee, & Essex, 1993; Gomez & Hope, 2006). Other observations suggest that Gag remains entirely monomeric or forms low-order multimers in the cytoplasm and Gag oligomerization occurs only at the cell membrane (Datta et al., 2007).

Interestingly, studies in macrophages initially contradicted the model of HIV particle assembly at the inner face of plasma membrane, as considerable amounts of HIV-1 Gag protein and/or mature virion particles were detected in late endosomes (Nydegger, Foti, Derdowski, Spearman, & Thali, 2003; Sherer et al., 2003; Pelchen-Matthews, Kramer, & Marsh, 2003). These findings suggested that at least in macrophages Gag was initially targeted to late endosomal membranes, virions were formed by budding into the endosomal lumen, and extracellular particles were liberated via an endosome-based secretory pathway (Nguyen, Booth, Gould, & Hildreth, 2003). Consistent with this view, virion budding requires the ESCRT proteins, which normally mediate the budding of vesicles into the late endosomal lumen (Bieniasz, 2006). However, later studies demonstrated that in this cell type, productive HIV-1 assembly occurs at the plasma membrane (Jouvenet et al., 2006). Large areas of the macrophage plasma membrane are sequestered within the cell, forming invaginations that have the appearance of an intracellular compartment. However, this "compartment" is bounded by a plasma membrane that is continuous with the conventional plasma membrane. Virions assembled in these intracellular pseudo-compartments bounded by the plasma membrane become trapped and accumulate therein, presenting an impression of enriched production at intracellular sites as compared to the conventional plasma membrane upon ultrastructural analysis. These intracellular pseudo-compartments may represent favored sites for HIV-1 assembly in macrophages, but it is not currently clear whether they are static or dynamic or whether they represent exocytic, endocytic, or phagocytic intermediates (Bieniasz, 2009).

HIV-1 genomic RNA is brought to the site of aggregation of Gag, GagPol, and Env complexes through an interaction between the *cis*-acting region of the viral RNA, RNA packaging (psi) sequence, and the zinc fingers of the Gag precursor NC domain (Cimarelli, Sandin, Hoglund, & Luban, 2000). The psi region consists of four stem-loop structures (SL1, SL2, SL3, and SL4) and is located between the 5'-LTR and the Gag initiation site (Lever, 2000). The NC domain also facilitates dimerization of the viral RNA, a process, which seems to be important for RNA packaging (Sakuragi, Ueda, Iwamoto, & Shioda, 2003). Once delivered to the plasma membrane, Gag, GagPol, and Env complexes start to interact and initiate encapsidation of two copies of viral genomic RNA and Lysine tRNA, which serves as a primer for reverse transcription.

The L domains of HIV-1 and other retroviruses bind directly or indirectly to components of the ESCRT pathway in order to complete assembly and separate the nascent virion envelope from cell membranes (Bieniasz, 2006; Morita & Sundquist, 2004). The HIV-1 L domains consist of peptide sequences PTAP and YPLTSL, which bind Tsg101 (a component of ESCRT-I) and ALIX (an ESCRT-I- and ESCRT-III-binding protein), respectively (Garrus et al., 2001; Bieniasz, 2006; Morita & Sundquist, 2004; Bieniasz, 2009). The ESCRT pathway is normally implicated in (1) selection of ubiquitin-tagged transmembrane protein cargos and their sorting into specified areas of endosomal membranes, (2) induction of membrane invagination away from the cytoplasm and toward the endosomal lumen, and (3) fusion of the neck of the induced membrane invagination to generate a vesicle within the endosomal lumen (Bieniasz, 2009). The model of recruitment of the ESCRT machinery components by the retroviruses to build a budding complex for particle release includes the following steps (reviewed in Pincetic & Leis, 2009). (1) The PTAP L domain of HIV-1 binds to Tsg101. Whether this initial interaction takes place in the cytosol or at the plasma membrane remains to be defined. (2) The Gag polyprotein is ubiquitylated by an unidentified E3 ubiquitin ligase. Some evidence suggests that Nedd4L may play a role since its over-expression rescues budding of HIV-1 Gag/ΔPTAP. (3) Gag oligomerization in the cytosol increases membrane avidity and rapidly targets Gag to sites of assembly/budding on the plasma membrane. (4) During the budding process, Gag may recruit additional ESCRT factors eventually leading to ESCRT-III polymerization at the base of a budding particle. (5) ESCRT-III subunits recruit the AAA ATPase, Vps4, to mediate the disassembly of membrane-bound ESCRT complexes and to provide the energy for membrane fission. (6) Immature viral particles are released from cellular membranes.

During or shortly after the budding of immature viral particles from the host cell, the noninfectious virions with a spherical core composed of $Pr55^{Gag}$ and $Pr160^{GagPol}$ polyproteins are transformed to infectious virions during a step termed maturation. During maturation, the *pol*-encoded HIV protease processes itself from GagPol precursor, forms homodimers and then cleaves the Gag of Pr55 and Pr160 polyprotein precursors into three major proteins (matrix, capsid, and nucleocapsid) and three polypeptides (p6, p2, and p1), and the Pol domain of the GagPol Pr160 precursor into the p51 and p66 subunits of reverse transcriptase and the integrase. These events lead to reorganization of the internal virion structure characterized by formation of conical-shaped cores (Gross et al., 2000). Gag cleavage and maturation are essential for infectivity.

CONCLUSION

Understanding of the mechanisms governing HIV-1 replication has lead to development of effective antiretroviral agents and is expected to provide new therapeutic leads in the future. Introduction of highly active antiretroviral therapy (HAART) dramatically improved patients' survival; however, its impact on the prevalence and the course of HAD and HAND remains debatable. While multiple studies suggest that the incidence rates of HAD and CNS opportunistic infections are decreasing, some research indicates that the prevalence of HAND is rising as people with HIV live longer (Kaul, 2009).

Since the introduction of HAART in 1996, the incidence of HAD has decreased by approximately 50%. However, as resistance of the virus to antiretroviral drugs develops and CD4 cell counts decline, the incidence of HAD may begin to rise again. Poor CNS penetration of many antiretroviral agents contributes to persistence of HIV-1 in the brain and explains, at least in part, the sanctuary status of the CNS for the ancestral viral variants (Van Marle et al., 2002; Nowacek & Gendelman, 2009; Liu et al., 2000). It is clear that new drugs, especially those that can cross the blood-brain barrier and are tailored to inhibit HIV-1 replication in macrophages, will be needed to eliminate HAND from the list of diseases associated with HIV infection.

REFERENCES

Alfano, M., Schmidtmayerova, H., Amella, C. A., Pushkarsky, T., & Bukrinsky, M. (1999). The B-oligomer of pertussis toxin deactivates CC chemokine receptor 5 and blocks entry of M-tropic HIV-1 strains. *J Exp Med*, *190*, 597–606.

Alkhatib, G., Combadiere, C., Broder, C. C., Feng, Y., Kennedy, P. E., Murphy, P. M., et al. (1996). CC CKR5: A RANTES, MIP-1alpha, MIP-1beta receptor as a fusion cofactor for macrophage-tropic HIV-1. *Science*, *272*, 1955–1958.

Andersen, J. L., DeHart, J. L., Zimmerman, E. S., Ardon, O., Kim, B., Jacquot, G., et al. (2006). HIV-1 Vpr-induced apoptosis is cell cycle dependent and requires Bax but not ANT. *PLoS Pathog*, *2*, e127.

Andersen, J. L., Le, R. E., & Planelles, V. (2008). HIV-1 Vpr: Mechanisms of G2 arrest and apoptosis. *Exp Mol Pathol*, *85*, 2–10.

Arhel, N., Munier, S., Souque, P., Mollier, K., & Charneau, P. (2006). Nuclear import defect of human immunodeficiency virus type 1 DNA flap mutants is not dependent on the viral strain or target cell type. *J Virol*, *80*, 10262–10269.

Arhel, N. J., Souquere-Besse, S., Munier, S., Souque, P., Guadagnini, S., Rutherford, S., et al. (2007). HIV-1 DNA flap formation promotes uncoating of the pre-integration complex at the nuclear pore. *EMBO J*, *26*, 3025–3037.

Arthos, J., Rubbert, A., Rabin, R. L., Cicala, C., Machado, E., Wildt, K., et al. (2000). CCR5 signal transduction in macrophages by human immunodeficiency virus and simian immunodeficiency virus envelopes. *J Virol*, *74*, 6418–6424.

Baltimore, D. (1970). RNA-dependent DNA polymerase in virions of RNA tumour viruses. *Nature*, *226*, 1209–1211.

Bannert, N., Schenten, D., Craig, S., & Sodroski, J. (2000). The level of CD4 expression limits infection of primary rhesus monkey macrophages by a T-tropic simian immunodeficiency virus and macrophagetropic human immunodeficiency viruses. *J Virol*, *74*, 10984–10993.

Barry, D. M. & Wente, S. R. (2000). Nuclear transport: Never-ending cycles of signals and receptors. *Essays Biochem*, *36*, 89–103.

Bartz, S. R., Rogel, M. E., & Emerman, M. (1996). Human immunodeficiency virus type 1 cell cycle control: Vpr is cytostatic and mediates G2 accumulation by a mechanism which differs from DNA damage checkpoint control. *J Virol*, *70*, 2324–2331.

Bergamaschi, A., Ayinde, D., David, A., Le, R. E., Morel, M., Collin, G., et al. (2009). The human immunodeficiency virus type 2 Vpx protein usurps the CUL4A-DDB1 DCAF1 ubiquitin ligase to overcome a postentry block in macrophage infection. *J Virol*, *83*, 4854–4860.

Berger, E. A. (1998). HIV entry and tropism. When one receptor is not enough. *Adv Exp Med Biol*, *452*:*151–7*, 151–157.

Bieniasz, P. D. (2004). Intrinsic immunity: Afront-line defense against viral attack. *Nat Immunol*, *5*, 1109–1115.

Bieniasz, P. D. (2006). Late budding domains and host proteins in enveloped virus release. *Virology*, *344*, 55–63.

Bieniasz, P. D. (2009). The cell biology of HIV-1 virion genesis. *Cell Host Microbe*, *5*, 550–558.

Blazkova, J., Trejbalova, K., Gondois-Rey, F., Halfon, P., Philibert, P., Guiguen, A., et al. (2009). CpG methylation controls reactivation of HIV from latency. *PLoS Pathog*, *5*, e1000554.

Bogerd, H. P. & Cullen, B. R. (2008). Single-stranded RNA facilitates nucleocapsid: APOBEC3G complex formation. *RNA*, *14*, 1228–1236.

Bolton, D. L. & Lenardo, M. J. (2007). Vpr cytopathicity independent of G2/M cell cycle arrest in human immunodeficiency virus type 1-infected CD4+ T cells. *J Virol*, *81*, 8878–8890.

Bouyac-Bertoia, M., Dvorin, J. D., Fouchier, R. A., Jenkins, Y., Meyer, B. E., Wu, L. I., et al. (2001). Hiv-1 infection requires a functional integrase nls. *Mol Cell*, *7*, 1025–1035.

Braaten, D. & Luban, J. (2001). Cyclophilin A regulates HIV-1 infectivity, as demonstrated by gene targeting in human T cells. *EMBO J*, *20*, 1300–1309.

Buckman, J. S., Bosche, W. J., & Gorelick, R. J. (2003). Human immunodeficiency virus type 1 nucleocapsid zn(2+) fingers are required for efficient reverse transcription, initial integration processes, and protection of newly synthesized viral DNA. *J Virol*, *77*, 1469–1480.

Bukrinskaya, A., Brichacek, B., Mann, A., & Stevenson, M. (1998). Establishment of a functional human immunodeficiency virus type 1 (HIV- 1) reverse transcription complex involves the cytoskeleton. *J Exp Med*, *188*, 2113–2125.

Bukrinsky, M. (2004). A hard way to the nucleus. *Mol Med*, *10*, 1–5.

Bukrinsky, M. & Adzhubei, A. (1999). Viral protein R of HIV-1. *Rev Med Virol*, *9*, 39–49.

Bukrinsky, M. I., Haggerty, S., Dempsey, M. P., Sharova, N., Adzhubel, A., Spitz, L., et al. (1993). A nuclear localization signal within HIV-1 matrix protein that governs infection of non-dividing cells. *Nature*, *365*, 666–669.

Bultmann, A., Muranyi, W., Seed, B., & Haas, J. (2001). Identification of two sequences in the cytoplasmic tail of the human immunodeficiency virus type 1 envelope glycoprotein that inhibit cell surface expression. *J Virol*, *75*, 5263–5276.

Campbell, E. M., Nunez, R., & Hope, T. J. (2004). Disruption of the actin cytoskeleton can complement the ability of Nef to enhance human immunodeficiency virus type 1 infectivity. *J Virol*, *78*, 5745–5755.

Cara, A., Guarnaccia, F., Reitz, M. S., Gallo, R. C., & Lori, F. (1995). Self-limiting, cell type-dependent replication of an integrase-defective human immunodeficiency virus type 1 in human primary macrophages but not T lymphocytes. *Virology*, *208*, 242–248.

Casper, C., Naver, L., Clevestig, P., Belfrage, E., Leitner, T., Albert, J., et al. (2002). Coreceptor change appears after immune deficiency is established in children infected with different HIV-1 subtypes. *AIDS Res Hum Retroviruses*, *18*, 343–352.

Charneau, P., Alizon, M., & Clavel, F. (1992). A second origin of DNA plus-strand synthesis is required for optimal human immunodeficiency virus replication. *J Virol*, *66*, 2814–2820.

Charneau, P., Mirambeau, G., Roux, P., Paulous, S., Buc, H., & Clavel, F. (1994). HIV-1 reverse transcription. A termination step at the center of the genome. *J Mol Biol*, *241*, 651–662.

Chaudhuri, R., Lindwasser, O. W., Smith, W. J., Hurley, J. H., & Bonifacino, J. S. (2007). Downregulation of CD4 by human immunodeficiency virus type 1 Nef is dependent on clathrin and involves direct interaction of Nef with the AP2 clathrin adaptor. *J Virol*, *81*, 3877–3890.

Checroune, F., Yao, X. J., Gottlinger, H. G., Bergeron, D., & Cohen, E. A. (1995). Incorporation of Vpr into human immunodeficiency virus type 1: Role of conserved regions within the P6 domain of Pr55gag. *J Acquir Immune Defic Syndr Hum Retrovirol*, *10*, 1–7.

Chen, M., Elder, R. T., Yu, M., O'Gorman, M. G., Selig, L., Benarous, R., et al. (1999). Mutational analysis of Vpr-induced G2 arrest, nuclear localization, and cell death in fission yeast. *J Virol*, *73*, 3236–3245.

Chen, S. S., Lee, S. F., & Wang, C. T. (2001). Cellular membrane-binding ability of the C-terminal cytoplasmic domain of human immunodeficiency virus type 1 envelope transmembrane protein gp41. *J Virol*, *75*, 9925–9938.

Christ, F., Thys, W., De, R. J., Gijsbers, R., Albanese, A., Arosio, D., et al. (2008). Transportin-SR2 imports HIV into the nucleus. *Curr Biol*, *18*, 1192–1202.

Chukkapalli, V., Hogue, I. B., Boyko, V., Hu, W. S., & Ono, A. (2008). Interaction between the human immunodeficiency virus type 1 Gag matrix domain and phosphatidylinositol-(4,5)-bisphosphate is essential for efficient gag membrane binding. *J Virol*, *82*, 2405–2417.

Cimarelli, A., Sandin, S., Hoglund, S., & Luban, J. (2000). Rescue of multiple viral functions by a second-site suppressor of a human immunodeficiency virus type 1 nucleocapsid mutation. *J Virol*, *74*, 4273–4283.

Collin, M. & Gordon, S. (1994). The kinetics of human immunodeficiency virus reverse transcription are slower in primary human macrophages than in a lymphoid cell line. *Virology*, *200*, 114–120.

Conti, L., Rainaldi, G., Matarrese, P., Varano, B., Rivabene, R., Columba, S., et al. (1998). The HIV-1 vpr protein acts as a negative regulator of apoptosis in a human lymphoblastoid T cell line: Possible implications for the pathogenesis of *AIDS J Exp Med*, *187*, 403–413.

Conticello, S. G., Harris, R. S., & Neuberger, M. S. (2003). The Vif protein of HIV triggers degradation of the human antiretroviral DNA deaminase APOBEC3G. *Curr Biol*, *13*, 2009–2013.

Crump, C. M., Xiang, Y., Thomas, L., Gu, F., Austin, C., Tooze, S. A., et al. (2001). PACS-1 binding to adaptors is required for acidic cluster motif-mediated protein traffic. *EMBO J*, *20*, 2191–2201.

Cullen, B. R. (1991). Regulation of HIV-1 gene expression. *FASEB J*, *5*, 2361–2368.

Cullen, B. R. (1998). Retroviruses as model systems for the study of nuclear RNA export pathways. *Virology*, *249*, 203–210.

Cullen, B. R. & Greene, W. C. (1989). Regulatory pathways governing HIV-1 replication. *Cell*, *58*, 423–426.

Datta, S. A., Zhao, Z., Clark, P. K., Tarasov, S., Alexandratos, J. N., Campbell, S. J., et al. (2007). Interactions between HIV-1 Gag molecules in solution: An inositol phosphate-mediated switch. *J Mol Biol*, *365*, 799–811.

Davis, C. B., Dikic, I., Unutmaz, D., Hill, C. M., Arthos, J., Siani, M. A., et al. (1997). Signal transduction due to HIV-1 envelope interactions with chemokine receptors CXCR4 or CCR5. *J Exp Med*, *186*, 1793–1798.

de Noronha, C. M., Sherman, M. P., Lin, H. W., Cavrois, M. V., Moir, R. D., Goldman, R. D., et al. (2001). Dynamic disruptions in nuclear envelope architecture and integrity induced by HIV-1 Vpr. *Science*, *294*, 1105–1108.

de Rocquigny, H., Petitjean, P., Tanchou, V., Decimo, D., Drouot, L., Delaunay, T., et al. (1997). The zinc fingers of HIV nucleocapsid protein NCp7 direct interactions with the viral regulatory protein Vpr. *J Biol Chem*, *272*, 30753–30759.

Deng, H., Liu, R., Ellmeier, W., Choe, S., Unutmaz, D., Burkhart, M., et al. (1996). Identification of a major co-receptor for primary isolates of HIV-1. *Nature*, *381*, 661–666.

Dimitrov, D. S., Norwood, D., Stantchev, T. S., Feng, Y., Xiao, X., & Broder, C. C. (1999). A mechanism of resistance to HIV-1 entry: Inefficient interactions of CXCR4 with CD4 and gp120 in macrophages. *Virology*, *259*, 1–6.

Dingwall, C. & Laskey, R. A. (1991). Nuclear targeting sequences—a consensus? *Trends Biochem Sci*, *16*, 478–481.

Doms, R. W. (2000). Beyond receptor expression: The influence of receptor conformation, density, and affinity in HIV-1 infection. *Virology*, *276*, 229–237.

Dragic, T., Litwin, V., Allaway, G. P., Martin, S. R., Huang, Y., Nagashima, K. A., et al. (1996). HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC- CKR-5. *Nature*, *381*, 667–673.

Dunn, B. M., Goodenow, M. M., Gustchina, A., & Wlodawer, A. (2002). Retroviral proteases. *Genome Biol*, *3*, REVIEWS3006.

Dyer, W. B., Geczy, A. F., Kent, S. J., McIntyre, L. B., Blasdall, S. A., Learmont, J. C., et al. (1997). Lymphoproliferative immune function in the Sydney Blood Bank Cohort, infected with natural nef/long terminal repeat mutants, and in other long-term survivors of transfusion-acquired HIV-1 infection. *AIDS*, *11*, 1565–1574.

Fackler, O. T. & Baur, A. S. (2002). Live and let die: Nef functions beyond HIV replication. Immunity. *16*, 493–497.

Fassati, A., Gorlich, D., Harrison, I., Zaytseva, L., & Mingot, J. M. (2003). Nuclear import of HIV-1 intracellular reverse transcription complexes is mediated by importin 7. *EMBO J*, *22*, 3675–3685.

Feng, Y. X., Copeland, T. D., Henderson, L. E., Gorelick, R. J., Bosche, W. J., Levin, J. G., et al. (1996). HIV-1 nucleocapsid protein induces "maturation" of dimeric retroviral RNA in vitro. *Proc Natl Acad Sci USA*, *93*, 7577–7581.

Fouchier, R. A., Meyer, B. E., Simon, J. H., Fischer, U., Albright, A. V., Gonzalez-Scarano, F., et al. (1998). Interaction of the human immunodeficiency virus type 1 Vpr protein with the nuclear pore complex. *J Virol*, *72*, 6004–6013.

Fouchier, R. A., Meyer, B. E., Simon, J. H., Fischer, U., & Malim, M. H. (1997). HIV-1 infection of non-dividing cells: Evidence that the amino-terminal basic region of the viral matrix protein is important for Gag processing but not for post-entry nuclear import. *EMBO J*, *16*, 4531–4539.

Francois, F. & Klotman, M. E. (2003). Phosphatidylinositol 3-kinase regulates human immunodeficiency virus type 1 replication following viral entry in primary CD4(+) T lymphocytes and macrophages. *J Virol*, *77*, 2539–2549.

Freed, E. O. (2001). HIV-1 replication. *Somat Cell Mol Genet*, *26*, 13–33.

Freed, E. O., Englund, G., & Martin, M. A. (1995). Role of the basic domain of human immunodeficiency virus type 1 matrix in macrophage infection. *J Virol*, *69*, 3949–3954.

Gabuzda, D. & Wang, J. (1999). Chemokine receptors and virus entry in the central nervous system. *J Neurovirol*, *5*, 643–658.

Gallay, P., Hope, T., Chin, D., & Trono, D. (1997). HIV-1 infection of nondividing cells through the recognition of integrase by the importin/karyopherin pathway. *Proc Natl Acad Sci USA*, *94*, 9825–9830.

Gallay, P., Stitt, V., Mundy, C., Oettinger, M., & Trono, D. (1996). Role of the karyopherin pathway in human immunodeficiency virus type 1 nuclear import. *J Virol*, *70*, 1027–1032.

Garrus, J. E., von Schwedler, U. K., Pornillos, O. W., Morham, S. G., Zavitz, K. H., Wang, H. E., et al. (2001). Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding. *Cell*, *107*, 55–65.

Geyer, M., Fackler, O. T., & Peterlin, B. M. (2001). Structure—function relationships in HIV-1 Nef. *EMBO Rep*, *2*, 580–585.

Goff, S. P. (2008). Knockdown screens to knockout HIV-1. *Cell*, *135*, 417–420.

Goh, W. C., Rogel, M. E., Kinsey, C. M., Michael, S. F., Fultz, P. N., Nowak, M. A., et al. (1998). HIV-1 Vpr increases viral expression by manipulation of the cell cycle: A mechanism for selection of Vpr in vivo. *Nat Med*, *4*, 65–71.

Gomez, C. Y. & Hope, T. J. (2006). Mobility of human immunodeficiency virus type 1 Pr55Gag in living cells. *J Virol*, *80*, 8796–8806.

Gorlich, D., Pante, N., Kutay, U., Aebi, U., & Bischoff, F. R. (1996). Identification of different roles for RanGDP and RanGTP in nuclear protein import. *EMBO J*, *15*, 5584–5594.

Gorry, P. R., Bristol, G., Zack, J. A., Ritola, K., Swanstrom, R., Birch, C. J., et al. (2001). Macrophage tropism of human immunodeficiency virus type 1 isolates from brain and lymphoid tissues predicts neurotropism independent of coreceptor specificity. *J Virol*, *75*, 10073–10089.

Gramberg, T., Sunseri, N., & Landau, N. R. (2010). Evidence for an activation domain at the amino terminus of simian immunodeficiency virus Vpx. *J Virol*, *84*, 1387–1396.

Grate, D. & Wilson, C. (1997). Role REVersal: understanding how RRE RNA binds its peptide ligand. *Structure*, *5*, 7–11.

Gray, F., Adle-Biassette, H., Chretien, F., Lorin, d. l. G., Force, G., & Keohane, C. (2001). Neuropathology and neurodegeneration in human immunodeficiency virus infection. Pathogenesis of HIV-induced lesions of the brain, correlations with HIV-associated disorders and modifications according to treatments. *Clin Neuropathol*, *20*, 146–155.

Greenbaum, N. L. (1996). How Tat targets TAR: Structure of the BIV peptide-RNA complex. *Structure*, *4*, 5–9.

Gross, I., Hohenberg, H., Wilk, T., Wiegers, K., Grattinger, M., Muller, B., et al. (2000). A conformational switch controlling HIV-1 morphogenesis. *EMBO J*, *19*, 103–113.

Gupta, K., Ott, D., Hope, T. J., Siliciano, R. F., & Boeke, J. D. (2000). A human nuclear shuttling protein that interacts with human immunodeficiency virus type 1 matrix is packaged into virions. *J Virol*, *74*, 11811–11824.

Haffar, O. K., Popov, S., Dubrovsky, L., Agostini, I., Tang, H., Pushkarsky, T., et al. (2000). Two nuclear localization signals in the HIV-1 matrix protein regulate nuclear import of the HIV-1 pre-integration complex. *J Mol Biol*, *299*, 359–368.

Harris, R. S. & Liddament, M. T. (2004). Retroviral restriction by APOBEC proteins. *Nat Rev Immunol*, *4*, 868–877.

He, G., Ylisastigui, L., & Margolis, D. M. (2002). The regulation of HIV-1 gene expression: The emerging role of chromatin. *DNA Cell Biol*, *21*, 697–705.

He, J., Chen, Y., Farzan, M., Choe, H., Ohagen, A., Gartner, S., et al. (1997). CCR3 and CCR5 are co-receptors for HIV-1 infection of microglia. *Nature*, *385*, 645–649.

Hearps, A. C. & Jans, D. A. (2006). HIV-1 integrase is capable of targeting DNA to the nucleus via an Importin alpha/beta-dependent mechanism. *Biochem J*, *398*, 475–484.

Heinzinger, N. K., Bukrinsky, M. I., Haggerty, S. A., Ragland, A. M., Kewalramani, V., Lee, M. et al. (1994). The Vpr protein of human immunodeficiency virus type 1 influences nuclear localization of viral nucleic acids in nondividing host cells. *Proc Natl Acad Sci USA*, *91*, 7311–7315.

Hermida-Matsumoto, L. & Resh, M. D. (2000). Localization of human immunodeficiency virus type 1 Gag and Env at the plasma membrane by confocal imaging. *J Virol*, *74*, 8670–8679.

Holmes, D., Knudsen, G., key-Cushman, S., & Su, L. (2007). FoxP3 enhances HIV-1 gene expression by modulating NFkappaB occupancy at the long terminal repeat in human T cells. *J Biol Chem*, *282*, 15973–15980.

Holmes, R. K., Malim, M. H., & Bishop, K. N. (2007). APOBEC-mediated viral restriction: Not simply editing? *Trends Biochem Sci*, *32*, 118–128.

Hrimech, M., Yao, X. J., Bachand, F., Rougeau, N., & Cohen, E. A. (1999). Human immunodeficiency virus type 1 (HIV-1) Vpr functions as an immediate-early protein during HIV-1 infection. *J Virol*, *73*, 4101–4109.

Jenkins, Y., McEntee, M., Weis, K., & Greene, W. C. (1998). Characterization of HIV-1 vpr nuclear import: Analysis of signals and pathways. *J Cell Biol*, *143*, 875–885.

Jonckheere, H., Anne, J., & De Clercq, E. (2000). The HIV-1 reverse transcription (RT) process as target for RT inhibitors. *Med Res Rev*, *20*, 129–154.

Jouvenet, N., Neil, S. J., Bess, C., Johnson, M. C., Virgen, C. A., Simon, S. M., et al. (2006). Plasma membrane is the site of productive HIV-1 particle assembly. *PLoS Biol*, *4*, e435.

Kaul, M. (2009). HIV-1 associated dementia: Update on pathological mechanisms and therapeutic approaches. *Curr Opin Neurol*, *22*, 315–320.

Kiernan, R. E., Ono, A., Englund, G., & Freed, E. O. (1998). Role of matrix in an early postentry step in the human immunodeficiency virus type 1 life cycle. *J Virol*, *72*, 4116–4126.

Klein, K. C., Reed, J. C., & Lingappa, J. R. (2007). Intracellular destinies: Degradation, targeting, assembly, and endocytosis of HIV Gag. *AIDS Rev*, *9*, 150–161.

Koh, K., Miyaura, M., Yoshida, A., Sakurai, A., Fujita, M., & Adachi, A. (2000). Cell-dependent gag mutants of HIV-1 are crucially defective at the stage of uncoating/reverse transcription in non-permissive cells. *Microbes Infect*, *2*, 1419–1423.

Kulkosky, J. & Skalka, A. M. (1994). Molecular mechanism of retroviral DNA integration. *Pharmacol Ther*, *61*, 185–203.

Kutay, U., Bischoff, F. R., Kostka, S., Kraft, R., & Gorlich, D. (1997). Export of importin alpha from the nucleus is mediated by a specific nuclear transport factor. *Cell*, *90*, 1061–1071.

Lavallee, C., Yao, X. J., Ladha, A., Gottlinger, H., Haseltine, W. A., & Cohen, E. A. (1994). Requirement of the Pr55gag precursor for incorporation of the Vpr product into human immunodeficiency virus type 1 viral particles. *J Virol*, *68*, 1926–1934.

Lawrence, D. C., Stover, C. C., Noznitsky, J., Wu, Z., & Summers, M. F. (2003). Structure of the intact stem and bulge of HIV-1 Psi-RNA stem-loop SL1. *J Mol Biol*, *326*, 529–542.

Le, R. E., Belaidouni, N., Estrabaud, E., Morel, M., Rain, J. C., Transy, C., et al. (2007). HIV1 Vpr arrests the cell cycle by recruiting DCAF1/VprBP, a receptor of the Cul4-DDB1 ubiquitin ligase. *Cell Cycle*, *6*, 182–188.

Lee, K., Ambrose, Z., Martin, T. D., Oztop, I., Mulky, A., Julias, J. G., et al. (2010). Flexible use of nuclear import pathways by HIV-1. *Cell Host Microbe*, *7*, 221–233.

Leung, K., Kim, J. O., Ganesh, L., Kabat, J., Schwartz, O., & Nabel, G. J. (2008). HIV-1 assembly: Viral glycoproteins segregate quantally to lipid rafts that associate individually with HIV-1 capsids and virions. *Cell Host Microbe*, *3*, 285–292.

Lever, A. M. (2000). HIV RNA packaging and lentivirus-based vectors. *Adv Pharmacol*, *48*, 1–28.

Levin, A., Loyter, A., & Bukrinsky, M. I. (2010). Strategies to inhibit viral protein nuclear import: HIV-1 as a target. *Biochim Biophys Acta* (in press).

Levin, A., rmon-Omer, A., Rosenbluh, J., Melamed-Book, N., Graessmann, A., Waigmann, E., et al. (2009). Inhibition of HIV-1 integrase nuclear import and replication by a peptide bearing integrase putative nuclear localization signal. *Retrovirology*, *6*, 112.

Li, S., Juarez, J., Alali, M., Dwyer, D., Collman, R., Cunningham, A., et al. (1999). Persistent CCR5 utilization and enhanced macrophage tropism by primary blood human immunodeficiency virus type 1 isolates from advanced stages of disease and comparison to tissue-derived isolates. *J Virol*, *73*, 9741–9755.

Liang, C. & Wainberg, M. A. (2002). The role of Tat in HIV-1 replication: An activator and/or a suppressor? *AIDS Rev*, *4*, 41–49.

Limon, A., Nakajima, N., Lu, R., Ghory, H. Z., & Engelman, A. (2002). Wild-type levels of nuclear localization and human immunodeficiency virus type 1 replication in the absence of the central DNA flap. *J Virol*, *76*, 12078–12086.

Lin, Y. L., Mettling, C., Portales, P., Reynes, J., Clot, J., & Corbeau, P. (2002). Cell surface CCR5 density determines the post-entry efficiency of R5 HIV-1 infection. *Proc Natl Acad Sci USA*, *99*, 15590–15595.

Liu, B., Sarkis, P. T., Luo, K., Yu, Y., & Yu, X. F. (2005). Regulation of Apobec3F and human immunodeficiency virus type 1 Vif by Vif-Cul5-ElonB/C E3 ubiquitin ligase. *J Virol*, *79*, 9579–9587.

Liu, R., Paxton, W. A., Choe, S., Ceradini, D., Martin, S. R., Horuk, R., et al. (1996). Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell*, *86*, 367–377.

Liu, Y., Tang, X. P., McArthur, J. C., Scott, J., & Gartner, S. (2000). Analysis of human immunodeficiency virus type 1 gp160 sequences from a patient with HIV dementia: Evidence for monocyte trafficking into brain. *J Neurovirol*, *6 Suppl 1*, S70–S81.

Lori, F., Malykh, A., Cara, A., Sun, D., Weinstein, J. N., Lisziewicz, J., &. (1994). Hydroxyurea as an inhibitor of human immunodeficiency virus-type 1 replication. *Science*, *266*, 801–805.

Louboutin, J. P., Agrawal, L., Reyes, B. A., Van Bockstaele, E. J., & Strayer, D. S. (2007). Protecting neurons from HIV-1 gp120-induced oxidant stress using both localized intracerebral and generalized intraventricular administration of antioxidant enzymes delivered by SV40-derived vectors. *Gene Ther*, *14*, 1650–1661.

Luban, J., Bossolt, K. L., Franke, E. K., Kalpana, G. V., & Goff, S. P. (1993). Human immunodeficiency virus type 1 Gag protein binds to cyclophilins A and B. *Cell*, *73*, 1067–1078.

Maertens, G., Cherepanov, P., Debyser, Z., Engelborghs, Y., & Engelman, A. (2004). Identification and characterization of a functional nuclear localization signal in the HIV-1 integrase interactor LEDGF/p75. *J Biol Chem*, *279*, 33421–33429.

Malim, M. H. & Emerman, M. (2008). HIV-1 accessory proteins—ensuring viral survival in a hostile environment. *Cell Host Microbe*, *3*, 388–398.

Marcello, A., Zoppe, M., & Giacca, M. (2001). Multiple modes of transcriptional regulation by the HIV-1 Tat transactivator. *IUBMB Life*, *51*, 175–181.

Marchand, C., Maddali, K., Metifiot, M., & Pommier, Y. (2009). HIV-1 IN inhibitors: 2010 update and perspectives. *Curr Top Med Chem*, *9*, 1016–1037.

Masuda, T., Kuroda, M. J., & Harada, S. (1998). Specific and independent recognition of U3 and U5 att sites by human immunodeficiency virus type 1 integrase in vivo. *J Virol*, *72*, 8396–8402.

Mattaj, I. W. & Englmeier, L. (1998). Nucleocytoplasmic transport: The soluble phase. *Annu Rev Biochem*, *67*, 265–306.

McDonald, D., Vodicka, M. A., Lucero, G., Svitkina, T. M., Borisy, G. G., Emerman, M., et al. (2002). Visualization of the intracellular behavior of HIV in living cells. *J Cell Biol*, *159*, 441–452.

McDonnell, N. B., De Guzman, R. N., Rice, W. G., Turpin, J. A., & Summers, M. F. (1997). Zinc ejection as a new rationale for the use of cystamine and related disulfide-containing antiviral agents in the treatment of AIDS. *J Med Chem*, *40*, 1969–1976.

Mehle, A., Strack, B., Ancuta, P., Zhang, C., McPike, M., & Gabuzda, D. (2004). Vif overcomes the innate antiviral activity of APOBEC3G by promoting its degradation in the ubiquitin-proteasome pathway. *J Biol Chem*, *279*, 7792–7798.

Melchior, F., Paschal, B., Evans, J., & Gerace, L. (1993). Inhibition of nuclear protein import by nonhydrolyzable analogues of GTP and identification of the small GTPase Ran/TC4 as an essential transport factor. *J Cell Biol*, *123*, 1649–1659.

Melikyan, G. B. (2008). Common principles and intermediates of viral protein-mediated fusion: The HIV-1 paradigm. *Retrovirology*, *5*, 111.

Meyerhans, A., Vartanian, J. P., Hultgren, C., Plikat, U., Karlsson, A., Wang, L., et al. (1994). Restriction and enhancement of human immunodeficiency virus type 1 replication by modulation of intracellular deoxynucleoside triphosphate pools. *J Virol*, *68*, 535–540.

Miller, M. D., Farnet, C. M., & Bushman, F. D. (1997). Human immunodeficiency virus type 1 preintegration complexes: Studies of organization and composition. *J Virol*, *71*, 5382–5390.

Miyauchi, K., Kim, Y., Latinovic, O., Morozov, V., & Melikyan, G. B. (2009). HIV enters cells via endocytosis and dynamin-dependent fusion with endosomes. *Cell*, *137*, 433–444.

Moore, M. S. & Blobel, G. (1993). The GTP-binding protein Ran/TC4 is required for protein import into the nucleus. *Nature*, *365*, 661–663.

Mori, K., Rosenzweig, M., & Desrosiers, R. C. (2000). Mechanisms for adaptation of simian immunodeficiency virus to replication in alveolar macrophages. *J Virol*, *74*, 10852–10859.

Morikawa, Y., Hockley, D. J., Nermut, M. V., & Jones, I. M. (2000). Roles of matrix, p2, and N-terminal myristoylation in human immunodeficiency virus type 1 Gag assembly. *J Virol*, *74*, 16–23.

Morita, E. & Sundquist, W. I. (2004). Retrovirus budding. *Annu Rev Cell Dev Biol*, *20*, 395–425.

Moroianu, J., Blobel, G., & Radu, A. (1995). Previously identified protein of uncertain function is karyopherin alpha and together with karyopherin beta docks import substrate at nuclear pore complexes. *Proc Natl Acad Sci USA*, *92*, 2008–2011.

Moulard, M. & Decroly, E. (2000). Maturation of HIV envelope glycoprotein precursors by cellular endoproteases. *Biochim Biophys Acta*, *1469*, 121–132.

Mujawar, Z., Rose, H., Morrow, M. P., Pushkarsky, T., Dubrovsky, L., Mukhamedova, N., et al. (2006). Human immunodeficiency virus impairs reverse cholesterol transport from macrophages. *PLoS Biol*, *4*, e365.

Nabel, G. & Baltimore, D. (1987). An inducible transcription factor activates expression of human immunodeficiency virus in T cells. *Nature*, *326*, 711–713.

Naghavi, M. H., Estable, M. C., Schwartz, S., Roeder, R. G., & Vahlne, A. (2001). Upstream stimulating factor affects human immunodeficiency virus type 1 (HIV-1) long terminal repeat-directed transcription in a cell-specific manner, independently of the HIV-1 subtype and the core-negative regulatory element. *J Gen Virol*, *82*, 547–559.

Neil, S. J., Sandrin, V., Sundquist, W. I., & Bieniasz, P. D. (2007). An interferon-alpha-induced tethering mechanism inhibits HIV-1 and Ebola virus particle release but is counteracted by the HIV-1 Vpu protein. *Cell Host Microbe*, *2*, 193–203.

Neil, S. J., Zang, T., & Bieniasz, P. D. (2008). Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. *Nature*, *451*, 425–430.

Nguyen, D. G., Booth, A., Gould, S. J., & Hildreth, J. E. (2003). Evidence that HIV budding in primary macrophages occurs through the exosome release pathway. *J Biol Chem*, *278*, 52347–52354.

Nie, Z., Bergeron, D., Subbramanian, R. A., Yao, X. J., Checroune, F., Rougeau, N., et al. (1998). The putative alpha helix 2 of human immunodeficiency virus type 1 Vpr contains a determinant which is responsible for the nuclear translocation of proviral DNA in growth-arrested cells. *J Virol*, *72*, 4104–4115.

Nishizawa, M., Kamata, M., Mojin, T., Nakai, Y., & Aida, Y. (2000). Induction of apoptosis by the Vpr protein of human immunodeficiency virus type 1 occurs independently of G(2) arrest of the cell cycle. *Virology*, *276*, 16–26.

Nitahara-Kasahara, Y., Kamata, M., Yamamoto, T., Zhang, X., Miyamoto, Y., Muneta, K., et al. (2007). Novel nuclear import of Vpr promoted by importin alpha is crucial for human immunodeficiency virus type 1 replication in macrophages. *J Virol*, *81*, 5284–5293.

Nowacek, A. & Gendelman, H. E. (2009). NanoART, neuroAIDS and CNS drug delivery. *Nanomed*, *4*, 557–574.

Nydegger, S., Foti, M., Derdowski, A., Spearman, P., & Thali, M. (2003). HIV-1 egress is gated through late endosomal membranes. *Traffic*, *4*, 902–910.

O'Brien, W. A., Namazi, A., Kalhor, H., Mao, S. H., Zack, J. A., & Chen, I. S. (1994). Kinetics of human immunodeficiency virus type 1 reverse transcription in blood mononuclear phagocytes are slowed by limitations of nucleotide precursors. *J Virol*, *68*, 1258–1263.

Ohagen, A., Ghosh, S., He, J., Huang, K., Chen, Y., Yuan, M., et al. (1999). Apoptosis induced by infection of primary brain cultures with diverse human immunodeficiency virus type 1 isolates: Evidence for a role of the envelope. *J Virol*, *73*, 897–906.

Ono, A., Ablan, S. D., Lockett, S. J., Nagashima, K., & Freed, E. O. (2004). Phosphatidylinositol (4,5) bisphosphate regulates HIV-1 Gag targeting to the plasma membrane. *Proc Natl Acad Sci USA*, *101*, 14889–14894.

Ono, A. & Freed, E. O. (1999). Binding of human immunodeficiency virus type 1 Gag to membrane: Role of the matrix amino terminus. *J Virol*, *73*, 4136–4144.

Ono, A. & Freed, E. O. (2001). Plasma membrane rafts play a critical role in HIV-1 assembly and release. *Proc Natl Acad Sci USA*, *98*, 13925–13930.

Ou, W. & Silver, J. (2005). Efficient trapping of HIV-1 envelope protein by hetero-oligomerization with an N-helix chimera. *Retrovirology*, *2*, 51.

Pelchen-Matthews, A., Kramer, B., & Marsh, M. (2003). Infectious HIV-1 assembles in late endosomes in primary macrophages. *J Cell Biol*, *162*, 443–455.

Pereira, L. A., Bentley, K., Peeters, A., Churchill, M. J., & Deacon, N. J. (2000). A compilation of cellular transcription factor interactions with the HIV-1 LTR promoter. *Nucleic Acids Res*, *28*, 663–668.

Perez-Caballero, D., Zang, T., Ebrahimi, A., McNatt, M. W., Gregory, D. A., Johnson, M. C., et al. (2009). Tetherin inhibits HIV-1 release by directly tethering virions to cells. *Cell*, *139*, 499–511.

Pierson, T. C., Kieffer, T. L., Ruff, C. T., Buck, C., Gange, S. J., & Siliciano, R. F. (2002). Intrinsic stability of episomal circles formed during human immunodeficiency virus type 1 replication. *J Virol*, *76*, 4138–4144.

Piguet, V., Wan, L., Borel, C., Mangasarian, A., Demaurex, N., Thomas, G., et al. (2000). HIV-1 Nef protein binds to the cellular protein PACS-1 to downregulate class I major histocompatibility complexes. *Nat Cell Biol*, *2*, 163–167.

Pincetic, A. & Leis, J. (2009). The mechanism of budding of retroviruses from cell membranes. *Adv Virol*, *2009*, 6239691–6239699.

Pizzato, M., Helander, A., Popova, E., Calistri, A., Zamborlini, A., Palu, G., et al. (2007). Dynamin 2 is required for the enhancement of HIV-1 infectivity by Nef. *Proc Natl Acad Sci USA*, *104*, 6812–6817.

Planelles, V. & Benichou, S. (2010). Vpr and its interactions with cellular proteins. *Curr Top Microbiol Immunol*, *339*, 177–200.

Platt, E. J., Wehrly, K., Kuhmann, S. E., Chesebro, B., & Kabat, D. (1998). Effects of CCR5 and CD4 cell surface concentrations on infections by macrophagetropic isolates of human immunodeficiency virus type 1. *J Virol*, *72*, 2855–2864.

Pluymers, W., De Clercq, E., & Debyser, Z. (2001). HIV-1 integration as a target for antiretroviral therapy: A review. *Curr Drug Targets Infect Disord*, *1*, 133–149.

Poon, B. & Chen, I. S. (2003). Human immunodeficiency virus type 1 (HIV-1) Vpr enhances expression from unintegrated HIV-1 DNA. *J Virol*, *77*, 3962–3972.

Popov, S., Rexach, M., Ratner, L., Blobel, G., & Bukrinsky, M. (1998a). Viral protein R regulates docking of the HIV-1 preintegration complex to the nuclear pore complex. *J Biol Chem*, *273*, 13347–13352.

Popov, S., Rexach, M., Zybarth, G., Reiling, N., Lee, M. A., Ratner, L., et al. (1998b). Viral protein R regulates nuclear import of the HIV-1 pre-integration complex. *EMBO J*, *17*, 909–917.

Premack, B. A. & Schall, T. J. (1996). Chemokine receptors: Gateways to inflammation and infection. *Nat Med*, *2*, 1174–1178.

Pruss, D., Bushman, F. D., & Wolffe, A. P. (1994a). Human immunodeficiency virus integrase directs integration to sites of severe DNA distortion within the nucleosome core. *Proc Natl Acad Sci USA*, *91*, 5913–5917.

Pruss, D., Reeves, R., Bushman, F. D., & Wolffe, A. P. (1994b). The influence of DNA and nucleosome structure on integration events directed by HIV integrase. *J Biol Chem*, *269*, 25031–25041.

Pryciak, P. M. & Varmus, H. E. (1992). Nucleosomes, DNA-binding proteins, and DNA sequence modulate retroviral integration target site selection. *Cell*, *69*, 769–780.

Pushkarsky, T., Dubrovsky, L., & Bukrinsky, M. (2001). Lipopolysaccharide stimulates HIV-1 entry and degradation in human macrophages. *J Endotoxin Res*, *7*, 271–276.

Rain, J. C., Cribier, A., Gerard, A., Emiliani, S., & Benarous, R. (2009). Yeast two-hybrid detection of integrase-host factor interactions. *Methods*, *47*, 291–297.

Reil, H., Bukovsky, A. A., Gelderblom, H. R., & Gottlinger, H. G. (1998). Efficient HIV-1 replication can occur in the absence of the viral matrix protein. *EMBO J*, *17*, 2699–2708.

Rexach, M. & Blobel, G. (1995). Protein import into nuclei: Association and dissociation reactions involving transport substrate, transport factors, and nucleoporins. *Cell*, *83*, 683–692.

Riviere, L., Darlix, J. L., & Cimarelli, A. (2010). Analysis of the viral elements required in the nuclear import of HIV-1 DNA. *J Virol*, *84*, 729–739.

Roe, T., Reynolds, T. C., Yu, G., & Brown, P. O. (1993). Integration of murine leukemia virus DNA depends on mitosis. *EMBO J*, *12*, 2099–2108.

Saad, J. S., Miller, J., Tai, J., Kim, A., Ghanam, R. H., & Summers, M. F. (2006). Structural basis for targeting HIV-1 Gag proteins to the plasma membrane for virus assembly. *Proc Natl Acad Sci USA*, *103*, 11364–11369.

Sakuragi, J. I., Ueda, S., Iwamoto, A., & Shioda, T. (2003). Possible role of dimerization in human immunodeficiency virus type 1 genome RNA packaging. *J Virol*, *77*, 4060–4069.

Schindler, M., Munch, J., Kutsch, O., Li, H., Santiago, M. L., Bibollet-Ruche, F., et al. (2006). Nef-mediated suppression of T cell activation was lost in a lentiviral lineage that gave rise to HIV-1. *Cell*, *125*, 1055–1067.

Schindler, M., Rajan, D., Banning, C., Wimmer, P., Koppensteiner, H., Iwanski, A., et al. (2010). Vpu serine 52 dependent counteraction of tetherin is required for HIV-1 replication in macrophages, but not in ex vivo human lymphoid tissue. *Retrovirology*, *7*, 1.

Schmidtmayerova, H., Nuovo, G. J., & Bukrinsky, M. (1997). Cell proliferation is not required for productive HIV-1 infection of macrophages. *Virology*, *232*, 379–384.

Schroder, A. R., Shinn, P., Chen, H., Berry, C., Ecker, J. R., & Bushman, F. (2002). HIV-1 integration in the human genome favors active genes and local hotspots. *Cell*, *110*, 521–529.

Schuitemaker, H., Kootstra, N. A., Fouchier, R. A., Hooibrink, B., & Miedema, F. (1994). Productive HIV-1 infection of macrophages restricted to the cell fraction with proliferative capacity. *EMBO J*, *13*, 5929–5936.

Segura-Totten, M. & Wilson, K. L. (2001). Virology. HIV—breaking the rules for nuclear entry. *Science*, *294*, 1016–1017.

Sharova, N., Wu, Y., Zhu, X., Stranska, R., Kaushik, R., Sharkey, M., et al. (2008). Primate lentiviral Vpx commandeers DDB1 to counteract a macrophage restriction. *PLoS Pathogens*, *4*, e1000057.

Sheehy, A. M., Gaddis, N. C., Choi, J. D., & Malim, M. H. (2002). Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. *Nature*, *418*, 646–650.

Sherer, N. M., Lehmann, M. J., Jimenez-Soto, L. F., Ingmundson, A., Horner, S. M., Cicchetti, G., et al. (2003). Visualization of retroviral replication in living cells reveals budding into multivesicular bodies. *Traffic*, *4*, 785–801.

Siebenlist, U., Franzoso, G., & Brown, K. (1994). Structure, regulation and function of NF-kappa B. *Annu Rev Cell Biol*, *10*, 405–455.

Smit, T. K., Wang, B., Ng, T., Osborne, R., Brew, B., & Saksena, N. K. (2001). Varied tropism of HIV-1 isolates derived from different regions of adult brain cortex discriminate between patients with and without AIDS dementia complex (ADC): Evidence for neurotropic HIV variants. *Virology*, *279*, 509–526.

Sodeik, B., Ebersold, M. W., & Helenius, A. (1997). Microtubule-mediated transport of incoming herpes simplex virus 1 capsids to the nucleus. *J Cell Biol*, *136*, 1007–1021.

Sokolskaja, E. & Luban, J. (2006). Cyclophilin, TRIM5, and innate immunity to HIV-1. *Curr Opin Microbiol*, *9*, 404–408.

Sonza, S., Mutimer, H. P., O'Brien, K., Ellery, P., Howard, J. L., Axelrod, J. H., et al. (2002). Selectively reduced tat mRNA heralds the decline in productive human immunodeficiency virus type 1 infection in monocyte-derived macrophages. *J Virol*, *76*, 12611–12621.

Soros, V. B., Yonemoto, W., & Greene, W. C. (2007). Newly synthesized APOBEC3G is incorporated into HIV virions, inhibited by HIV RNA, and subsequently activated by RNase H. *PLoS Pathog*, *3*, e15.

Srivastava, S., Swanson, S. K., Manel, N., Florens, L., Washburn, M. P., & Skowronski, J. (2008). Lentiviral Vpx accessory factor targets VprBP/DCAF1 substrate adaptor for cullin 4 E3 ubiquitin ligase to enable macrophage infection. *PLoS Pathog*, *4*, e1000059.

Stevenson, M. & Gendelman, H. E. (1994). Cellular and viral determinants that regulate HIV-1 infection in macrophages. *J Leukoc Biol*, *56*, 278–288.

Subbramanian, R. A., Kessous-Elbaz, A., Lodge, R., Forget, J., Yao, X. J., Bergeron, D., et al. (1998). Human immunodeficiency virus type 1 Vpr is a positive regulator of viral transcription and infectivity in primary human macrophages. *J Exp Med*, *187*, 1103–1111.

Suhasini, M. & Reddy, T. R. (2009). Cellular proteins and HIV-1 Rev function. *Curr HIV Res*, *7*, 91–100.

Suomalainen, M., Nakano, M. Y., Keller, S., Boucke, K., Stidwill, R. P., & Greber, U. F. (1999). Microtubule-dependent plus- and minus end-directed motilities are competing processes for nuclear targeting of adenovirus. *J Cell Biol*, *144*, 657–672.

Swigut, T., Shohdy, N., & Skowronski, J. (2001). Mechanism for down-regulation of CD28 by Nef. *EMBO J*, *20*, 1593–1604.

Taunton, J. (2001). Actin filament nucleation by endosomes, lysosomes and secretory vesicles. *Curr Opin Cell Biol*, *13*, 85–91.

Terry, L. J. & Wente, S. R. (2009). Flexible gates: Dynamic topologies and functions for FG nucleoporins in nucleocytoplasmic transport. *Eukaryot Cell*, *8*, 1814–1827.

Tristem, M., Purvis, A., & Quicke, D. L. (1998). Complex evolutionary history of primate lentiviral vpr genes. *Virology*, *240*, 232–237.

Turchan, J., Pocernich, C. B., Gairola, C., Chauhan, A., Schifitto, G., Butterfield, D. A., et al. (2003). Oxidative stress in HIV demented patients and protection ex vivo with novel antioxidants. *Neurology*, *60*, 307–314.

Van Damme, N., Goff, D., Katsura, C., Jorgenson, R. L., Mitchell, R., Johnson, M. C., et al. (2008). The interferon-induced protein BST-2 restricts HIV-1 release and is downregulated from the cell surface by the viral Vpu protein. *Cell Host Microbe*, *3*, 245–252.

Van Marle, G., Rourke, S. B., Zhang, K., Silva, C., Ethier, J., Gill, M. J., et al. (2002). HIV dementia patients exhibit reduced viral neutralization and increased envelope sequence diversity in blood and brain. *AIDS*, *16*, 1905–1914.

Vodicka, M. A., Koepp, D. M., Silver, P. A., & Emerman, M. (1998). HIV-1 Vpr interacts with the nuclear transport pathway to promote macrophage infection. *Genes Dev*, *12*, 175–185.

Weinberg, J. B., Matthews, T. J., Cullen, B. R., & Malim, M. H. (1991). Productive human immunodeficiency virus type 1 (HIV-1) infection of nonproliferating human monocytes. *J Exp Med*, *174*, 1477–1482.

Woodward, C. L., Prakobwanakit, S., Mosessian, S., & Chow, S. A. (2009). Integrase interacts with nucleoporin NUP153 to mediate the nuclear import of human immunodeficiency virus type 1. *J Virol*, *83*, 6522–6533.

Wu, Y. & Marsh, J. W. (2003). Early transcription from nonintegrated DNA in human immunodeficiency virus infection. *J Virol*, *77*, 10376–10382.

Wu, Y., Yoder, A., Yu, D., Wang, W., Liu, J., Barrett, T., et al. (2008). Cofilin activation in peripheral CD4 T cells of HIV-1 infected patients: A pilot study. *Retrovirology*, *5*, 95.

Yamashita, M. & Emerman, M. (2006). Retroviral infection of non-dividing cells: Old and new perspectives. *Virology*, *344*, 88–93.

Yamashita, M., Perez, O., Hope, T. J., & Emerman, M. (2007). Evidence for direct involvement of the capsid protein in HIV infection of non-dividing cells. *PLoS Pathog*, *3*, 1502–1510.

Yi, Y., Chen, W., Frank, I., Cutilli, J., Singh, A., Starr-Spires, L.,et al. (2003). An unusual syncytia-inducing human immunodeficiency virus type 1 primary isolate from the central nervous system that is restricted to CXCR4, replicates efficiently in macrophages, and induces neuronal apoptosis. *J Neurovirol*, *9*, 432–441.

Yoder, A., Yu, D., Dong, L., Iyer, S. R., Xu, X., Kelly, J., et al. (2008). HIV Envelope-CXCR4 signaling activates cofilin to overcome cortical actin restriction in resting CD4 T cells. *Cell*, *134*, 782–792.

Yuan, X., Yu, X., Lee, T. H., & Essex, M. (1993). Mutations in the N-terminal region of human immunodeficiency virus type 1 matrix protein block intracellular transport of the Gag precursor. *J Virol*, *67*, 6387–6394.

Zack, J. A., Arrigo, S. J., Weitsman, S. R., Go, A. S., Haislip, A., & Chen, I. S. (1990). HIV-1 entry into quiescent primary lymphocytes: Molecular analysis reveals a labile, latent viral structure. *Cell*, *61*, 213–222.

Zennou, V., Perez-Caballero, D., Gottlinger, H., & Bieniasz, P. D. (2004). APOBEC3G incorporation into human immunodeficiency virus type 1 particles. *J Virol*, *78*, 12058–12061.

Zennou, V., Petit, C., Guetard, D., Nerhbass, U., Montagnier, L., & Charneau, P. (2000). HIV-1 genome nuclear import is mediated by a central DNA flap. *Cell*, *101*, 173–185.

Zhang, H., Dornadula, G., & Pomerantz, R. J. (1996). Endogenous reverse transcription of human immunodeficiency virus type 1 in physiological microenviroments: An important stage for viral infection of nondividing cells. *J Virol*, *70*, 2809–2824.

Zhang, H., Dornadula, G., & Pomerantz, R. J. (1998). Natural endogenous reverse transcription of HIV type 1. *AIDS Res Hum Retroviruses*, *14 Suppl 1*:*S93–5*, S93–S95.

Zheng, J., Thylin, M. R., Ghorpade, A., Xiong, H., Persidsky, Y., Cotter, R., et al. (1999). Intracellular CXCR4 signaling, neuronal apoptosis and neuropathogenic mechanisms of HIV-1-associated dementia. *J Neuroimmunol*, *98*, 185–200.

Zybarth, G., Reiling, N., Schmidtmayerova, H., Sherry, B., & Bukrinsky, M. (1999). Activation-induced resistance of human macrophages to HIV-1 infection in vitro. *J Immunol*, *162*, 400–406.

1.2

HIV-1 IMMUNOLOGY

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This chapter serves as a bridge between the molecular and structural biology of the human immunodeficiency virus type one (HIV-1) and the immunological consequences that viral infection produces. The innate and adaptive immune systems and their responses play a critical role in the control of HIV replication. Unfortunately, immune dysfunction is common in HIV-infected individuals and the virus as well is capable of developing strategies to evade host defenses. Thus, in most untreated individuals, if viral replication is not fully contained, persistent viremia is sustained, eventually resulting in progressive disease and the development of acquired immunodeficiency syndrome (AIDS).

GENETIC ORGANIZATION OF HIV-1

HIV primarily targets the immune system and therefore the clinical consequences are manifested because of immune dysfunction and depletion. HIV-1 is a member of the primate lentivirus subgroup of retroviruses (Weiss et al., 1986, 2008; Burton & Weiss, 2010 Kellam & Weiss, 2006), and is a close relative of HIV-2 and simian immunodeficiency viruses (SIV). The HIV-1 particle is composed of two identical (+) strand RNA copies of the viral genome (Weiss et al., 1986, 2009). Following infection of host cell, the RNA genome is reversed transcribed into DNA or a provirus. The 5' and the 3' ends of the provirus contain 634 nucleotides of long terminal repeat structures (Figure 1.2.1). While the 5' LTR regulates the initiation of RNA transcription, the 3'LTR regulates RNA termination and polyadenylation. Gag and env genes encode the structural protein of the virus particle. Virus entry is regulated by the envelope proteins which are embedded in the lipid bilayer and mediate receptor binding and membrane fusion. The pol gene encodes the viral protease, reverse transcriptase, ribonuclease H, and integrase. The regulatory proteins Tat and Rev influence the replication of HIV by mediating their effects through the Tat response element and the Rev response element (RRE), respectively. Accessory proteins, which are at least partially dispensable for virus replication in vitro, are encoded by Vif, Vpr, Vpu, and Nef. Vif, a virion infectivity factor, promotes infectivity of virus particles but is not packaged to a significant extent in the virus particle. Vpr, a viral protein R, is homologous to Vpx, a viral protein X, of HIV-2 and SIVs, and is packaged into the virus particle, but its function is incompletely described. Vpu, viral protein U, is required for efficient virus budding and CD4 downregulation. Nef, that is, "negative factor," has multiple effects on virus replication and T cell activation. The envelope proteins play an important role in interactions with cellular receptor membrane, in membrane fusion effects, and in initiation of signal transduction into cells. The presentation of viral proteins to T cells is a critical early step in the immune response to HIV-1. Class 1 and class II restricted epitopes are located throughout the HIV-1 env protein. Analysis of these epitopes has provided new insights into antigen processing pathways and vaccine design strategies.

EARLY EVENTS FOLLOWING HIV-1 INFECTION

Attachment of the virus particle to the cell surface is the initial step in the infection process. Fusion of the viral and cellular membranes allows for the delivery of the viral core into the cytoplasm of the cell. The process of attachment and fusion is mediated by the interaction of viral glycoprotein spikes with cell surface receptors. HIV-l glycoprotein spikes specifically interact with the CD4 receptor and a seven-transmembrane G-protein coupled co-receptor on the cell surface. There is 1:1 binding stoichiometry of a monomer of gp120 with CD4. The binding of one CD4 molecule by a single gp120 in the trimeric spike is sufficient to induce conformational changes in all three glycoprotein subunits of the trimer (Salzwedel & Berger, 2000). The interaction of CD4 and co-receptor with the gp120 causes a rearrangement in gp41

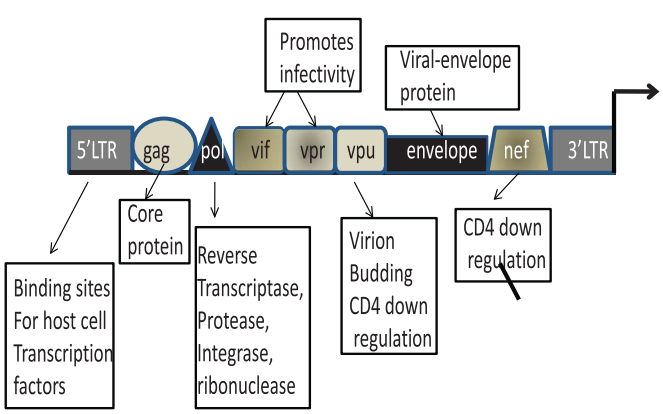

Figure 1.2.1 Genetic organization of the HIV-1 genome. Location of each gene are shown together with their function.

exposing the hydrophobic fusion domain, which then comes in contact with the cell membrane and becomes embedded into it, leading to viral entry (Figure 1.2.2).

During the viral entry process, the viral envelope disassociates from the virion, initiating the process of uncoating. Once inside the cell, further uncoating involves the disintegration of the viral capsid core and shedding of the capsid proteins (Figure 1.2.3).

The uncoating process involves the cellular protein cyclophilin A, which interacts with HIV-1 Gag polyprotein and is incorporated into the virion prior to reverse transcription (Franke, Yuan, & Luban, 1994; Thali, Bukovsky, & Kondo, 1994; Braaten, Franke, & Luban, 1996). The main early event in the life cycle of HIV-l is the reverse transcription of viral RNA into double-stranded DNA. This is catalyzed in the cytoplasm of the infected cell by the virus-encoded reverse transcriptase through the use of a cellular lysine tRNA molecule as a primer (Cen et al., 2001). Several viral and cellular factors are involved in the formation of the replication complex. The replication complex consists of the viral DNA/RNA, reverse transcriptase, integrase, viral matrix protein p17, viral protease, the accessory protein Vpr, and cellular histones and non-histone proteins. During the formation of the replication complex, various core viral proteins are lost. This replication complex, also termed the pre-integration complex, is subsequently transported into the nucleus.

The entry of the HIV-l pre-integration complex into the nucleus is mediated by the interaction between the cellular receptor karyopherin alpha and the nuclear localization signals (NLS) present on viral proteins, matrix, and integrase (Bukrinsky & Haffar, 1999). The viral protein Vpr increases the affinity of interaction between the viral NLS and the cellular receptor karyopherin alpha, facilitating nuclear transport of the pre-integration complex (Popov et al., 1998). Additional factors contribute to the nuclear transport of the pre-integration complex, including a triple-stranded intermediate produced during reverse transcription, called the DNA flap (Sherman & Greene, 2002). The viral integrase then catalyzes a series of reactions whereby the intact viral DNA is inserted into the cellular chromosomal DNA forming the provirus. The first step of the integration process is the removal of two nucleotides from the 3' ends of the viral DNA. In the second step, the host chromosome is randomly cut producing a 5-base staggered cut. This is followed by the insertion of the viral DNA. The third and final step consists of removal of unpaired bases at the 5' end of viral DNA, gap filling, and ligation. The first two steps are catalyzed by integrase, but the repair and ligation steps are thought to be mediated by cellular enzymes (Craigie, 2001).

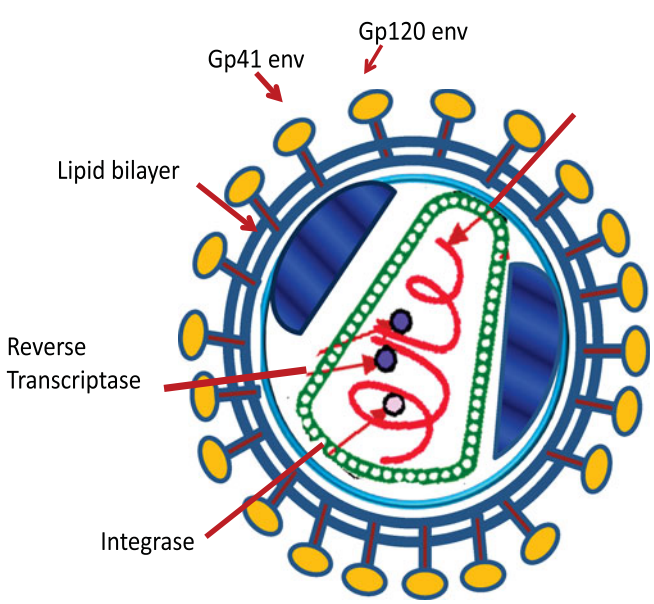

Figure 1.2.2 Structure of the HIV-1 virion. The envelope subunit proteins (gp41 and gp120), matrix protein (p18), viral capsid protein (p24), viral genomic RNA (RNA), and the reverse transcriptase enzyme CRT) are illustrated.

The early phase of the viral replication cycle ends following DNA integration and the virus enters the late phase of its life cycle and begins to multiply. The provirus is far more efficient in transcription of viral RNA than the free unintegrated HIV-1 viral DNA. The provirus produces multiple copies of the progeny viral RNA and mRNAs that are later translated into viral proteins. HIV-1 proviral transcription is greatly influenced by the activation state of the host cell and is regulated by sequences in the 5' long terminal repeat (LTR) of the viral genome. A large number of these regulatory sequences are able to specifically bind cellular transcription factors, thus enabling the HIV-1 provirus to use the cellular transcription machinery to replicate.

The HIV-1 primary transcript is the 9.2 kb mRNA, which is the full-length transcript encoding the Gag and Pol proteins. Translation initiation starts with the Gag open reading frame. The major translation product is the Gag polyprotein (p55), which is first translated as a precursor polyprotein and later cleaved by the viral protease. The Gag precursor polyprotein (p55) is also modified by myristoylation. The mature proteins derived from the gag precursor polyprotein consist of the matrix protein (MA) p17, the capsid protein (CA) p24, the nucleocapsid protein (NC) p7, and the p6 protein that binds to the Vpr protein. The minor translation product is the Gag-Pol precursor p160. The Pol gene products derived from the Gag-Pol polyprotein are the viral protease (Pr) p11, reverse transcriptase (RT) p66, and integrase (IN) p31. The primary transcript also serves as genomic RNA for the production of progeny virions after being capped at the 5' end and polyadenylated at the 3' end. The 4 kb transcript contains mRNAs encoding the Env proteins, Vif, Vpr, and Vpu proteins, as well as mRNAs for the minor forms of the Tat protein. The 4 kb transcript coding for the envelope proteins is first translated as a polyprotein and subsequently glycosylated. The glycosylated precursor polyprotein (gp160) is transported to the rough endoplasmic reticulum where it forms oligomers and enters the secretory pathway. A cellular enzyme in the Golgi apparatus then cleaves these oligomers to produce the surface glycoprotein (gp120) and the transmembrane glycoprotein (gp4l). The oligomeric forms of gp120 and gp41 are finally transported to the plasma membrane. The 2 kb transcript contains mRNAs encoding Nef, Rev, and the major forms of Tat proteins

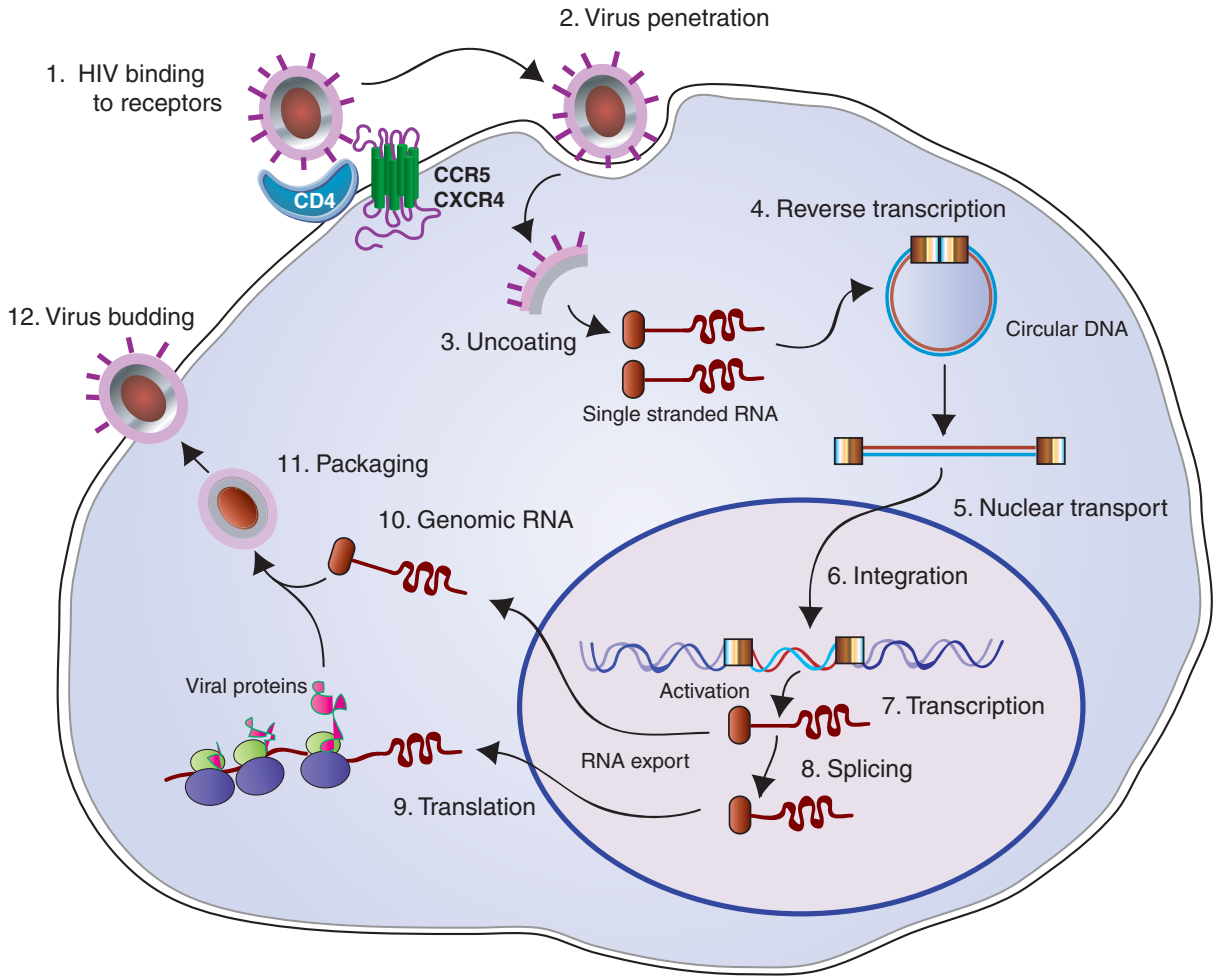

Figure 1.2.3 The replication cycle of the HIV-1 virion. HIV binds to CD4 receptors on the surface of T cells or macrophages and to one of two co-receptors, CCR5 and CXCR4. The next event involves the fusion of the viral protein gp41 to the host cell membranes. After the fusion event, the virus capsid is partially uncoated to form a ribonucleoprotein complex capable of reverse transcription. Viral genomic RNA is reverse transcribed to yield double stranded viral DNA. Viral DNA forms the pre-integration complex and is transported to the cell nucleus where it is integrated in to the cellular chromosome to form the provirus. From the provirus, the viral RNAs and proteins are expressed. Viral assembly and budding occur at the cell membrane forming the progeny virus.

(Purcell & Martin, 1993). All HIV-l RNA transcripts contain a transcription regulation sequence called TAR (transactivation-responsive or Tat-activation responsive) sequence. The 9 kb and 4 kb HIV-l RNA transcripts also contain a post-transcription regulatory sequence called an RRE (Rev-responsive element) sequence. These sequences are involved in the activation and regulation of viral RNA expression through interactions with the viral regulatory proteins Tat and Rev.

The exact mechanism of viral assembly and maturation is poorly understood. Viral assembly is energy dependent and probably involves unidentified cellular factors (Treitel & Resh, 2001). Viral assembly involves the transportation of the Gag and Gag-Pol precursor polyproteins and the envelope glycoproteins to the plasma membrane followed by the assembly of the viral capsid proteins and finally the packaging of HIV-l genomic RNA. Budding, the final stage of progeny virus production, is by the process of exocytosis. The Gag precursor proteins and viral components form the major components of the budding virus. In addition, cellular components including tRNAs and proteins are also incorporated into the budding virions.

Viral budding occurs at the plasma membrane of infected T cells, whereas in infected macrophages budding occurs at intracellular membrane sites, forming vacuoles containing the viral progeny (Gelderblom, 1991). During the maturation stage, the Gag and Gag-Pol precursor polyproteins are cleaved to yield mature viral proteins, the capsid core is formed, and the dimerization of viral RNA takes place. These events are believed to occur after budding has taken place.

An alternate route of HIV-l infection is by cell-to-cell transmission. One mechanism is the fusion of HIV-l infected cells with uninfected susceptible cells. The fusion event brings the infectious viral components into the uninfected cell, forming multinucleated giant cells (syncytia). This process is very efficient and does not involve cell-free virus. The other mechanism involves the unidirectional budding of HIV-l at the site of cell-to-cell contact.

IMMUNE RESPONSE FOLLOWING HIV-1 INFECTION

HIV infection disrupts the immune system through generalized immune activation and CD4+ T cell depletion. HIV infection can generally be broken down into four distinct stages: primary infection, a clinically asymptomatic stage, symptomatic HIV infection, and progression from HIV to AIDS, with individual variation in length, symptoms, and severity (Fauci, 1993). Primary HIV infection is the first stage of HIV disease, typically lasting only a week or two, when the virus first establishes itself in the body (Figure 1.2.4).

Some researchers use the term acute HIV infection to describe the period of time between when a person is first infected with HIV and when antibodies against the virus are produced (usually 6 to 12 weeks) and can be detected by an HIV test. This stage is associated with high levels of viral replication followed by a loss of CD4+ T cells in the gut and later in the blood (Hasse, 1999). This phase is characterized by the replication and infection of cells that express both CD4+CCR5+ T cells and remains localized in genital/rectal mucosa and draining lymph nodes (Gasper-Smith et. al, 2008; McMichael et al., 2010). Virus then spreads via the blood to other lymphoid tissue, especially in the gut. Even at an early stage of infection, the CD 4+T lymphocytes appear to be the major targets (Haase, 1999). There, it replicates profusely and the level of free virus in the blood rises exponentially and reaches a peak, often millions of virus copies per milliliter of plasma, 21–28 days after infection. Virus population doubles every 6 to 10 hours and the infected cell can productively infect 20 new cells (Nowak et al., 1997; Little, McLean, Spina, Richman, & Havlir, 1999). Virus levels then fall, rapidly at first, until a stable level is reached. This high level of viremia at the primary stage may be responsible for the systemic infection of the peripheral lymphoid organs. Within a few weeks of the initial viremia, the level of the virus decreases in the blood. This decrease coincides with the development of an immune response to HIV-l. Virus-specific cytolytic T lymphocytes (CTL) appear early and may play an important role in down-regulation of virus replication (Borrow et al., 1991 Pantaleo et al., 1994; Schmitz et al., 1999). HIV-1 infection may go undetected for long periods due to the nonspecific nature of the symptoms (Weber, 2001). Early CD8+ T cell responses are generally credited for controlling the initial peak of viral replication and establishment of a viral set point and partial recovery of CD4+ T cells in the periphery (Koup, Safrit, & Cao, 1994; Goonetilleke et al., 2009; Almeida et al., 2007). When CD4+ T cell numbers decline to critical levels, cell-mediated immunity is compromised, and the patient develops AIDS.

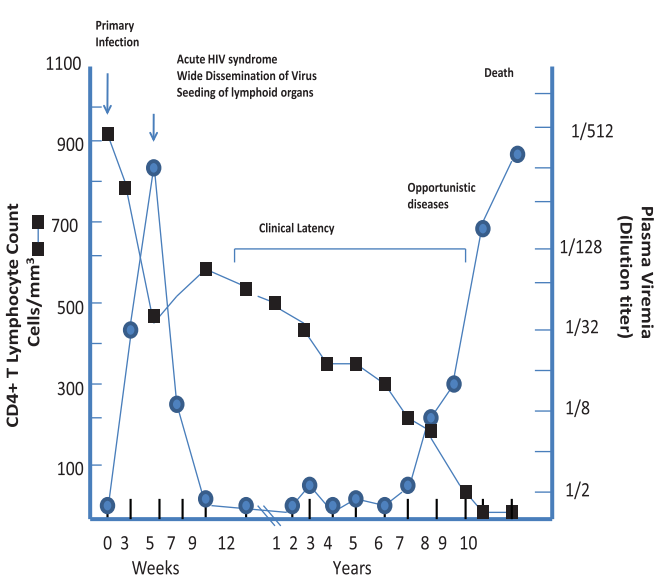

Figure 1.2.4 Time course of HIV-1 infection (redrawn from Pantaleo et al., 1993, with permission).

It is uncertain how the peak viremia of acute HIV-1 infection is controlled. Some mathematical models (Phillips, 1996; Davenport et al., 2007; Petravic, 2008) suggest that the rampant early infection results in the massive destruction of CD4+ T cells in the gut (Brenchley, 2004; Guadalupe et al., 2003) and elsewhere such that the cell substrate becomes limiting. However, studies in rhesus macaques infected with simian immunodeficiency virus (SIV) show that reduction of peak viremia is dependent on the presence of CD8+ cells (Schmitz et al., 1999) and either T or NK cells, or both. In HIV-1 infection, virus-specific CD8+ T cells first appear in the blood just before the viremia peaks and then expand and contract as virus load falls (Borrow et al., 1991 Koup, Safrit, & Cao, 1994; Wilson et al., 2000). HIV-1–specific CD8+ T cells are detectable before the development of detectable specific antibodies to HIV-1 and long before neutralizing antibodies (Huber & Trkola, 2007) are detected.

Three mechanisms are proposed by which CD8+ T cells suppress viral replication. In the first mechanism, IFN-gamma, an antiviral cytokine, is produced, which in turn inhibits HIV-1 replication (Meylan, Guatelli, Munis, Richman, & Kornbluth, 1993; Emilie, Maillot, Nicholas, Fior, & Galanaud, 1992). In addition, the chemokines MIP-1 -alpha, MIP-1 beta, and RANTES are secreted to suppress HIV-1 replication by competing or downregulating the cellular co-receptor CCR5. Downregulation of the co-receptor protects uninfected cells by limiting the availability of virus co-receptor (Wagner, Yang, & Garcia-Zepeda, 1998; Price et al., 1998). The second mechanism involves the lysis of virus-infected 'target' cells via Fas–FasL interaction, and FAS mediated apoptosis. Inhibition of HIV-1 replication in the third mechanism involves lysis of the cell through secretion of perforins and granzymes (Kagi, Seiler, & Pavlovic, 1995). CD8+ CTLs recognize viral antigens that have been intracellularly processed into peptides. These viral peptides, which are typically 8–11 amino acids in length, are presented on the infected cell together with a class I major histocompatibility complex (MHC) molecule and β2 microglobulin. The receptor on the CD8+ T cells recognizes the MHC class I molecule, β2 microglobulin, and the viral peptide complex. This recognition by the CTLs triggers the lysis of the infected cells. Cultured HIV-1 specific CTLs have been shown to lyse CD8+ CTL

cells in vitro (Yang, Kalams, & Rosenzweig, 1996). The virus-specific CD8+ T-cell responses are narrowly directed against a limited number of CTL epitopes despite exposure to a high level of viral antigens (Yu et al., 2002). The most frequently recognized viral epitopes are derived from the Gag, Pol, Env, and Nef proteins (Goulder et al 1996). Interestingly, epitopes derived from Tat, Rev, and Vif proteins are encountered at a lower frequency (Lamhamedi-Cherradi, Culman-Penciolelli, & Guy, 1995). In addition, viral replication is downregulated by the expression of membrane-bound Fas ligands that induce apoptosis in Fas-expressing cells (Kagi et al., 1995*).*

In addition to the CTL responses, cellular immune response consists of specific CD4+ T helper (Th-I) cell responses. Viral proteins that are endocytosed by antigen-presenting cells are cleaved into peptide antigens triggering the CD4+ T cell response. The antigens that are presented on the surface of the antigen-presenting cells, together with the MHC class II molecules are recognized by the CD4+ T cell receptor, which triggers the activation and differentiation of the CD4+T cells. Activated Th-1 cells produce cytokines such as interleukin-2 (IL-2) and IFN-γ (Th-1 response), which help to sustain the CTL response. Th-2 effector T cells secrete interleukins IL-4, IL-5, IL-6 and IL-1 0, which help in promoting B-cell responses to infection. In addition, the Th-1 cells activate antigen-presenting cells, such as dendritic cells, which further promote an effective immune response.

The combined effects of CTL and other elements of immune response cause the viral load in blood to decrease drastically. The cellular immune response is followed by an effective humoral response (Koup, Safrit, & Cao, 1994). In the primary acute phase of HIV-1 infection, partial viral clearance occurs before the specific antibody response is generated. Despite the early appearance of a vigorous immune response, this response ultimately fails to eliminate the virus. Several virological characteristics, such as the replication and mutation rate, proviral latency, and sequestration of viral reservoirs, play an important role in the immune evasion of HIV-1 (McMichael & Rowland-Jones, 2001). Transition of virus from R5 to X4 type, when the co-receptor requirement is changed from CCR5 to CXCR4 makes the virus resistant to inhibition by chemokines released by activated T cells. Other factors, like the downregulation of MHC molecules (Collins et al., 1998) and the upregulation of Fas ligands (Xu et al., 1999*),* contribute to the immune evasion and persistence of HIV-1 infection leading to disease progression.

The second phase of HIV-1 infection is the long asymptomatic period between the acute primary infection phase and the development of clinical immunodeficiency (AIDS). During this stage, HIV-1 continuously replicates in the infected cells and can be readily detected in the lymphoid tissues. This persistent viral replication and the associated chronic immune stimulation have been proposed to be responsible for the progressive destruction of lymphoid tissue and deterioration of the immune system. The immunological hallmark of this asymptomatic period leading to progression of AIDS is the gradual *loss* of CD4+T cells. The rate of CD4+ T-cell loss correlates with the level of ongoing viral replication; however, the exact mechanism by which HIV-I causes the depletion of CD4+ T cells is not known. There is increasing evidence that T cell activation, accelerated cell turnover, and imbalance of cytokines induces "cell cycle dysregulation" (CCD), which can result in the depletion of both CD4+ and CD8+ uninfected T cells (Gallati & Bocchino, 2007). A series of recent studies have proposed that the depletion of CD4+ T cells is more rapid and severe at the level of gut-associated lymphoid tissue than in peripheral blood and secondary lymphoid organs (i.e., lymph nodes and spleen) (Haase, 2005; Paiardini, Frank, Pandrea, Apetrei, & Silvestri, 2008). Identifying distinctions between pathogenic HIV and simian immunodeficiency virus (SIV) infections and nonprogressive SIV in natural African primate hosts might provide key insights into HIV pathogenesis. Similar to pathogenic HIV infection in humans, natural SIV infections result in high viral replication and massive acute depletion of mucosal CD4+ T cells. A key distinction of natural SIV infections is a rapidly developing anti-inflammatory milieu that prevents chronic activation, apoptosis, and proliferation of T cells, and preserves the function of other immune cell subsets, thus contributing to the integrity of the mucosal barrier and the lack of microbial translocation from the gut to the peritoneum. Immunologic features observed during natural SIV infections suggest approaches for designing new strategies for producing novel second-generation vaccines and therapeutic approaches to inhibit disease progression in HIV-infected humans (Pandrea et al., 2008).

More recently, the FOXP3(+)/CD8(+) T (Treg) population has been implicated to play a key role in controlling the magnitude and duration of adaptive immune responses through suppression of T cell activation (Torheim et al., 2009). A rapid expansion of CD25(+)/FOXP3(+)/CD8(+) regulatory T cells (Tregs) in the blood, lymphoid and colorectal mucosal tissues, preferential sites of virus replication, has been demonstrated following a pathogenic SIV infection in rhesus macaques (Nigam et al., 2010). Expression of molecules associated with immune suppressor function such as CTLA-4 and CD39 were associated with these Tregs with suppressed proliferation of SIV-specific T cells in vitro. These also express low levels of granzyme B and perforin, suggesting that these cells do not possess killing potential. Expansion of CD8+ Tregs correlated directly with acute phase viremia and inversely with the magnitude of antiviral T cell response. Expansion was observed in HIV-infected humans but not in SIV-infected sooty mangabeys with high viremia, suggesting a direct role for hyperimmune activation and an indirect role for viremia in the induction of these cells. These results suggest an important but previously unappreciated role for CD8+Tregs in suppressing antiviral immunity during immunodeficiency virus infections. These results also suggest that CD8+ Tregs expand in pathogenic immunodeficiency virus infections in the non-natural hosts and that therapeutic strategies that prevent expansion of these cells may enhance control of HIV infection.

The final phase of infection (symptomatic HIV infection, and progression from HIV to AIDS) is characterized by the onset of clinical immunodeficiency commonly known as acquired immunodeficiency syndrome (AIDS). Prior to the

onset of clinical immunodeficiency, there is a rapid decline in CD4+ T cell count and an overall increase in viral load (Connor, Mohri, Cao, & Ho, 1993). Viral replication occurs in many sites in addition to the lymphoid tissue (Reinhart et al., 1997). When the CD4+ T cell counts fall below 200 cells/ml, opportunistic infections begin to surface. The average period for the development of AIDS after initial infection varies among infected individuals. Prospective studies involving large cohorts of HIV-infected individuals clearly indicate that while some individuals remain asymptomatic for as long as 10 years after initial infection (long-term non-progressors; LTNPs), others develop AIDS within 24 months (rapid progressors; RPs). Among these individuals are some who progress to AIDS similarly to rapid progressors, but in whom both clinical and laboratory parameters remain stable for an unusually long period of time once the disease progression has occurred (slow progressors; SPs).

Between the multitude of factors that govern the natural history and pathogenesis of HIV-1 infection, viral and host factors and their complex interactions are considered crucial determinants of disease outcome (Fauci, 1993; Schnittman & Fauci, 1994; Pantaleo, Graziosi, & Fauci, 1997; Burinsky, Stanwick, & Dempsey, 1991; Wahl & Orenstein, 1997; Haynes, 1996; McCune, 1995;; Pantaleo & Fauci, 1994). For instance, LTNPs appear to have predominantly Th1 -T cell profiles, strong CD8+ lymphocyte antiviral responses, absence of enhancing antibodies, low viral loads, and predominantly non-syncytium-inducing (NSI) viral phenotypes, whereas RPs have high viral loads and predominantly syncytium-inducing (S1) viral phenotypes. RPs are believed to be infected with more rapidly replicating virulent HIV strains, whereas NPs may be infected with less pathogenic HIV variants. Thus, preservation of immune function and low viral replication are common findings in HIV-infected subjects with non-progressive disease, whereas loss of immune function and high viremia are characteristic features for rapid progression (Haynes et al., 1996). However, with the advent of combination antiretroviral therapies, there are some changes in the natural history of HIV disease (Feinberg & McLean, 1997).

CLINICAL PROFILE OF HIV-1 INFECTION

Acute HIV-l infection is symptomatic in 90% of the infected individuals (Bell et al., 2010). The clinical manifestations of acute HIV-l infection appear in some cases within days, but most often 2–6 weeks after exposure to the virus. The period of illness associated with the acute phase is generally 10 days, but may also vary from 3 to 25 days (Pedersen, Lindhardt, & Jensen, 1989). The symptoms commonly include fever, night sweats, fatigue, headache, weight loss, and a mononucleosis-like illness consisting of fever, pharyngitis, and adenopathy. In addition, an erythematous, maculopapular, non-pruritic rash distributed on the face and trunks may also be evident. In general, oral candidiasis is not seen in patients with acute infection, but it can occur as a manifestation of initial infection. Oral or genital lesions may also occur. The central nervous system (CNS) may also manifest symptoms resembling a syndrome of meningoencephalitis (Tambussi et al., 2000). Laboratory findings in patients with acute HIV-l infection may include thrombocytopenia, leukopenia, and elevated liver enzyme values; however, none of these are diagnostic. During this early symptomatic phase, antibodies are not yet generated and a standard diagnostic test based on antibody reactivity will be negative (Busch & Satten, 1997). However, plasma viral RNA detection methods and assays detecting the p24 antigen in plasma, in general, give positive results during this symptomatic early phase of infection (Bollinger, Brookmeyer, & Mehendale, 1997). Since none of the clinical or laboratory findings are distinctive for the acute retroviral syndrome, the main factor that facilitates diagnosis is a history of exposure. All patients who present with a compatible syndrome should be questioned about their risk for HIV-l infection and, if suspicion is high, a presumptive diagnosis can be made. During the chronic asymptomatic phase of HIV-l infection, persistent generalized lymphadenopathy is observed in individuals who are otherwise well. HIV-l-related lymphadenopathy persists for at least 3 months. The progression of HIV-l infection is a result of a decline in immunocompetence that occurs due to increased replication of HIV-l from previously latent sites.

As the disease progresses, infected individuals may suffer from constitutional symptoms, such as weight loss, nausea and vomiting, and hematological disorders. Administering antiretroviral drugs, which can abrogate viral replication, can control or reverse these symptoms of disease.

Initial disease symptoms that follow the asymptomatic period are a sudden and unexplained loss in body weight, also known as wasting syndrome, usually accompanied by diarrhea; persistence of generalized lymphadenopathy; and the onset of neurological disease. The infected individual also suffers from dangerously high fevers, causing night sweats. Furthermore, HIV can spread to the brain and cause neurological damage by itself or by creating an immunocompromised environment that is permissive for other opportunistic infectious agents. About one-third of all AIDS patients exhibit some form of the following neurological symptoms: dementia caused by brain damage leading to loss of mental function; myelopathy leading to the weakness of limbs or paralysis; and a sensory neuropathy causing numbness, especially in the feet, and sensations of burning or stinging in the hands or feet.

As the disease progresses into the symptomatic phase, the immune system is severely compromised, resulting in a wide range of adverse immunological clinical conditions. The two major consequences of immunological damage are the occurrence of opportunistic infections, caused by infectious agents that seldom cause disease in healthy individuals, and the development of cancers.

INNATE IMMUNE RESPONSES FOLLOWING HIV-1 INFECTION

The early immune response to HIV-1 infection is an important factor in determining the clinical course of disease. The innate immune system is the first line of defense against

invading organisms that rapidly functions to limit bacterial and viral infection before the activation of the adaptive immune system can occur. The elements of the innate (non-specific) immune system include anatomical barriers, secretory molecules, and cellular components. Among the mechanical anatomical barriers are the skin and internal epithelial layers, the movement of the intestines, and the oscillation of bronchopulmonary cilia. Associated with these protective surfaces are chemical and biological agents. The anatomical barriers are very effective in preventing colonization of tissues by microorganisms. However, when there is damage to tissues the anatomical barriers are breached and infection ensues. Both soluble and cellular components contribute to the innate immune response. Dendritic cells, macrophages, interferon-producing cells, natural killer (NK) cells, neutrophils, eosinophils, γδ T cells, NK T cells, CD8+ T cells with non-cytotoxic antiviral activity, and B 1 cells are the cellular components of the innate immune system (Levy, 2001). These cells are normally rapidly recruited and/or activated at the site of virus infection. The key soluble molecules of innate immunity include cytokines, chemokines, complement, defensins, acute phase reactants, mannan-binding lectins, and C-reactive proteins (Levy, 2001). HIV-1 infection causes a wide range of abnormalities in the innate immune system, contributing to the lung pathology observed in HIV-l-infected individuals (McMichael et al., 2010; Hewson et al., 1999; Agostini & Semenzato, 1996), downregulation of the normal continuous production of nitric oxide (NO) by the lung epithelium, upregulation of NO production by tissue macrophages, aberrations in systemic and pulmonary glutathione metabolism, and alteration in macrophage function. (Voelkel et al 2008 Adams et al., 1993).

Neutrophils, DCs, NK cells, NK T cells, γδ T cells, CD8+ T cells, and B1 cells are the other important cellular components of the innate immune system. Neutrophils are rapidly recruited to the site of viral infection by chemokines secreted by activated macrophages and virus-infected cells (Chang and Altfeld 2010). Neutrophils release proteins and inflammatory cytokines that help in controlling infection by pathogens. HIV-1 infection can cause a decrease in neutrophil function (Szelc, McMicheltree, Roberts, & Stiehm, 1992). Dendritic cells are highly specialized in capturing and presenting antigens to T cells and stimulating the differentiation and proliferation of B cells (Banchereau & Steinman, 2007;). They are thought to be key modulators of adaptive immune response during viral infections. Upon activation, dendritic cells are known to secrete a number of antiviral and immunoregulatory cytokines such as interferons; tumor necrosis factor alpha (TNF-α); and interleukins IL-l, IL-6, IL-12 and IL-18 (Stockwin, McGonagle, Martin, & Blair, 2000). DCs express chemokine receptors used for HIV-1 entry. Chemokine receptors facilitate the migration of DCs to areas of inflammation where they can secrete cytokines and type II interferons, which can suppress HIV-l infection. In addition, the NK cells can be rapidly recruited into infected organs and tissues by chemoattractant factors produced by virally infected cells and activated macrophages. NK cells can eliminate HIV-l-infected cells by cell-dependent cytolysis and secreted cytokines such as IFN-α, TNF-alpha, granulocyte-macrophage colony-stimulating factor (GM-CSF), and β-chemokines. The γδ T cells generally present in mucosal surfaces exhibit a more restricted repertoire compared with that of αβ T cells. These cells interact directly with non-peptide antigens or cellular stress proteins and serve as a link between innate and adaptive immune systems (Medzhitov & Janeway, 2000). Furthermore, these cells have the ability to lyse HIV-1 infected cells (Wallace et al., 1996). Monocytes from infected individuals exhibit decreased phagocytosis, chemotaxis, intracellular killing, and cytokine expression. Similarly, the granulocyte subset of neutrophils exhibit decreased phagocytosis and intracellular killing.

Mannose-binding lectins and complement are among the soluble components of innate immunity that exhibit anti HIV-1 activity. These soluble products can bind to HIV-1 and bring about the lysis of the virus or help macrophages to efficiently engulf them by phagocytosis (Sato et al., 2011). Studies have shown that individuals with low levels of circulating mannose-binding lectins are more susceptible to HIV-l infection and enhanced disease progression (Garred, Madsen, & Balslev, 1997). Complement can also cause the rapid clearance and destruction of HIV-l. It has been shown that complement lyses HIV-l in the presence of antiviral antibodies (Sullivan et al., 1996). In addition, it also serves as an opsonin for phagocytosis of HIV-1 virus (Spear, Sullivan, Landay, & Lint, 1990). Thus, the complement system is involved in both the innate immune system and the adaptive immune system. Other soluble components of the innate immune system such as cytokines and chemokines are released following interaction of pathogens with cells. Cytokines like IL-4, IL-6 and IL-12 secreted by the innate immune response can determine whether a Th-I or Th2-type adaptive immunity prevails. Furthermore, other cytokines like TNF-α and interferons can modulate HIV-l replication. Similarly, the production of chemokines can function as chemoattractants recruiting NK cells, T cells, and macrophages to sites of infection.

T CELL RESPONSES FOLLOWING HIV-1 INFECTION

CD4 + T cells are the major reservoirs of both actively replicating and latent HIV-1 throughout the course of disease. While both naive and memory T cells have the capacity to be infected with HIV-l, only activated memory T cells preferentially replicate virus (Woods, Roberts, Butera, & Follzs, 1997; Chun et al., 1995). Naive T cells do not have the ability to replicate HIV-l, even after activation (Roederer et al., 1997). These data lend support to the theory that antigen-specific interactions between infected macrophage or dendritic cells and uninfected memory CD4+ T cells leads to dissemination of HIV-1 and explain, in part, why chronic immune activation in the context of antigen presentation and cell activation such as seen in people from sub-Saharan Africa, may accelerate HIV-l disease progression.

T-cell abnormalities such as CD4+ T cell lymphopenia, characterized by decreased lymphoproliferation and a decreased number of cells having naive phenotype, are seen in HIV-1-infected individuals. In addition, infected individuals demonstrate a decreased delayed hypersensitivity skin test response to recall antigens. CD8+ T cells exhibit impaired cytokine production and cytolysis. Other viral immunomodulation strategies by the virus include chemokine and cytokine modulation, inhibition of apoptosis, inhibition of NK cell activity, and interference with MHC class I and class II antigen presentation.

The acute primary HIV-l infection is characterized by a TH 1 profile including the secretion of pro-inflammatory cytokines IL-l, IL-2, IL-6, TNF-α, IFN-gamma (Graziosi et al., 1996; Rinaldo et al., 1990), as well as the other cytokines IL-4, IL-7, IL-I0, IL-12 and IL-13 (Chehimi, Starr, & Frank, 1994; Chehimi et al., 1996; Than et al., 1997; Patella, Florio, Petraroli, & Marone, 2000). Several viral proteins, such as gp120, Tat, Nef, and Vpr, are able to induce the secretion of a number of cytokines. The Th-1 type cytokines help to induce the strong cellular response that is responsible for the initial control of viremia. The progression of disease in HIV-l-infected individuals is associated with the switch of cytokine response from Th-1 to Th-2. The exact mechanism that causes this shift during AIDS progression is not known. However, reports suggest that HIV-I viral proteins may contribute to the shift in Th-2 immune response. The viral protein Nef has been reported to decrease the production of IFN-gamma and IL-2 (Collette et al., 1996) and impair the signaling of Th-1 cytokines (Collette, Dutartre, Benziane, & Olive, 1997). In addition, Tat induces the expression of the IL 4 receptor alpha chain (Husain, Leland, Aggarwal, & Puri et al., 1996) and inhibits lL-2 production (Ito, Ishida, & He, 1998). These functions of the HIV-1 proteins may contribute to the shift in cytokine profile as the infection progresses.

A major host defense against viruses is induction of apoptosis of infected cells. However, many viruses can counteract these cellular responses by targeting specific stages of the downstream apoptotic pathways by using proteins that often mimic or counteract host cell functions. While apoptosis plays an important role in the destruction of HIV-I-infected T cells, other cell types such as myeloid cells become chronically or latently infected and serve as reservoirs for HIV-1. Anti-apoptotic pathways are likely to play an important role in the establishment and maintenance of latency by preventing infected cells from being rapidly killed. HIV-1 can inhibit TNF-alpha-mediated apoptosis by using mechanisms that persistently activate NF-κB, thus protecting myeloid cells from destruction (DeLuca, Kwon, Pelletier, Wainberg, & Hiscott, 1998). In addition, HIV-1 Tat protein can apparently upregulate cellular Bcl-2 in different cell types, and Vpr protein can inhibit NF-κB activation leading to suppression of T-cell receptor (TCR)-mediated apoptosis in resting T cells (Roulston, Marcellus, & Branton, 1999).

HIV-I expresses two proteins, Nef and Vpu, that have been shown to downregulate the expression of surface MHC class I molecule (Piguet et al., 1999). HIV-I glycoprotein, Nef, downregulates the expression of class I HLA -A2 protein on the surface of infected cells. Vpu prevents the cell surface expression of class I molecules by interfering with the processing of these molecules and destabilizing them (Kerkau, Bacik, & Bennink, 1997). This downregulation of the MHC class I molecule protects HIV-I infected cells from NK-cell-mediated lysis (Collins et al., 1998).

Although HIV-1 does not directly reduce expression of MHC class II molecules in infected cells, it can markedly impair MHC class II antigen-specific pathways. HIV-I viral proteins, Vpu, Env, and Nef, cooperate in the degradation of CD4, leading to a significant reduction of CD4 expression on T cells (Aiken, Konner, Landau, Lenburg, & Trono, 1994; Anderson, Lenburg, Landau, & Garcia, 1994; Fujita, Omura, & Silver, 1997). CD4 serves as a co-receptor during antigen recognition by the TCR on T cells, and ligation of CD4 increases the sensitivity of a T cell antigen presented by MHC class II molecules. The reduction of CD4 expression on T cells contributes to the gradual loss of responsiveness of T lymphocytes to MHC class II-restricted antigens (Louie, Wahl, Hewlett, Epstein, & Dhawan, 1996).

B CELL RESPONSES IN HIV-1 INFECTION

Although HIV-l does not replicate in B cells, it produces severe B-cell abnormalities eventually leading to B-cell depletion (Patke & Shearer, 2000; Rodriguez, Thomas, O'Rourke, Stiehm, & Plaeger, 1996). The B-cell abnormalities detected in HIV-1 infected individuals are a decrease in B-cell number, detection of circulating immune complexes, elevated levels of autoantibodies, and increased production of nonspecific immunoglobulins G, A and M. Impaired production of specific antibodies to new and recall antigens is also seen. This includes both T-dependent and -independent antigens (Shirai, Cosentino, Leitman-Klinman, & Klinman, 1992; Yarchoan, Redfield, & Broder, 1986; Borkowsky et al., 1992; Gibb et al., 1995). Recent reports have identified a subpopulation of B cells with low CD21 expression in high viremia patients. These cells, which are poor antibody responders and low secretors of immunoglobulins, could be partly responsible for causing the humoral defects seen in HIV-l-infected individuals (Moir, Malaspina, & Ogwaro, 2001). Furthermore, HIV-l gene products like gpl20 can modulate B-cell function apparently by binding to the VB3 domain of membrane immunoglobulin (Goodglick, Zevit, Neshat, & Braun, 1995).

HIV-l activates complement through alternative and classic pathways (Montefiori, Robinson, & Mitchell, 1989). Although there is deposition of C3 on viral surfaces, there is decreased activity of the complement C5-C9 membrane attack complex. In addition, HIV-I downregulates cell surface complement receptors after infection (Munson, Scott, Landay, & Spear, 1995). HIV-1 infection decreases CRI expression on B cells and erythrocytes by proteolytic cleavage of the receptor (Jouvi, Rozenbaum, Russo, & Kazatchkine, 1987). HIV-1 infection also downregulates CR2 expression by altered transcription (Larcher, Schultz, & Hofbauer, 1990). HIV-1 gp120 decreases

C5a receptor expression and thus impairs the chemotatic response of monocytes to inflammatory stimuli (Wahl et al., 1989). In some cases, HlV-1 may infect cells using complement receptors. Villous processes of follicular dendritic cells expressing high levels of complement receptors and Fc receptors trap numerous particles of opsonized HIV-1 virus particles that are highly infectious (Heath, Tew, Szakal, & Burton, 1995).

ROLE OF CHEMOKINES HIV-1 PATHOGENESIS

Chemokines and their receptors play a critical role in HIV-1 infection and disease pathogenesis (reviewed in Reinhart et al., 2009). The seven transmembrane G-protein coupled receptors CXCR4 and CCR5 act as coreceptors for HIV-1 entry into immune cells (Feng, Broder, Kennedy, & Berger, 1996, Deng et al., 1996). Interestingly, SIVs predominantly use CCR5 for entry (Edinger et al., 1997). Deletion in CCR5 is associated with increased protection from both acquisition of HIV-1 and subsequent disease. Secondary lymphoid tissues are critical sites of soluble and cell-associated antigen sampling of peripheral tissues, and they are key compartments for the generation of cellular and humoral immune responses. Chemokines are major mediators of cell trafficking during immune inductive and effector activities, and changes in their expression patterns in lymphoid tissues could contribute to the pathogenesis of HIV-1 and SIV in multiple ways. Infection of rhesus macaques with pathogenic SIV leads to the induction of multiple inflammatory chemokines in lymph nodes and spleen including: CXCL8, CXCL9, CXCL10, and CXCL11, CCL3, CCL4, CCL5, CCL2 CCL19 and CCL20. In a study of host responses to oral transmission of SIV, high CXCL9 and CXCL10 levels in lymph nodes after infection were associated with rapid progression of disease, whereas high levels of these chemokines in the oral mucosa were associated with slow progression of disease (Milush et al., 2007). When chemokine expression in peripheral blood mononuclear cells (PBMCs) were examined from SIV- or SHIV-infected nonhuman primates, CCL3, CCL4 and CCL5 were found to be upregulated (reviewed in Reinhart et al., 2009). In contrast, the homeostatic lymphoid chemokine CCL21 and the Th2 recruiting chemokines CCL17 and CCL22 and the anti-apoptotic chemokines CCL25 and CXCL12 chemokines are downregulated during SIV infection. The overall implications of the findings regarding chemokine expression associated with lymphoid tissues from SIV-infected macaques suggest that SIV infection leads to development of a Th-1 polarized, inflammatory milieu. These modified environments in lymphoid tissues are polarized toward type 1, IFN-gamma production due to increased recruitment of T cells expressing CCR5 and CXCR3, which are found predominantly on Th-1 cells and due to decreased recruitment of T cells expressing CCR4, which is found predominantly on Th-2 T cells and Tregs. Although it would be reasonable to expect that increased local expression of CCR5 ligands would decrease viral replication, this does not appear to be the case in these tissues.

NEUROPATHOGENESIS IN HIV-1 INFECTION—ROLE OF PERIPHERAL IMMUNE CELLS

HIV-1-associated dementia (HIV-D) is a syndrome of motor and cognitive dysfunction observed in approximately 5% to 10% of patients infected with HIV-1 (McArthur et al., 1993; Sacktor et al., 2010). Although the neuropathogenesis of HIV-D is not completely understood, the role of immune cells in its manifestation is well demonstrated (Fischer-Smith, Bell, Croul, Lewis, & Rappaport, 2008). CNS infection of HIV-1 has been shown early during the acute phase of infection; however, the action of cytotoxic T cells eliminates productively infected cells. At later phase of the disease, productive infection of the CNS sets in, with the concomitant development of CNS disease (Fischer-Smith & Rappaport, 2008). The source and mechanism of this latter infection of the CNS has been a matter of considerable debate, with two divergent models. In the first model, the re-emergence of the virus from a latent reservoir (Trojan horse model) is thought to be the mechanism for the productive infection. In the second model, new invasion of the CNS with virus-infected cells (late invasion model; Fischer-Smith & Rappaport, 2008) is proposed to be the causative factor. In the Trojan horse model, it is proposed that the virus enters the CNS early, and replicates at low levels as a reservoir separated from the periphery. During the course of the disease, a more virulent CNS phenotype of the virus emerges, leading to the manifestation of disease pathogenesis. In support of the Trojan horse model, several studies suggest CNS compartmentalization of the HIV virus through comparisons of viral quasispecies found in the plasma compared with those virus found in cerebrospinal fluid (CSF) (Clements et al., 2005, 2008; Cunningham, 2000; Stingele et al., 2001; Strain et al., 2005; Tashima et al., 2002). These studies demonstrate suppression of virus replication in the CNS as early as 21 days, without loss of CNS viral DNA (Barber, 2004). However, macrophage trafficking is also observed in this model, negating microglial activation as the sole contributor to CNS disease. Although these studies do not preclude the contribution of infiltrating macrophages, the constant level of viral DNA in CNS suggested a latent CNS reservoir as a major contributor.

Despite the early viral entry, several lines of evidence also support the late invasion model. According to this model, virus entry into the CNS is due largely to the trafficking of HIV-1–infected monocytes/macrophages from the systemic circulation into the CNS (Meltzer et al., 1990). The number of total brain macrophages is dramatically increased in HIV encephalopathy (HIVE), the pathogenic manifestation of HIV, without additional evidence of local proliferation of these cells (Fischer-Smith et al., 2004;). Additional studies have shown that macrophage/microglia represent the principle productive reservoir of HIV-1 infection in the CNS (Kure et al., 1991; Porwit et al.,1989;). The contribution of infected macrophage and microglial cells to neuronal injury through secretion of viral and host factors has been the subject of

numerous reviews (Fischer-Smith & Rappaport, 2005; Gonzalez-Scarano & Martin-Garcia, 2005).

The late invasion model proposes that the peripheral immune compartment plays a prominent role and contributes to the development of CNS disease. Previous studies comparing HIV-1 gp120 sequences have demonstrated the greatest similarity between envelope sequences derived from the brain with those derived from bone marrow and blood (Gartner et al., 1997)). The role of monocytes/macrophage trafficking from the periphery into the CNS is further supported by the beneficial effects of highly active antiretroviral therapy (HAART) despite poor CNS penetration of most of these antiretroviral compounds (Vehmas et al., 2004). Although conclusively discriminating between resident microglia and perivascular macrophages is difficult, combined CD markers have been used to make this distinction. The combination of antigenic markers for perivascular macrophage positive for CD14 (lipopolysaccharide [LPS] receptor) and CD45 (leukocyte common antigen [LCA]), neither of which are expressed on microglia, has been used to identify two populations of activated macrophages in the CNS of patients with HIVE (Fischer-Smith et al., 2001). Macrophages accumulating perivascularly with a CD14+/CD45(LCA)+/CD16+ (FcγIII receptor) phenotype appear to be the principal reservoir of productive HIV-1 infection in the CNS. Similar observations were also reported in SIVE (Williams et al., 2001. Importantly, CD16+ monocytes preferentially harbor HIV in vivo, are more permissive to HIV infection than CD16− monocytes, and are likely important as reservoirs of infection and tissue dissemination (Ellery et al., 2007; Joworoski et al., 2007). In support of the latter hypothesis, the increase in total brain macrophages that bear antigenic markers of the peripheral compartment appears to be due to trafficking of monocytes/macrophage into the CNS from the periphery, rather than local microglial proliferation (Fischer-Smith et al., 2004).

The passage of monocyte and leukocyte into the CNS would not occur without the complex chemokine gradient that is established during HIV-1 infection. Chemokine involvement in HIV-1 neuropathogenesis is well recognized because of their abilities to: (i) recruit HIV-1-infected immune cells into the brain, (ii) serve as mediators for inflammatory responses, and (iii) serve as ligands for HIV-1 coreceptors, specifically CXCR4 and CCR5 (Hesselgesser et al., 1998). CCL2 is considered to be a critical factor involved in the infiltration of monocytes and lymphocytes across the BBB during CNS inflammation. Numerous studies strongly suggest that increased CCL2 expression in the CNS is associated with enhanced progression of HIVE (Dhillon et al., 2008). Chemokines can also promote virus replication and contribute to injury and eventual loss of neurons (Asensio & Campbell, 1999; Miller & Meucci, 1999). In addition to CCL2, another chemokine, CXCL10 (interferon1-inducible peptide) has also been detected in the CSF of individuals with HIV-1 infection (Kolb et al., 1999). Understanding the underlying mechanisms that lead to the migration of HIV-1-infected monocytes from the periphery into the CNS are important future directions in therapeutic strategies (Kraft-Terry et al., 2009).

CONCLUSION

The complete eradication of HIV-1 from infected patients remains problematic, although the use of HAART has improved the prognosis of patients who are HIV-positive. A major obstacle to total viral eradication is the persistence of viral reservoirs and continuous rounds of de novo virus infection of host cells with rapid turnover of both free virus and virus-producing cells. It is likely that various immune activation stimuli, including most pathogens, can potentially affect the infectivity, transmissibility and pathogenesis of HIV by influencing several aspects of HIV biology. First, infection of monocytes/macrophages by many pathogens can induce immune activation of monocytes and T lymphocytes, thus increasing the infectivity of these cells to HIV. Second, these infections can also promote disease progression by creating a cytokine environment that favors faster replication of HIV. Third, these infectious agents can modulate the expression of coreceptors on the surfaces of permissive cellular targets, thus altering the cellular tropism. Such immune pressures can lead to emergence of viral variants that not only use multiple coreceptors, but also are more virulent. Collectively, all these factors are likely to increase HIV pathogenesis.

REFERENCES

Almeida, J. R., Price, D. A., Papagno, L., Arkoub, Z. A., Sauce, D., Bornstein, E., et al. (2007). Superior control of HIV-1 replication by CD8+ T cells is reflected by their avidity, polyfunctionality, and clonal turnover. *J Exp Med*, *204*(10), 2473–85.

Aiken, C., Konner, J., Landau, N., Lenburg, M., & Trono, D. (1994). Nef induces CD4 endocytosis: Requirement for a critical dileucine motif in the membrane-proximal CD4 cytoplasmic domain. *Ce1l*, *76*, 853–64.

Agostini C, Semenzato G. (1996). Immunologic effects of HIV in the lung. *Clin Chest Med*. 1996 Dec;17(4):633–45.

Anderson, S., Lenburg, M., Landau, N., & Garcia, J. (1994). 111e cytoplasmic domain of CD4 is sufficient for its down-regulation from the cell surface by human immunodeficiency virus type I *Nef J Virol*, *68*, 3092–101.

Asensio, V. C. & Campbell, I. L. (1999). Chemokines in the CNS: Plurifunctional mediators in 501 diverse states. *Trends Neurosci*, *22*, 504–512.

Barber, C. G. (2004). CCR5 antagonists for the treatment of HIV. *Curr Opin Investig Drugs*, *8*, 851–61. Review.

Bell, S. K., Little, S. J., Rosenberg, E. S. (2010). Clinical management of acute HIV infection: best practice remains unknown. *J Infect Dis.* 202 Suppl 2:S278–88. Review.

Bollinger, R. C., Brookmeyer, R. S., & Mehendale, S. (1997). Risk factors and clinical presentation of acute primary HIV infection in India. *J Am Med Ass*, *278*, 2085.

Bonavia, R., Bajello, A., Barbero, S., Albini, A., Noonan, D. M., & Schettini, G. (2001). HIV-1 Tat causes apoptotic death and calcium homeostasis alterations in rat neurons. *Biochem Biophys Res Comm*, *288*, 301–8.

Borkowsky, W., Rigaud M., Krasinski, K., Moore, T., Lawrence, R. & Pollack H. (1992). Cell-mediated and humoral immune responses in

children infected with human immunodeficiency virus during the first 4 years of life. *J Pediatr*, *120*, 371–5.
Borrow, P., Lewicki, H., Hahn, B.1-I., Shaw, G. M., & Oldstone M. (1991). Virus-specific C8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type j infection. *J Virol*, *68*, 6103–6110.
Braaten, D., Franke, E. K., & Luban, J. (1996). Cyclophillin A is required for an early step in the life cycle of human immunodeficiency virus type I before initiation of reverse transcription. *J Virol*, *70*, 3551–60.
Brenchley, J. M., Schacker, T. W., Ruff, L. E., Price, D. A., Taylor, J. H. Beilman, G. J., et al. (2004). CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. *J Exp Med*, *200*, 749–759.
Bukrinsky, M. I., Stanwick, T. L., & Dempsey, M. P. (1991). Quiescent T lymphocytes as an inducible virus reservoir in HIV-l infection. *Science*, *254*, 423–7.
Burton, D. R., Weiss, R. A. (2010). AIDS/HIV. A boost for HIV vaccine design. *Science*. 13;329(5993):770–3.
Busch, M. P., Satten G. A. (1997) Time course of viremia and antibody seroconversion following human immunodeficiency virus exposure. *Am J Med.*;102(5B):117–24; discussion 125–6.
Cen, S., Khorchid, A., Lavanbakht, 1-1., Gabor, J., Stello, T, & Shiba, K. (2001). Incorporation of lysyl-tRNA synthethase into human immunodeficiency virus type I. *J Virol*, *75*, 5043–8.
Chang, J.J., Altfeld, M. (2010). Innate immune activation in primary HIV-1 infection. *J Infect Dis.* Oct 15;202 Suppl 2:S297–301.
Chehimi, J., Ma, X., Chouaib, S., et al. (1996). Differential production of interleukin 10 during human immunodeficiency virus infection. *AIDS Res Human Retroviruses*, *12*, 4053–61.
Chehimi, J., Starr, S.E., & Frank, I. (1994). Impaired interleukin-12 production in human immunodeficiency virus-infected patients. *J Exp Med*, *141*, 99–104.
Chun, T.-W., Finzi, D., Margolick, J., Chadwick, K., Schwartz, D., & Siliciano, R. F. (1995). The in vivo fate of HIV-I-infected T cells: Quantitative analysis of the transition to stable latency. *Nat Med*, *I*, 1284–90.
Chun, T. W., Chadwick, K., Margolick, J., & Siliciano, R. F. (1970). Differential susceptibility of naive and memory CD4+ T cells to the cytopathic effects of infection with human immunodeficiency virus type I strain. *J Virol*, *71*, 4436–44.
Clements, J. E., Mankowski, J. L., Gama, L., & Zink, M. C. (2008). The accelerated simian immunodeficiency virus macaque model of human immunodeficiency virus-associated neurological disease: From mechanism to treatment. *J Neurovirol*, *14*(4), 309–17. Review.
Clements, J. E., Li, M., Gama, L., Bullock, B., Carruth, L. M., Mankowski, J. L., et al. (2005). The central nervous system is a viral reservoir in simian immunodeficiency virus—infected macaques on combined antiretroviral therapy: A model for human immunodeficiency virus patients on highly active antiretroviral therapy. *J Neurovirol*, *11*(2), 180–9.
Cunningham A. (2000). African Americans and AIDS: highlights of 2nd annual Washington conference. *AIDS Treat News*, *17*(339), 8.
Collette, Y., Chang, H. L., & Cerdan, H., et al. (1996). Specific Thl cytokine down-regulation associated with primary clinically derived human immunodeficiency virus type I Nef gene-induced expression. *J Immunol*, *156*, 360–70.
Collette, Y., Dutartre, H., Benziane, A., & Olive, D. (1997). The role of HIV-2 Nef in T-cell activation: Nef impairs induction of Th I cytokines and interacts with Src family tyrosine kinase Lck. *Res Virol*, *148*, 52–8.
Collins, K. L., Chen, B. K., Kalams, S. A., Walker, B. D., & Baltimore D. (1998). HIV-I Nef protein protects infected primary cells against killing by cytotoxic T lymphocytes. *Nature*, *391*, 397–401.
Connor, R. I., Mohri, H., Cao, Y., & Ho, D. D. (1993). Increased viral burden and cytopathicity correlate temporally with CD4+ T-lymphocyte decline and clinical progression in human immunodeficiency virus type I-infected individuals. *J Virol*, *67*, 1772–7.
Connor, R. I., Sheridan, I. C. E., Ceradini, D., Choe, S., & Landau, N. R. (1997). Change in coreceptor use correlates with disease progression in HIV-I infected individuals. *J Exp Med*, *185*, 621–8.
Craigie, R. (200l). HIV integrase, a brief overview from chemistry to therapeutics. *J BiolChem*, *266*, 23213–16.
Davenport, M. P., Ribeiro, R. M., Zhang, L., Wilson, D. P., & Perelson A. S. (2007). Understanding the mechanisms and limitations of immune control of HIV. *Immunol Rev*, *216*, 164–175.
DeLuca, C., Kwon, H., Pelletier, N., Wainberg, M., & Hiscott, J. (1998). NF-KB protects HIV-I infected myeloid cells from apoptosis. *Virology*, *244*, 27–38.
Deng, H., Liu, R., Ellmeier, W., et al. (1996). Identification of a major coreceptor for primary isolates of HIV-1. *Nature*, *381*, 661–6.
Dhillon, N. K., et al. (2008). Roles of MCP-1 in development of HIV-dementia. *Front Biosci*, *560 13*, 3913–3918.
Ellery, P. J., Tippett, E., Chiu, Y. L., Paukovics G., Cameron, P. U., Solomon, A., Lewin, S. R., Gorry, P. R., Jaworowski, A., Greene, W. C., Sonza, S., Crowe, S.M. (2007). The CD16+ monocyte subset is more permissive to infection and preferentially harbors HIV-1 in vivo. *J Immunol.* 178(10):6581–9.
Edinger, A. L., Amedee, A., Miller, K., et al. (1997). Differential utilization of CCR5 by macrophage and T cell tropic simian immunodeficiency virus strains. *Proc Natl Acad Sci USA*, *94*, 4005–10.
Emilie, D., Maillot, M. C., Nicholas, J. E, Fior, R., & Galanaud P. (1992). Antagonistic effect of interferon gamma on Tat-induced transactivation of HIV long terminal repeats. *J BioI Chem*, *267*, 20565–70.
Fauci, A. S. (1993). Multifactorial nature of human immunodeficiency virus disease: Implication for therapy. *Science*, *262*, 1011–18.
Feinberg, M. B., McLean, A. R. (1997). AIDS: decline and fall of immune surveillance? *Curr Biol*.7(3):R136–40. Review.
Feng, Y., Broder, C. C., Kennedy, P. E., & Berger, E. A. (1996). HIV-1 entry cofactor: Functional cDNA cloning of a seven-transmembrane, G protein- coupled receptor. *Science*, *272*, 872–7.
Fischer-Smith, T, Croul S, Adeniyi A, Rybicka K, Morgello S, Khalili K, Rappaport J. (2004). Macrophage/microglial accumulation and proliferating cell nuclear antigen expression in the central nervous system in human immunodeficiency virus encephalopathy. *Am J Pathol*. 164(6):2089–99.
Fischer-Smith, T, Rappaport J. (2005). Evolving paradigms in the pathogenesis of HIV-1-associated dementia. *Expert Rev Mol Med*. 7(27): 1–26.
Fischer-Smith, T., Bell, C., Croul, S., Lewis, M., & Rappaport, J. (2008). Monocyte/macrophage trafficking in acquired immunodeficiency syndrome encephalitis: Lessons from human and nonhuman primate studies. *J Neurovirol*, *14*(4), 318–26. Review.
Fischer-Smith, T., Tedaldi, E. M., & Rappaport, J. (2008). CD163/CD16 co-expression by circulating monocytes/macrophages in HIV: Potential biomarkers for HIV infection and AIDS progression. *AIDS Res Hum Retroviruses*, *24*(3), 417–21.
Franke, E. K., Yuan, H. E. H., & Luban, J. (1994). Specific incorporation of cyclophilin A into HIV-1 virion. *Nature*, *372*, 359–62.
Fujita, K., Omura, S., & Silver, J. (1997). Rapid degradation of CD4 in cells expressing human immunodeficiency virus type I Env and Vpu is blocked by proteasome inhibitors. *J Gen Virol*, *78*, 619–725.
Galati D, Bocchino M. (2007). New insights on the perturbations of T cell cycle during HIV infection. *Curr Med Chem*., 14(18):1920–4. Review.
Garred, P., Madsen, H. O., & Balslev, U (1997). Susceptibility of HIV infection and progression of AIDS in relation to variant alleles of mannose-binding lectin. *Lancet*, *349*, 236–40.
Gartner S, McDonald RA, Hunter EA, Bouwman F, Liu Y, Popovic M. (1997). Gp120 sequence variation in brain and in T-lymphocyte human immunodeficiency virus type 1 primary isolates. *J Hum Virol*. Nov-Dec;1(1):3–18.
Gasper-Smith N, Crossman D. M., Whitesides, J. F., Mensali, N., Ottinger J. S., Plonk SG, et al. (2008). Induction of plasma (TRAIL), TNFR-2, Fas ligand, and plasma microparticles after human immunodeficiency virus type 1 (HIV-1) transmission: Implications for HIV-1 vaccine design. *J Virol*.;82(15):7700–10.
Gelderblom, H. R. (1991). Assembly and morphology of HIV: potential effect of structure on viral function. *AIDS*. 5(6):617–37.
Gibb, D., Spoulou, V., Giacomelli, A., Griffiths, H., Masters, J., & Misbah, S. (1995). Antibody response to *Haemophilus inlfluenzae* type b and

Streptoccocus pneumoniae vaccines in children with human immunodeficiency virus infection. *Ped Inect Dis J*, *14*, 129–35.
Glass, W. G., McDermott, D. H., Lim, J. K., Lekhong, S., Yu, S. F., Frank, W. A., et al. (2006). CCR5 deficiency increases risk of symptomatic West Nile virus infection. *J Exp Med*, *203*(1), 35–40.
Goodglick, L., Zevit, N., Neshat, M., & Braun, J. (1995). Mapping the Ig super-antigen-binding site of HIV gp120. *J Immunol*, *155*, 5151–9.
González-Scarano, F. & Martín-García, J. (2005). The neuropathogenesis of AIDS. *Nat Rev Immunol*, *5*(1), 69–81. Review.
Goonetilleke, N., Liu, M. K., Salazar-Gonzalez, J. F., Ferrari, G., Giorgi, E., Ganusov, V. V., et al. (2009). The first T cell response to transmitted/founder virus contributes to the control of acute viremia in HIV-1 infection. *J Exp Med*, *206*(6), 1253–72.
Goulder PJ, Bunce M, Krausa P, McIntyre K, Crowley S, Morgan B, Edwards A, Giangrande P, Phillips RE, McMichael AJ. (1996) Novel, cross-restricted, conserved, and immunodominant cytotoxic T lymphocyte epitopes in slow progressors in HIV type 1 infection. *AIDS Res Hum Retroviruses.* 12(18):1691–8.
Graziosi C, Gantt KR, Vaccarezza M, Demarest JF, Daucher M, et al (1996). Kinetics of cytokine expression during primary human immunodeficiency virus type 1 infection. Proc Natl Acad Sci U S A. 1996 Apr 30;93(9):4386–91.
Guadalupe, M., Reay, E., Sankaran, S., Prindiville, T., Flamm, J., McNeil, A., et al. (2003). Severe CD4+ T-cell depletion in gut lymphoid tissue during primary human immunodeficiency virus type 1 infection and substantial delay in restoration following highly active antiretroviral therapy. *J Virol*, *77*, 11708–11717.
Haase, A. T. (1999). Population biology of HIV-I infection: Viral demographics and dynamics in lymphatic tissues. *Ann Rev Immunol Rev*, *17*, 625–56.
Haase, A. T., Henry, K., Zupancic, M., et al. (1996). Quantitative image analysis of HIV-I infection in lymphoid tissue. *Science*, *274*, 985–9.
Haase, A. (2005). Pencil at mucosal front lines for HIV and SIV and their hosts. *Nat Rev Immunol*, *5*, 783–89.
Heath, S. L., Tew, J. G., Szakal, A. K., & Burton, G. E (1995). Follicular dendritic cells and human immunodeficiency virus infectivity. *Nature*, *377*, 740–4.
Hesselgesser, J., et al. (1998). Identification and characterization of the CXCR4 chemokine 614 receptor in human T cell lines: Ligand binding, biological activity, and HIV-1 615 infectivity. *J Immunol*, *160*, 877–883.
Haynes, B. F. (1996). HIV vaccines: Where we are and where we are going. *Lancet*, *348*(9032), 933–7. Review.
Huber, M. & Trkola, A. (2007). Humoral immunity to HIV-1: Neutralization and beyond. *J Intern Med*, *262*, 5–25.
Husain, S. R., Leland, P., Aggarwal, B. B, & Puri, R. K. (1996). Transcriptional upregulation of interleukin 4 receptors by human immunodeficiency virus type I Tat gene. *AIDS Res Hum Retroviruses*, *12*, 1349–59.
Ito, M., Ishida, T., & He, L. (1998). HIV-I type 1 TAT protein inhibits interleukin•12 production by human peripheral blood mononuclear cells. *AIDS Res Hum Retrovirus*, *14*, 845–9.
Jaworowski A, Kamwendo DD, Ellery P, Sonza S, Mwapasa V, Tadesse E, Molyneux ME, Rogerson SJ, Meshnick SR, Crowe SM. (2007). CD16+ monocyte subset preferentially harbors HIV-1 and is expanded in pregnant Malawian women with Plasmodium falciparum malaria and HIV-1 infection. *J Infect Dis.* 196(1):38–42.
Jouvin, M. H., Rozenbaum, R., Russo, & Kazatchkine, M. D. (1987). Decreased expression of C3b/C4b complement receptor (CRI) in AIDS and AIDS-related syndromes correlates with clinical subpopulations of patients with HIV-1 infection. *AIDS*, *1*, 89–94.
Kagi, D., Seiler, P., & Pavlovic, I., (1995). The roles of perforin and Fas-dependent cytotoxicity in protection against cytopathic and non-cytopathic viruses. *Eur J Immunol*, *25*, 3256–62.
Kellam P, Weiss R. A. (2006). Infectogenomics: insights from the host genome into infectious diseases. Cell. 2006 Feb 24;124(4): 695–7.
Kerkau, L., Bacik, I., & Bennink, I. R. (1997). The human immunodeficiency virus type 1 (HIV-I) Vpu protein interferes with an early step in the biosynthesis of major histocompatibility. (MHC) class I molecules. *J Exp Med*, *185*, 1295–305.
Kolb, S. A., et al. (1999). Identification of a T cell chemotactic factor in the cerebrospinal fluid of HIV-1-infected individuals as interferon-gamma inducible protein. *J Neuroimmunol*, *93*, 172.
Koup, R. A, Safrit, J. A., & Cao, Y., (1994). Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type I syndrome. *J Immunol. 68*, 4650–5.
Kraft-Terry, S. D., Stothert, A. R., Buch, S., & Gendelman, H. E. (2010). HIV-1 neuroimmunity in the era of antiretroviral therapy, *Neurobiol Dis*.
Kraft-Terry, S. D., Buch, S. J., Fox, H. S., & Gendelman, H. E. (2009). A coat of many colors: Neuroimmune crosstalk in human immunodeficiency virus infection. *Neuron*, *64*, 33–45.
Kure, K., Llena, J. F., Lyman, W. D., Soeiro, R., Weidenheim, K. M., Hirano, A., et al. (1991). Human immunodeficiency virus-1 infection of the nervous system: An autopsy study of 268 adult, pediatric, and fetal brains. *Hum Pathol*, *22*(7), 700–10.
Lamhamedi-Cherradi, S., Culman-Penciolelli, B., & Guy, B. (1995). Different patterns of HIV-I specific cytotoxic T-lymphocyte activity after primary infection. *AIDS*, *9*, 421–6.
Larcher, C., Schultz, T. E., & Hofbauer, P. (1990). Expression of the OC//EBV receptor and of' other cell membrane surface markers is altered upon HIV-I infection of myeloid T and B cells. *J Acq Immune Defic Syndrome*, *3*, 103–8.
Levy, I. A. (2001). The importance of the innate immune system in controlling HIV infection and disease. *Trends Immunol*, *22*, 312–I5.
Levy, I. A., Mackewicz, C. E., & Barker, E. (1996). Controlling HIV-1 pathogenesis: The role of noncytotoxic anti-HIV activity of'CD8+ cells. *Immunol Today*, *17*, 217–24
Little, S. J., McLean, A. F., Spina, C. F, Richman, D. D., & Havlir D. V. (1999). Viral dynamics of acute HIV-I infection. *J Exp Med*, *190*, 841–50.
Li, Qingsheng, et al. (2009). Microarray analysis of lymphatic tissue reveals stage-specific, gene expression signatures in HIV-1 infection. *J Immunol*, *183*(3), 1975–1982.
Louie, A. T., Wahl, L. M., Hewlett, J. K., Epstein, I., & Dhawan, S. (1996). Impaired antigen presentation to CD4+ T-cells by HIV-infected monocytes is related to down-modulation of CD 4 expression on helper T cells: Possible involvement of H1V-induced cellular factors. *FEBS Lett*, *398*, 1–6.
McArthur, J. C., Hoover, D. R., Bacellar, H., Miller, E. N., Cohen, B. A., Becker, J. T., et al. (1993). Dementia in AIDS patients: Incidence and risk factors. Multicenter AIDS Cohort Study. *Neurology*, *43*(11), 2245–52.
McCune, J. M. (1995). Viral latency in HIV diseases. *Cell*, *82*, 183–8.
McMichael, A. J. & Rowland-Jones, S. L. (2001). Cellular immune responses to HIV-1. *Nature*, *10*, 980–7.
McMichael, A. J. & Walker, D. B. (1991). Cytotoxic T lymphocyte epitopes: Implications of rHIV vaccines. *AIDS*, *8*(suppI), Suppl-73.
McMichael AJ, Borrow P, Tomaras GD, Goonetilleke N, Haynes BF. (2010) The immune response during acute HIV-1 infection: clues for vaccine development. *Nat Rev Immunol*, *10*(1):11–23.
Medzhitov, R. & Janeway, C. (l998). Innate immune recognition and control of adaptive immune responses. *Scand Immunol*, *10*, 351–3.
Medzhitov, R. & Janeway, C. (2000). Innate immunity. *New Eng J Med*, *343*, 338–44.
Meltzer, M. S., Nakamura, M., Hansen, B. D., Turpin, J. A., Kalter, D. C., & Gendelman, H. E. (1990). Macrophages as susceptible targets for HIV infection, persistent viral reservoirs in tissue, and key immunoregulatory cells that control levels of virus replication and extent of disease. *AIDS Res Hum Retroviruses*, *6*(8), 967–71. Review.
Meucci, O., et al. (1998). Chemokines regulate hippocampal neuronal signaling and gp120 neurotoxicity. *Proc Natl Acad Sci USA*, *95*, 14500–14505.
Meucci, O., et al. (2000). Expression of CX3CR1 chemokine receptors on neurons and their role in neuronal survival. *Proc Natl Acad Sci USA*, *97*, 8075–8080.

Meylan, P. R., Guatelli, J. C., Munis, J. R., Richman, D. D., & Kornbluth, H. S. (1993). lvlechanisms for the inhibition of HIV replication by interferons-alpha., beta, gamma primary human macrophages. *Virology*, *193*, 138–48.

Milush, J. M., Stefano-Cole, K., Schmidt, K., Durudas, A., Pandrea, I., & Sodora, D. L. (2007). Mucosal innate immune response associated with a timely humoral immune response and slower disease progression after oral transmission of simian immunodeficiency virus to rhesus macaques. *J Virol*, *81*, 6175–86.

Miller RJ, Meucci O. (1999) AIDS and the brain: is there a chemokine connection? *Trends Neurosci*., 22(10):471–9.

Moir, S., Malaspina, A., & Ogwaro, K. M. (2001). HIV-l induced phenotypic and functional perturbations of B-cells in chronically infected individuals. *Proc Natl Acad Sci USA*, *98*, 10362–7.

Montefiori, D. C., Robinson, W. E., & Mitchell, W. M. (1989). Antibody independent, complement-mediated enhancement of HIV-l infection by mannosidase I and II inhibitors. *Antiviral Res*, *11*, 137–46.

Munson, L. G., Scott, M. E., Landay, A. L., & Spear, G. T. (1995). Decreased levels of complement receptor 1 (CD35) on B lymphocytes in persons with HIV infection. *Clin Immunol Immunopath*, *75*, 20–5.

Nigam, P., Velu, V., Kannanganat, S., Chennareddi, L., Kwa, S., Siddiqui, M., et al. (2010). Expansion of FOXP3+ CD8 T cells with suppressive potential in colorectal mucosa following a pathogenic simian immunodeficiency virus infection correlates with diminished antiviral T cell response and viral control. *J Immunol*, *184*(4), 1690–701. Epub 2010 Jan 6.

Nowak, M. A., Bonhoeffer, S., Shaw, G. M., May, R. M. (1997) Anti-viral drug treatment: dynamics of resistance in free virus and infected cell populations. *J Theor Biol.* 21;184(2):203–17.

Pandrea, I., Sodora, D. L., Silvestri, G., Apetrei, C. (2008) Into the wild: simian immunodeficiency virus (SIV) infection in natural hosts. *Trends Immunol*. 2008 Sep;29(9):419–28. Review.

Pantaleo, G. & Fauci, A. S. (1994). Tracking HIV during disease progression. *Curr Opin Immunol*, *6*, 600–64.

Pantaleo, G., Demarest, J. E, Soudeyns, H., et al. (1994). Major expansion of CD8+ T cells with a predominant VB during the primary immune response to HIV. *Nature*, *370*, 463–7.

Pantaleo, G., Graziosi, C., & Demarest, J. F. (1993). HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. *Nature*, *362*, 355–8.

Pantaleo, G., Graziosi, E., & Fauci, A. S. (1997). Virologic and immunologic events in primary HIV infection. *Springer Seminal Immunopathol*, *18*, 257–66.

Pascual, G., Danielsson, C., Steiger, G., & Schifferli, J. A. (1994). Proteolytic cleavage of CR1 on human erythrocytes in vivo: Evidence for enhanced cleavage in AIDS. *Eur J Immunol*, *24*, 702–8.

Pastinen, T., Liitsola, K., Niini, P., Salminen, M., & Syvanen, A. C. (1998). Contribution of the CCR5 and MBL genes to susceptibility to HIV type I infection in the Finnish population. *AIDS Res HIV Retroviruses*, *14*, 695–8.

Patella, V., Florio, G., Petraroli, A., & Marone, G. (2000). HIV-1 gp120 induces IL-1 and IL-13 release from human FcgR1+ cells through interaction with the VH3 region of IgE. *J Immunol*, *164*, 589–95.

Patke, C. L. & Shearer, W. T. (2000). Gp120 and TNF-alpha-induced modulation of human II cell function: Proliferation, cyclic AMP generation, IgG production, and B cell receptor expression. *J Allergy Clin Immunol*, *105*, 975–82.

Patterson, S., English, N. R., Longhurst, H., et al. (1998). Analysis of human immunodeficiency virus type 1 (HIV-1) variants and levels of infection in dendritic and T cells from symptomatic HIV-1 infected patients. *J Gen Virol*, *79*, 247–57.

Pedersen, C., Lindhardt, B. O., & Jensen, B. L. (1989). Clinical course of primary HIV infection: Consequences for subsequent course of infection. *Br Med J*, *299*, 154–7.

Pereira, L., Bentley, K., Peeters, A., Churchill, M. J., & Deacon, N. (2000). A compilation of cellular transcription factor interactions with the HIV-I LTR promoter. *Nucleic Acids Res*, *28*, 663–8.

Petravic, J., Loh, L., Kent, S. J., & Davenport, M. P. (2008). CD4+ target cell availability determines the dynamics of immune escape and reversion in vivo. *J Virol*, *82*, 4091–4101.

Phillips, A. N. (1996). Reduction of HIV concentration during acute infection: Independence from a specific immune response. *Science*, *271*, 497–499.

Piguet, V., Schwartz, O., Le Gall, S., & Trono, D. (1999). The downregulation of CD4 and MHC-l by primate lentiviruses: A paradigm for the modulation of cell surface receptors. *Immunol Rev*, *168*, 51–63.

Pleskoff, O., Sol, N., & Labrosse, B. (1997a). Human immunodeficiency virus strains differ in their ability to infect CD4+ cells expressing the rat homolog of CXCR-4 (Fusin). *J Virol*, *71*, 3259–62.

Pleskoff, O., Treboute, C., & Brelot, A. (1997b). Identification of a chemokine receptor encoded by human cytomegalovirus as a cofactor for HIV-l entry. *Science*, *276*, 1874–8.

Poccia, F., Battistini, L., Cipriani, B., Mancino, G., Martini, F., Gougeon, M. L., et al. (1999). Phosphoantigen-reactive Vgamma9Vdelta2 T lymphocytes suppress in vitro human immunodeficiency virus type 1 replication by cell-released antiviral factors including CC chemokines. *J Infect Dis*, *180*(3), 858–61.

Poli, G., Bressler, P., & Kinter, E. (1990a). Interleukin-6 induces human immunodeficiency virus expression in infected monocytes alone and in synergy with tumor necrosis factor alpha by transcriptional and post-transcriptional mechanisms. *J Exp Med*, *172*, 253–6.

Popov, S., Rexach, M., & Zybarth, G. (1998). Viral protein R regulates nuclear import of the HIV-l preintegration complex. *EMBO J*, *17*, 909–17.

Porwit, A., Böttiger, B., Pallesen, G., Bodner, A., & Biberfeld, P. (1989a). Follicular involution in HIV lymphadenopathy. A morphometric study. *APMIS 97*(2), 153–65.

Porwit, A., Parravicini, C., Petren, A. L., Barkhem, T., Costanzi, G., Josephs, S., et al. (1989b). Cell association of HIV in AIDS-related encephalopathy and dementia. *APMIS* 97(1), 79–90.

Price, D. A., Sewell, A. K., Dong, T., et al. (1998). Antigen-specific release of beta-chemokines by anti-HIV-I cytotoxic T-lymphocytes. *Curro Biol*, *8*, 355–8.

Paiardini, M., Frank, I., Pandrea, I., Apetrei, C., & Silvestri, G. (2008). Mucosal immune dysfunction in AIDS pathogenesis *AIDS Review*, 10, 36–46, 4.

Reinhart, T. A., Rogan, M. J., Huddleston, D., Rausch, D. M., Eiden, L. E., & Haase, A. T. (1997). Sinlian immunodeficiency virus burden in tissues and cellular compartments during cellular latency and AIDS. *J Infect Dis*, *176*, 1198–208.

Reinhart, T. A., Qin, S., & Sui, Y. (2009). Multiple roles for chemokines in the pathogenesis of SIV infection. *Curr HIV Res*, *7*(1), 73–82. Review.

Rinaldo, C. R. Jr., Armstrong, J. A., Kingsley, L. A., Zhou, S., & Ho, M. (1990). Relation of alpha and gamma interferon levels to development of AIDS in homosexual men. *J Exp Pathol*, *5*, 127–32.

Roederer M, Raju PA, Mitra DK, Herzenberg LA, Herzenberg LA. (1997) HIV does not replicate in naive CD4 T cells stimulated with CD3/CD28. *J Clin Invest.* 99(7):1555–64.

Rodriguez, C., Thomas, J. K., O'Rourke, S., Stiehm, E. R., & Plaeger, S. (1996). HIV disease in children is associated with selective decrease in CD23 and CD62L B-cells. *Clin Immunol Immunopathol*, *81*, 191–9.

Roulston, A., Lin, R., Beuparlant, P., Weinberger, M. A., & Hiscott, J. (1995). Regulation of human immunodeficiency virus type I and cytokine gene expression in myeloid cells by NF-κB/Rel transcription factors. *Microbiol Rev*, *59*, 481–505.

Roulston, A., Marcellus, R. C., & Branton, P. E. (1999). Viruses and apoptosis. *Annu Rev Microbiol*, *11*, 578–608.

Sacktor N, Skolasky RL, Cox C, Selnes O, Becker JT, Cohen B, Martin E, Miller EN; Multicenter AIDS Cohort Study (MACS). (2010) Longitudinal psychomotor speed performance in human immunodeficiency virus-seropositive individuals: impact of age and serostatus. *J Neurovirol*, 16(5):335–41.

Salzwedel, K. & Berger, E. A. (2000). Cooperative subunit interactions within the oligomeric envelope glycoproteins of HIV-l: Functional complementation of specific defects in gp120 and gp41. *Proc Natl Acad Sci USA*, *97*, 12794–9.

Sato, Y., Hirayama, M., Morimoto, K., Yamamoto, N., Okuyama, S., Hori, K. (2011), High mannose-binding lectin with preference for the cluster of {alpha}1-2 mannose from the green alga Boodlea coacta

is a potent entry inhibitor of HIV-1 and influenza viruses. *J Biol Chem*, 2011 Apr 1.
Schmitz, J. E., Kuroda, M. J., Santra, S. Sasseville, V. G., Simon, M. A., Lifton, P., et al. (1999). Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. *Science*, *283*, 857–860.
Schnittman, S. M. & Fauci, A. S. (1994). Human immunodeficiency virus and acquired immunodeficiency syndrome: An update. *Adv Intern Med*, *39*, 305–55.
Sherman, M. P. & Greene, W. C. (2002). Slipping through the door: HIV entry into the nucleus. *Microbes Infection*, *4*, 67–73.
Shirai, A, Cosentino, M., Leitman-Klinman, S., & Klinman, D. (1992). Human immunodeficiency virus infection induces both polyclonal and virus specific B-cell activation. *J Clin Invest*, *89*, 561–6.
Spear, T, Sullivan, B. L., Landay, A. L., & Lint, T. F. (1990). Neutralization of human immunodeficiency virus type 1 by complement occurs by viral lysis. *J Virol*, *64*, 5869–73.
Spira, A. I., Marx, P. A., & Patterson, B. K. (1996). Cellular targets of infection and route of viral dissemination after an intravaginal inoculation of simian immunodeficiency virus into rhesus macaques. *J Exp Med*, *183*, 215–25.
Steinman, R. M. (1991). The dendritic cell system and its role in immunogenicity. *Annu Rev Immunol*, *9*, 271–96.
Steinman RM, Banchereau J. (2007) Taking dendritic cells into medicine. Nature. 27;449(7161):419–26.
Stevenson M, Gendelman HE. (1994) Cellular and viral determinants that regulate HIV-1 infection in macrophages. *J Leukoc Biol.* 56(3):278–88.
Stockwin, L. H, McGonagle, D., Martin, I. G., & Blair, G. E. (2000). Dendritic cells: Immunological sentinels with a central role in health and disease. *Immunol Cell Biol*, *78*, 91–102.
Stingele, K., Haas, J., Zimmermann, T., Stingele, R., Hübsch-Müller, C., Freitag, M., et al. (2001). Independent HIV replication in paired CSF and blood viral isolates during antiretroviral therapy. *Neurology*, *56*(3), 355–61.
Strain, M. C., Letendre, S., Pillai, S. K., Russell, T., Ignacio, C. C., Günthard, H. F., et al. (2005). Genetic composition of human immunodeficiency virus type 1 in cerebrospinal fluid and blood without treatment and during failing antiretroviral therapy. *J Virol*, *79*(3), 1772–88.
Sullivan, B. L., Knopoff, E. J., Saifuddin, M., et al. (1996). Susceptibility of HIV-l plasma virus to complement-mediated lysis. *J Immunol*, *157*, 1791–98.
Szelc, E. M., McMicheltree, E., Roberts, R. L., & Stiehm, E. R. (1992). Deficient polymorphonuclear cell and mononuclear cell antibody-dependent cellular cytotoxicity in pediatric and adult human immunodeficiency virus infection. *J Infect Dis*, *166*, 486–93.
Tambussi, G., Gori, A., Capiluppi, B., et al. (2000). Neurological symptoms during primary human immunodeficiency virus infection correlate with high levels of HN RNA in cerebrospinal fluid. *Clin Infect Dis*, *20*, 962–5.
Tashima, K. T., Flanigan, T. P., Kurpewski, J., Melanson, S. M., & Skolnik, P. R. (2002). Discordant human immunodeficiency virus type 1 drug resistance mutations, including K103N, observed in cerebrospinal fluid and plasma. *Clin Infect Dis*, *35*(1), 82–3.
Thali, M., Bukovsky, A., & Kondo, E. (1994). Functional association of cyclophilin A with HIV virions. *Nature*, *372*, 363–5.
Than, S., Hu, R., Oyaizu, N., et al. (1997). Cytokine pattern in relation to disease progression in human immunodeficiency virus-infected children. *J Infect Dis*, *179*, 47–56.
Torheim, E. A., et al. (2009). Interleukin-10-secreting T cells defines a suppressive subset 748 within the HIV-1-specific T-cell population. *Eur J Immunol*, *39*, 1280–1287.
Treitel, M. & Resh, M. D. (2001). The late stage of immunodeficiency virus type 1 assembly is an energy dependent process. *J Virol*, *75*, 5473–81.
Vehmas A, Lieu J, Pardo CA, McArthur JC, Gartner S. (2004). Amyloid precursor protein expression in circulating monocytes and brain macrophages from patients with HIV-associated cognitive impairment. *J Neuroimmunol.* 157(1–2):99–110.
Voelkel, N. F., Cool, C. D., Flores, S. (2008) From viral infection to pulmonary arterial hypertension: a role for viral proteins? *AIDS.* Suppl 3:S49–53.
Wagner, L., Yang, O. O., & Garcia-Zepeda, E. A. (1998). B-chemokines are released from HIV-l specific cytolytic T-cell granules complexed to proteglycans. *Nature*, *391*, 1811–15.
Wahl, S. M. & Orenstein, J. M. (1997). Immune stimulation and HIV-l viral replication. *J Leuco Biol*, *62*, 67–71.
Wahl, S. M., Allen, J. B., Gartner, S., et al. (1989). HN-l and its envelope glycoproteins downregulate chemotatic ligand receptors and chemotatic function of peripheral blood monocytes. *J Immunol*, *142*, 3553–9.
Walker, C. M., Moody, D. J., Stites, D. P., & Levy J. A. (1986). CD8+ lymphocytes can control HN infection in vitro by suppressing virus replication. *Science*, *234*, 1563–66.
Wallace, M., Bartz, S. R., Chang, W. L., Mackenzie, D. A., Pauza, C. D., & Malkovsky, M. (1996). gd T lymphocyte responses to HIV. *Clin Exp Immunol*, *103*, 177–84.
Weber, R., Bossart, W., Cone, R., Luethy, R., Moelling, K. (2001) Phase I clinical trial with HIV-1 gp160 plasmid vaccine in HIV-1-infected asymptomatic subjects. *Eur J Clin Microbiol Infect Dis*. 20(11):800–3.
Wei, X., Gosh, S., & Taylor, M (1995). Viral dynamics in human immunodeficiency virus type 1 infection. *Nature*, *373*, 117–22.
Weichold, E. F., Zelia, D., & Barabitskaja, O. (1998). Neither human immunodeficiency virus-l (HIV-1) nor HIV-2 infects most-primitive human hematopoietic stem cells as assessed in long-term bone marrow cultures. *Blood*, *91*, 907–15.
Weiss, R. A. (2008). On viruses, discovery, and recognition. *Cell*, *135*(6), 983–6.
Weiss, R. A (1996). HIV receptors and the pathogenesis of AIDS. *Science*, *272*, 1885–6.
Weiss, R. A. & Clapham, P. R. (1996). AIDS: Hot fusion of HIV-1. *Nature*, *381*, 647–8.
Weiss, R. A., Clapham, P., Nagy, I. C., & Hosino, H. (1990). Oligomeric organization of gp120 on infectious human immunodeficiency virus type 1 particles. *J Virol*, *64*, 5674–7.
Weiss, R. A., Clapham, P. R., Weber, J. N., Dalgleish, A. G., Lasky, L. A., & Berman, P. W. (1986). Variable and conserved neutralization antigens of human immunodeficiency virus. *Nature*, *324*(6097), 572–5.
Williams KC, Corey S, Westmoreland SV, Pauley D, Knight H, deBakker C, Alvarez X, Lackner AA. (2001) Perivascular macrophages are the primary cell type productively infected by simian immunodeficiency virus in the brains of macaques: implications for the neuropathogenesis of AIDS. *J Exp Med.* 193(8):905–15.
Wilson, J. D., Ogg, G. S., Allen, R. L., Davis, C., Shaunak, S., Downie, J., et al. (2000). Direct visualization of HIV-1-specific cytotoxic T lymphocytes during primary infection. *AIDS*, *14*, 225–233.
Woods, T. E., Roberts, B. D., Butera, S. T., & Follzs, T. M. (1997). Loss of inducible virus in CD45RA naive cells after human immunodeficiency virus-1 entry accounts for preferential viral replication in CD45RO memory cells. *Blood*, *89*, 1635–41.
Wyatt, R. & Sodroski, J. (1998). The HIV-l envelope glycoproteins: Fusogens, antigens, and immunogens. *Science*, *280*, 1884–8.
Xu, X. N., Laffert, B., Screaton, G. R., et al. (1999). Induction of Fas ligand expression by HIV-I involves the interaction of Nef with the T cell receptor zeta chain. *J Exp Med*, *189*, 1489–96.
Yang, O. O, Kalams, S. A., & Rosenzweig, M. (1996). Efficient lysis of human immunodeficiency virus type I-infected cells by cytotoxic T lymphocytes. *J Virol*, *70*, 5799–806.
Yang, X. & Gabuzda, D. (1999). Regulation of human immunodeficiency virus type-l infectivity by the ERK mitogen-activated protein kinase signaling pathway. *J Virol*, *73*, 3460–6.
Yarchoan, R., Redfield, R., & Broder, S. (1986). Mechanism of cell activation in patients with acquired immunodeficiency syndrome and related disorders. *J Clin Invest*, *78*, 439–47.
Yu, X. G., Addo, M. M., Rosenberg, E. S., et al. (2002). Consistent patterns in the development and immunodominance of human immunodeficiency virus -l (HIV-1)-specific CD8+ T-cell responses following acute HIV-I infection. *J Virol*, *76*, 8690–701.

1.3

CHEMOKINES

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HIV-1-related central and peripheral nervous system problems are highly prevalent but the details of their pathogenesis are poorly understood. A widely held hypothesis is that infected cells in the nervous system secrete neurotoxins that exert direct and indirect deleterious effects. Candidate neurotoxins include excitotoxins, inflammatory mediators, and HIV-1 viral proteins. There is evidence that chemokines and chemokine receptors, which are expressed in all major cell types in the brain, may play pivotal roles in many of these diverse neurotoxic processes. This chapter explores these possible roles. Close attention is paid to a several proposed mechanisms, including those involving the CXCR4 chemokine receptor and the HIV gp120 protein, whose effects may be partly mediated by cytokine-related processes. To help provide a framework for understanding these complex pathogenic phenomena, the role of chemokines and chemokine receptors in normal development and function is discussed.

INTRODUCTION

It is a truth, universally acknowledged, that the consequences of HIV-1 infection encompass not only the immune system but the nervous system as well. The majority of individuals infected by HIV-1 display neurological symptoms and associated neuropathology involving both the central (CNS) and peripheral (PNS) nervous systems. (Kaul, Garden, & Lipton, 2001; Kolson & Gonzales-Scarano, 2001). The serious and incapacitating nature of these syndromes means that neurological problems make a significant contribution to the overall clinical picture in AIDS.

In spite of the fact that HIV-1-related neurological problems are so prevalent, there is little real understanding as to the mechanisms underlying HIV-1-related damage to the nervous system. Indeed, in many respects the situation is less straightforward than that associated with HIV-1-related problems with the immune system. The destruction of leukocytes by HIV-1 is associated with infection of these cells by the virus and its subsequent replication. On the other hand, this is not the case for HIV-1-related neuropathology (Kaul et al., 2001; Kolson & Gonzales-Scarano, 2001). The brains of HIV-1-infected individuals exhibit morphological changes to microglia, astrocytes, and neuronal populations, although it is widely believed that the virus only productively infects microglia (Gabuzda & Wang, 2000; Garden, 2002; Kaul et al., 2001; Kolson & Gonzales-Scarano, 2001). It is therefore not at all obvious how such widespread neuropathology might be produced. A generally held hypothesis suggests that infected cells in the nervous system such as microglia must secrete one or more neurotoxins that elicit a cascade of deleterious effects (Kaul et al., 2001). Although such an idea seems reasonable, the precise nature of these secondary neurotoxins remains obscure, even though there is a long list of possibilities. Indeed, it is likely that HIV-1-related neuropathology stems from more than one process. For example, problems resulting from the presence of inflammatory reactions in the brain (HIV encephalitis, HIVE) or from opportunistic infections may be important (Williams & Hickey, 2002). However, other mechanisms directly related to the effects of the virus within the nervous system should also be considered.

In order to understand how HIV-1 compromises the nervous system, it is useful to first examine its mechanism of action in the immune system. HIV-1 infects target cells by binding to receptors expressed in their membranes. The major coat protein of the virus, gp120, binds with high affinity to one of the chemokine receptors, usually in conjunction with the hCD4 molecule (Rossi & Zlotnik, 2000). Conformational changes ensuing from this interaction allow the virus to fuse its membrane with that of its target and insert its DNA into the host cell. However, as we shall discuss, chemokine receptors normally function as important signaling elements in cells. Indeed, an increasing number of papers have now reported that binding of HIV-1 to these receptors may do more than just allow fusion of the virus with its target and may also elicit important signaling events that regulate the fate of target cells (Davis et al., 1997; Gabuzda & Wang, 2000). Are these observations relevant for attempts to explain HIV-1-related neuropathology? It is now clear that all of the major cell types in the brain are capable of expressing both chemokines and chemokine receptors, including those chemokine receptors like CXCR4 that normally act as binding sites for HIV-1 (Bajetto et al., 2001; Bajetto, Bonavia, Barbero, & Schettini, 2002; Kolson & Gonzalez-Scarano, 2001; Martin-Garcia et al., 2002). An important question, therefore, is whether, as in the immune system, chemokine receptors in the brain are important for HIV-1-related neuropathology and, if so, which chemokine receptors and why.

CHEMOKINES AND THEIR RECEPTORS

In order to be able to answer such questions, we should discuss the nature of chemokines and their receptors as well as their

normal functions in the brain. Chemokines (the name is a contraction of the words CHEMOtactic cytoKINE) are a family of small proteins, originally shown to play a pivotal role in the control of leukocyte trafficking (Rossi & Zlotnik, 2000). Although this is still their best-studied function, chemokines have now been shown to be of key importance in the overall organization of the entire hematopoietic/lymphopoietic system, including the regulation of stem cell maturation, the formation of secondary lymphoid tissues, and angiogenesis (Ma, Jones, & Springer, 1999; Schwartz & Farber, 2002; Szekanecz & Koch, 2001; Nagasawa et al., 1996). Moreover, because chemokines and their receptors are also intimately involved in the orchestration of inflammatory responses and in the pathogenesis of AIDS (Berger, Murphy, & Farber, 1999), they are considered to be important potential therapeutic targets in these diseases (Proudfoot, 2002). Based on recent findings that chemokines and their receptors are involved in controlling organogenesis, as well the maturation and migration of different types of stem cells (Knaut, Werz, Gelser, & Nusslein-Volhard, 2002; Doitsidou et al., 2002; McGrath, Koniski, Maltby, McGann, & Palis, 1999; Braun et al., 2002; Moepps et al., 2000; Tachibana et al., 1998), it is now becoming clear that chemokine-mediated signaling may have a far wider influence than originally anticipated. Thus, aside from the hematopoietic/lymphopoietic system, chemokines probably have important roles to play in the development and homeostasis of all tissues.

What then, if anything, do chemokines have to do with the nervous system? It is well known that chemokines and their receptors have an important part to play in neuroinflammatory disease (DeGroot & Woodroofe, 2001). In keeping with their traditionally defined roles as organizers of the immune system, chemokines produced by resident brain cells have been shown to be of central importance in guiding leukocytes to sites of inflammation within the brain. In addition, however, recent work has established that all of the major cell types in the brain, including microglia, glia, neurons and neural stem cells, can express different chemokine receptors, making them all potential targets for the actions of chemokines. Furthermore, it is known that these different cell types are capable of synthesizing chemokines, suggesting that a complete chemokine ligand/receptor system exists independently within the brain. Thus, it now appears that chemokines may play a much more complex role in the nervous system, extending far beyond their known role as local mediators of immune and inflammatory responses.

Although these observations are intriguing, they also raise many questions. For instance, do the effects of chemokines in the brain merely recapitulate their well-established effects on leukocytes or is something else afoot? Indeed, we now know that chemokines and their receptors participate in the development of the cerebellum, hippocampus, and many other parts of the nervous system; regulate oligodendrocyte maturation and myelination in the spinal cord; and influence axonal growth, neuronal survival, and a host of other interesting phenomena. Many of the effects described so far have involved the CXCR4 chemokine receptor and its unique ligand, the chemokine CXCL12/SDF-1. However, there are numerous indications that other chemokines and their receptors are important as well.

Over 50 chemokines have been identified to date (Berger, Murphy, & Farber, 1999; Murphy et al., 2000; Murphy, 2002). All of these molecules share certain similarities in their primary sequences and, more importantly, a similar overall tertiary structure (Proudfoot, 2002). Chemokines can be tentatively grouped into subfamilies according to different structural criteria, particularly the position of a pair of cysteines located near the N terminal of each protein (Rossi & Zlotnik, 2000; Proudfoot, 2002; Proudfoot, Shaw, Power, & Wells, 2002). If a single amino acid separates the two cysteines the chemokine is designated an α-chemokine. If the two cysteines directly follow one another, then the chemokine is designated a β-chemokine. Moreover, the presence of only a single cysteine residue, such as in lymphotactin, places the chemokine in the δ-chemokine family, whereas two cysteines separated by three amino acids as in the case of fractalkine, places it in the γ-chemokine family. The majority of chemokines are in the α or β families. There is only one member in the γ and δ-families known at this time. In general, chemokines are secreted from cells. However in rare cases, such as the δ-chemokine fractalkine and CXCL16, the unique ligand for the CXCR6 receptor, the chemokine moiety is found tethered to a transmembrane mucin–like stalk. Fractalkine can act in this tethered mode or else be cleaved from its mucin stalk and act at remote targets. Each chemokine possesses a "trivial" name, such as stromal cell-derived factor 1 (SDF-1), as well as a systematic name, such as CXCL12. In this chapter I will generally use the trivial name.

All of the known effects of chemokines are transduced through the activation of G-protein-coupled receptors (GPCRs), whereby 6 GPCRs mediate the effects of α- and 10 GPCRs mediate the effects of β-chemokines. As for the γ-and δ-chemokines, their effects are mediated by only one GPCR. Two major patterns of chemokine/receptor selectivity are apparent: Some chemokine receptors can be activated by multiple chemokines, whereas others, such as the CXCR4 receptor, have only one known agonist, in this case the chemokine SDF-1. Recently, a second receptor for SDF-1 has been identified and named CXCR7 (Thelen & Thelen, 2008). This receptor, which is widely expressed in the developing and adult nervous systems (Fig 1.3.1), also recognizes CXCL11/ITAC (Interferon-inducible T-cell Alpha Chemoattractant). This is a very interesting receptor as although it has a typical GPCR structure it does not seem to signal in a typical GPCR manner. Thus, rather than activating a G-protein, signaling resulting from the activation of CXCR7 receptors appears to exclusively utilize the β-arrestin signaling pathway (Rajagopal et al., 2010). How CXCR7 contributes to the biological effects of SDF-1 is not yet understood. However, the phenotype of CXCR7 knockout mice is clearly not identical to that of CXCR4 knockout mice. Indeed the former are viable whereas the latter die during embryogenesis (Sierro et al., 2007). Hence, it is clear that CXCR4 and CXCR7 must have predominantly separate functions.

Although a rapid expansion in the number of chemokines and their receptors is phylogenetically associated with the

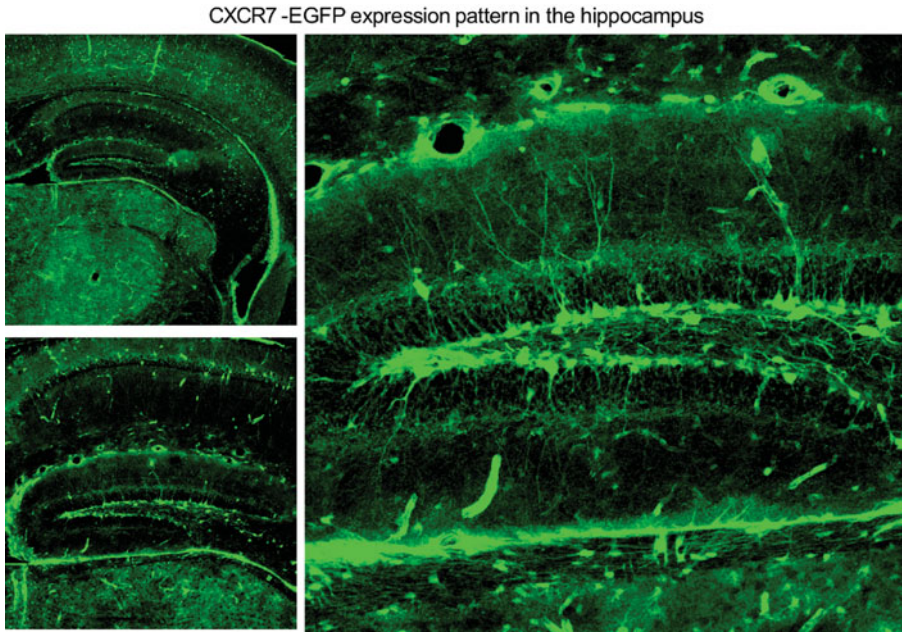

Figure 1.3.1 Expression of CXCR7 receptors in the hippocampus of a 6 week old CXCR7-EGFP transgenic mouse. Note the expression of CXCR7 by numerous blood vessels and neurons (unpublished observations).

development of vertebrates, homologues of these molecules have been identified in simpler systems. For instance the genomes of several viruses encode chemokines and their receptors, and these genes are of importance for viral pathogenesis (Proudfoot et al., 2002). An observation of considerable significance was the finding that certain chemokine receptors, particularly CCR5 and CXCR4, are used by HIV-1 to infect target cells, as discussed above (Berger et al., 1999). Because the sequence of the HIV-1 coat protein gp120 varies with each viral strain, different viruses exhibit greater affinity for either CCR5 or CXCR4 receptors, which are predominantly expressed by different subsets of leukocytes. Hence, these observations also help to explain HIV-1 tropism—the reason why different viral strains selectively infect different types of cells.

BRAIN CHEMOKINE RECEPTORS IN HIV-1-RELATED DEMENTIA

As discussed above, the view is widely held that HIV-1-related neuropathology results from the secretion of toxic factors from infected cells in the brain. The infected cells might be microglia or other macrophage-like cells (perivascular macrophages) that enter the brain in association with HIVE. Furthermore, it is quite possible that more than one type of neurotoxin might be involved. There has been no shortage of suggestions as to the nature of these secreted toxins. For example, glutamate-induced excitotoxicity has long been thought to play a role in HIV-1-related effects in the brain and drugs that block glutamate receptors have been suggested as potential therapeutic agents in HAD (Kaul, Garden, & Lipton, 2001; Kolson & Gonzalez-Scarano, 2001). Inflammatory cytokines, such as TNF-α, IL-1, and IL-6 have also been frequently suggested as playing an important role (Kaul et al., 2001; Kolson & Gonzalez-Scarano, 2001). Finally, it is also widely believed that viral proteins, shed from replicating viruses in microglia or other cells, may act as toxins in the brain. These could be proteins such as gp120, the HIV-1 coat protein, or other viral proteins such as Tat. Indeed, there is evidence suggesting a role for many of these proteins as potential neurotoxins (Kaul et al., 2001; Kolson & Gonzalez-Scarano, 2001; Nath, 2002). The participation of gp120 has been the focus of considerable attention. The idea that gp120 could be an endogenous neurotoxin in HAD comes from a variety of sources. For example, purified gp120 injected into the brains of rodents (Corasaniti, 2001), or incubated with neurons in culture, clearly induces neuronal death as well as activation of microglia and astrocytes, indicating that the protein could be directly toxic to neurons or kill neurons by inducing the release of toxins from cells such as microglia and astrocytes (Kaul et al., 2001; Garden, 2002; Meucci et al., 1998; Meucci & Miller, 1996). Furthermore, transgenic mice which overexpress gp120 develop pathological signs that are reminiscent of those in HAD. (Toggas et al., 1994) If, as these data suggest, gp120 is a significant participant in HIV-1 related events in the brain, then this raises the issue of how it produces its effects. As we know that chemokine receptors are the major binding sites for gp120, one would imagine that these receptors in the brain would mediate the effects of the protein. One consideration is that although many types of chemokine receptors are expressed by cells in the brain, not all of these are susceptible to interactions with gp120. The major receptors of importance are likely to be CXCR4 and CCR5 receptors and possibly CCR3 receptors that are known to be highly expressed by microglia. Neural stem cells in neurogenic

regions of the adult brain and some neurons also express CXCR4 receptors (Bhattacharrya et al., 2008; Tran, Ren, Chenn, & Miller, 2007). It is therefore interesting to examine data on which strains of the HIV-1 virus are most likely to be associated with HAD. The development of HIV-1 in the brain occurs separately from virus in the periphery. Currently, it is thought that viruses with selectivity for the CXCR4 receptor or those able to bind to both CXCR4 and CCR5 are the most likely to produce neurological symptoms (Gorry et al., 2001). Such selectivity is only part of the story, as some brain-derived viruses with selectivity for CXCR4 do not produce neuronal apoptosis when tested in vitro (Gorry et al., 2001). Nevertheless, binding of virus/gp120 to the CXCR4 receptor would seem to be an important factor in the genesis of HAD. It should be noted in passing that many of the studies that have demonstrated the neurotoxic potential of gp120 have been performed in rodents (Meucci et al., 1998; Meucci & Miller, 1996; Toggas et al., 1994; Corasaniti et al., 2001). Given the fact that interactions of gp120 with chemokine receptors normally require the participation of the human (rodent won't do) CD4 molecule (Berger et al., 1999), one may wonder how this is achieved. Several studies have demonstrated that gp120s of various types can interact with neurons and other cells in the nervous system in a "CD4 independent manner" (Hesselgesser et al., 1998). How this occurs is not at all clear at this time. One possibility is that these cells express a molecule that can be used as a co-receptor instead of the CD4 molecule. Alternatively, much of the HIV-1-related neuropathology may involve the action of the virus at sites such as Toll-like receptors (Heil et al., 2004).

THE NORMAL FUNCTIONS OF BRAIN CHEMOKINE RECEPTORS

Prior to examining the role of chemokine receptors in the nervous system in the neuropathological aspects of HIV-1 infection, it is important to understand the normal functions of these receptors in the nervous system. Thus, if CXCR4 and CCR5 play important roles in the normal physiology of the nervous system it would be quite possible that these might be disrupted by HIV-1. The first papers demonstrating that neurons and other types of resident brain cells normally express chemokine receptors began to appear around 1997. Subsequently, several studies have been published describing the detailed distribution of certain chemokine receptors in the nervous system (Jazin, Soderstrom, Ebendal, & Larhammer, 1997; Lavi et al., 1997; Moepps, Frodl, Rodewald, Bagglioni, & Gierschik, 1997; Horuk et al., 1997; reviewed in Bajetto, Bonavia, Barbero, & Schettini, 2002; Bajetto et al., 2001). In those instances where the question has been examined, it appears that individual neurons can express complex combinations of different chemokine receptors (Meucci et al., 1998; Oh et al., 2001; Gillard, Mastracci, & Miller, 2001). Although the significance of this diversity of chemokine receptor expression is not fully understood, specific functions for particular chemokine receptors in the nervous system have now been clearly identified. In particular, the CXCR4 receptor and its sole known ligand SDF-1 are extensively expressed in the developing embryo from very early times, including at very high levels in the developing nervous system (McGrath et al., 1999; Braun et al., 2002; Moepps et al., 2000; Jazin et al., 1997). Patterns of CXCR4/SDF-1 expression are complementary and dynamic throughout embryogenesis, suggesting that CXCR4 receptors might be important in the development of the nervous system. This possibility was confirmed in 1998 when two groups described the phenotypes of mice in which the genes for the CXCR4 receptor or SDF-1 had been deleted (Zou, Kottmann, Kuroda, Taniuchi, & Littman, 1998, Ma et al., 1998). These mutant mice usually die around the time of birth and display various defects in organogenesis, including significant alterations in the development of the brain (Tachibana et al., 1998, Zou et al., 1998, Ma et al., 1998). The first neurological problem to be described concerned the cerebellum. During the normal course of cerebellar development, precursors for cerebellar granule neurons proliferate extensively during the early postnatal period (approx 4 weeks in rodents and 2 years in man) in a specialized zone known as the external granule layer (EGL). The EGL lies immediately beneath a layer of externally situated meningeal cells (pia mater), localized on the cerebellar surface. Following proliferation, postmitotic immature granule cells move to the inner aspect of the EGL and then migrate inwards along the processes of Bergmann glial cells to form the internal granule layer (IGL), consisting of mature granule cells. This orderly process was shown to be disrupted in the cerebella of CXCR4 and SDF-1 knockout (ko) mice. It was observed that the cerebella from mutant embryos contained groups of ectopically situated granule neurons within or beneath the Purkinje cell layer, suggesting aberrant early migration of granule cells and/or their precursors out of the EGL. However, other aspects of cerebellar development appeared to be normal. These results make perfect sense when one considers the normal spatial and temporal expression patterns of CXCR4 receptors and SDF-1 in the cerebellum at this time. (Klein et al., 2001, Reiss, Mentlein, Sievers, & Hartmann, 2002) SDF-1 is not expressed by cerebellar neurons, but is strongly expressed by the meningeal cells in the external pial layer. In contrast, CXCR4 receptors are expressed by dividing cells, presumably granule cell progenitors, in the EGL. Moreover, it had been previously demonstrated that the normal development of the IGL was dependent on the integrity of the pial layer and it had been suggested that these cells secreted some factor that regulated granule cell migration by attracting as well as maintaining granule cell progenitors within the EGL (Hartmann, Schulze, & Sievers, 1998). Indeed, the role of SDF-1 in this regard has now been demonstrated by showing that isolated granule cell progenitors or progenitors within EGL explants will migrate toward a source of exogenously provided SDF-1 (Klein et al., 2001, Zou et al., 1998). Furthermore, the observations that granule cell progenitors will normally migrate toward cerebellar meningeal cells (Klein et al., 2001, Reiss et al., 2002, Hartmann et al., 1998, Zhu et al., 2002), and that this migration is impeded in SDF-1 ko mice indicates that SDF-1 is the only, or at least the major, factor that maintains granule cell progenitors within the EGL (Zhu et al., 2002). Thus, granule

progenitors are maintained in the proliferative environment of the EGL through the chemoattractant effects of SDF-1 secreted from the overlying pia mater. Moreover, as demonstrated by Klein et al. (2001), SDF-1 not only serves to localize progenitors within the EGL, but also plays a role in ensuring their proliferation through facilitatory interactions with sonic hedgehog (SHH), an important mitogen for granule cell precursors also found within the EGL. Although the synthesis of SDF-1 is maintained during development, at some point, perhaps because of interactions with other receptor signaling systems, granule cell progenitors downregulate CXCR4 expression and/or signaling and stop dividing. They move to the inner aspect of the EGL and then migrate to the IGL, presumably attracted there by other chemo attractants such as BDNF (Borghesani et al., 2002). An echo of these observations can be found in the known role of CXCR4/SDF-1 signaling in lymphocyte development. SDF-1 is expressed by cells in secondary lymphoid organs and serves to attract and maintain CXCR4 expressing precursor cells in this proliferative environment (Bleul, Schultze, & Springer, 1998). Moreover, deletion of the genes for SDF-1 or CXCR4 results in inappropriate redistribution of stem cells into the circulation prior to their maturation (Nagasawa et al., 1996, Zou et al., 1998, Ma et al., 1998).

When one examines the properties of embryonic cerebellar neurons, it is clear that they express many types of chemokine receptors (Gillard et al., 2001), suggesting that in addition to SDF-1, other chemokines and their receptors may also play distinct roles in cerebellar development. In support of this contention, a recent study has described the changing expression pattern of CCR1 receptors, as well as the CCR1 activating chemokine MIP-1α, during the development of the cerebellum (Cowell & Silverstein, 2003). It was observed that during the first three weeks after birth, CCR1 receptors are transiently expressed by all of the major cell types in the cerebellum, including granule cells, Purkinje and Golgi neurons, astrocytes, Bergmann glia, and microglia. However, the times at which all of these cell types express CCR1 receptors differ. Interestingly, during the times of peak receptor expression each cell type exhibits close interactions with Purkinje cells. Furthermore, the Purkinje cells themselves appear to synthesize the chemokine MIP-1α, a major ligand for CCR1 receptors. It has therefore been suggested that MIP-1α/CCR1 signaling may be of importance in the maturation of neurites and synapse formation during this crucial period of cerebellar development (Cowell & Silverstein 2003). Indeed, chemokines and their receptors may play a widespread role in the regulation of axonal growth. A recent study has demonstrated that SDF-1 can either attract or repel the growth cones of developing cerebellar granule neurons or Xenopus spinal neurons in culture, depending on the circumstances (Xiang et al., 2002). When levels of cGMP are low, SDF-1 produces growth cone repulsion, but this is transformed into attraction when the levels of this cyclic nucleotide are high. Thus, it is possible that CCR1 receptor signaling may play a role of this kind in the cerebellum.

In addition to the cerebellum, the widespread expression pattern for SDF-1/CXCR4 in the embryonic nervous system (McGrath et al., 1999) suggests that chemokine signaling may be important in the development of other brain areas as well. Indeed, it has now been demonstrated that CXCR4 receptors play an essential role in the development of the hippocampal dentate gyrus. Studies conducted by Lu et al. (2002) and Bagri et al. (2002) have shown that in CXCR4 KO mice the granule cell layer of the dentate gyrus fails to develop properly. Normally, precursors that are destined to form these granule cells originate in the dentate neuroepithelium (Altman & Bayer, 1990, Sievers, Hartmann, Pehlemann, & Berry, 1992). As they migrate away from this proliferative zone ("primary germinal matrix"), they form a stream of migratory cells that continue to proliferate (secondary germinal matrix), and which eventually populate the developing blades of the dentate gyrus, producing its typical V-shaped pattern. Subsequently, precursor cells continue to proliferate within the hilar region of the dentate gyrus (tertiary germinal matrix), producing very large numbers of dentate granule neurons in the early postnatal period. Eventually, reduced numbers of granule cell precursors settle in a narrow region between the granule cell layer and the hilus, and these precursors continue to generate new neurons throughout adult life. Adult neurogenesis in the hippocampal dentate gyrus is thought to be of great importance for the consolidation of memories, and disruption of this process may also be a key event in the generation of seizures and other abnormal brain activity (Gould & Gross, 2002). Thus, a clear understanding of the molecular events that underlie the formation and maintenance of the dentate gyrus has many important implications. Using a variety of molecular markers, cells that can be identified as dentate granule neurons can be observed in CXCR4 ko mice, although they appear to occur in reduced numbers (Lu, Grove, & Miller, 2002; Bagri et al., 2002). This implies that, as in the case of the cerebellum, SDF-1 may have proliferative effects on dentate granule cell precursors. Moreover, those granule cells that do occur are mostly found localized ectopically within the migratory stream. In other words, it appears as if reduced numbers of dentate granule cells can go through their normal developmental program, but do so without ever reaching their normal destination. As in the case of the cerebellum, these results can be understood by considering the normal localization of CXCR4\SDF-1 in the developing hippocampus (Fig 1.3.2) (Lu et al., 2002; Bagri et al., 2002). During embryogenesis in rodents, from around E14 until birth, SDF-1 is highly expressed in the meninges overlying the hippocampus as well as in Cajal-Retzius cells. These latter cells also express the molecule reelin, which has been shown to play a critical role in dentate granule cell migration. In contrast to the expression of SDF-1, CXCR4 receptors are strongly expressed over the same time period, but by cells in the migratory stream as well as the developing dentate gyrus, presumably immature granule cells and their precursors. (Fig 1.3.2) Thus, SDF-1secreted by meningeal cells is in an excellent position to provide a guiding chemoattractant cue for CXCR4-expressing cells as they migrate towards the dentate gyrus. Therefore, in many respects, this situation represents an inversion of that in the developing cerebellum, where SDF-1 serves to maintain precursors in the EGL prior to migration. It is interesting to note that in adult

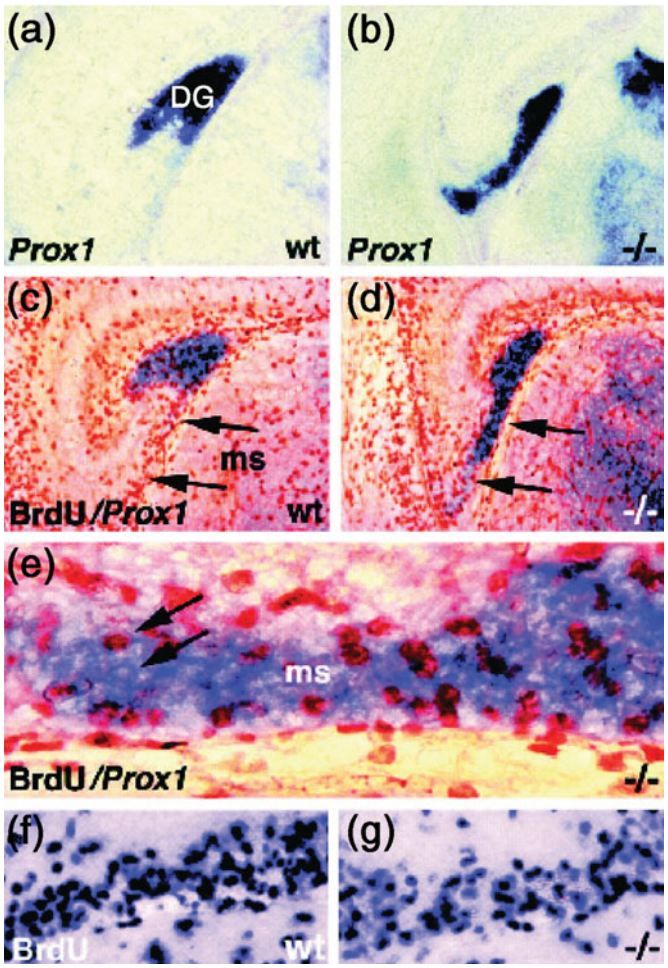

Figure 1.3.2 Defects in secondary proliferative cell population that forms the dentate gyrus (DG). (A-D) Coronal sections through the E18.5 hippocampus of wild type (A and C) ir CXCR4 mutant mice (B and D), processed to show expression of Prox1 blue or Prox1 together with BrdUrd-labeled dividing cells (orange). In the wild type mouse, Prox1 is expressed the forming dg (A and C). By contrast, in the mutant, Prox1 is expressed in the vestigal DG but also along the migratory stream (ms) (arrows in D) of dividing cells running along the ventral surface of the hippocampus into the dg. BrdUrd-labeled cells of the ms can be stream of cells shown in D. Numerous blue, Prox1-expressing appear among the brown, BrdUrd-labeled cells, but the populations appear largely distinct. (E) Higher magnification views of BrdUrd-labeled dividing cells (dark Blue) coursing through the ms in a wild-type (F) and a CXCR4 mutant mouse (G). About 30% fewer BrdUrd-labeled cells appear in the mutant (G) than in the wild type (F). From Lu et al., 2002.

animals, SDF-1 and CXCR4 continue to be expressed in the dentate gyrus, but by granule neurons and progenitor cells in the intragranular zone respectively. This suggests that CXCR4 signaling may be of continued importance in the production of new dentate granule neurons throughout life (Lu et al., 2002). SDF-1 is also produced by meningeal cells covering the surface of the entire cortex and might therefore be expected to play a role in cortical development as well. Intriguingly, many types of lissencephalic disease (i.e. neuronal migrational defects that lead to abnormal sulci in the cerebral cortex) are associated with hypertrophy of the meningeal layer and abnormal "overmigration" of developing cortical neurons, consistent with some chemoattractant role for SDF-1 in the migration of these cells (Hartmann et al., 1999). Meningeal expression of CXCL12 controls positioning and migration of Cajal-Retzius cells via CXCR4 signaling (Borrell & Marin, 2006; Paredes, Li, Berger, Baraban, & Pleasure, 2006). Furthermore, SDF-1/CXCR4 signaling controls cortical interneuron migration by focusing the cells within migratory streams and controlling their position within the cortical plate (Li et al., 2008; Lopez-Bendito et al., 2008; Stumm et al., 2003; Tiveron et al., 2006).

In summary, therefore, there is good evidence for both chemotactic and proliferative effects of chemokine signaling, particularly through the CXCR4 receptor, in association with several important aspects of neuronal development. Additional studies have indicated that different chemokines may act as chemotactic cues for other neuronal populations, (Bolin et al., 1998) so a widespread role for chemokines in the orchestration of neuronal development is certainly possible.

Aside from neurons, chemokine receptor signaling also appears to be important in the development of glia. Signaling via CXCR2 receptors, which are also widely expressed in the nervous system throughout embryogenesis (Luan, Furuta, Du, & Richmond, 2001), has been shown to direct the development of oligodendrocytes and myelination in the spinal cord (Tsai et al., 2002; Robinson et al., 1998; Wu, Miller, Ransohoff, Robinson, & Nishayama, 2000). In the first week after birth, oligodendrocyte precursor cells (OPCs) develop in the ventral ventricular zone of the embryonic spinal cord and these cells express CXCR2 receptors. At the same time, the chemokine GRO-α, which acts as an agonist at CXCR2 receptors, is synthesized by astrocytes in this region. GRO-α has two effects on OPCs. First, somewhat surprisingly for a chemokine, it inhibits their migration. This effect is achieved by enhancing adhesive interactions between OPCs and the extra cellular matrix. Secondly, GRO-α cooperates with platelet-derived growth factor (PDGF), a widely expressed mitogen for OPCs, which enhances proliferation of OPCs in the ventral cord. These cells subsequently differentiate and myelinate axons in this part of the cord. At later times the synthesis of GRO-α in the ventral cord wanes, and as a result the migration of OPCs is no longer inhibited, allowing OPCs to migrate into the dorsal portion of the cord. Simultaneously, the synthesis of GRO-α by astrocytes in the dorsal cord is upregulated. Thus, the proliferation of OPCs is now stimulated in the dorsal cord and myelination of axons in this region of cord can proceed. In addition to CXCR2, it has also been reported that oligodendrocyte progenitors express CXCR4 receptors and that interference with SDF-1/CXCR4 signaling may disrupt the development of oligodendrocytes in the spinal cord. Hence, the development of oligodendrocytes may be under the control of several chemokine signaling systems.

Chemokine receptors may also play a key role in the control of myelination in the peripheral nervous system (Kury, Greiner-Petter, Cornely, Jurgens, & Muller, 2002). Following peripheral nerve injury, axons distal to the lesion degenerate and are broken down in a process known as Wallerian degeneration. As a result, new axons can now regenerate from the remaining stump. During the process of nerve regeneration,

Schwann cells, the glial cells that are responsible for myelination in the peripheral nervous system, actively proliferate, and it has been demonstrated that these cells express the β helix-loop-helix transcription factor, Mash2. Furthermore, this transcription factor is downregulated after peripheral nerve injury and appears to be a negative regulator of Schwann cell proliferation. Interestingly, two of the genes that are downstream of Mash2 are the chemokine Mob-1 (the mouse homologue of the chemokine IP-10) and the CXCR4 receptor. It is interesting to note that Schwann cells in adult peripheral nerve have been shown to co-express Mash2 and CXCR4 receptors. Because these cells also express SDF-1, it seems highly likely that SDF-1 is involved in the paracrine regulation of Schwann cell development.

In summary, two major themes emerge from the reported effects of chemokines in the developing nervous system. The first is that chemokines are clearly important in directing the movement of progenitor cells to different locales—something that one might expect based on their well-established chemoattractant effects on leukocytes. Interestingly, however, there appear to be multiple variations on this theme, with chemokines acting as either "stop" or "go" signals depending on the circumstances. The second major theme to emerge is the apparent role of chemokines in ensuring the continued proliferation of progenitor cell populations, something that has been observed in the cerebellum, hippocampus, and spinal cord. In such cases, chemokines usually seem to play the role of facilitating the effects of another major mitogen such as SHH or PDGF. Thus, chemokines may act as generally important regulators of neural progenitor cell development—something that is clearly worth investigating further. It is an intriguing possibility that interference of some of the developmentally important functions of CXCR4 receptors by HIV-1 could contribute to its neuropathological effects.

CHEMOKINE RECEPTOR FUNCTION IN ADULT NEURONS

As we have discussed, many of the effects of chemokines that have been described in the developing nervous system are analogous to those observed in the developing hematopoietic/lymphopoietic system. According to some reports, chemokine receptors are also widely expressed in the adult nervous system by neurons, glia, and microglia. Data from several species, including rodents and primates, have demonstrated expression of many types of chemokine receptors by different subsets of mature neurons and glia under normal conditions (reviewed in Bajetto et al., 2002; Bajetto et al., 2001; Oh et al., 2001). Intriguingly, many of these receptors are up- or down-regulated under pathological conditions as well as in response to stressful stimuli (Bajetto et al., 2002; Bajetto et al., 2001; Oh et al., 2001). However, roles for chemokine receptor signaling in adult neurons have not been completely defined at this time, although the data so far suggests some strong possibilities.

GPCRs are extremely versatile molecules and can signal in a wide variety of modes (Pierce et al., 2002). Classically, receptor activation affords signaling through the subunits of heterotrimeric G protein. In addition, however, activation of GPCRs can also lead to signaling through several other pathways. For example, the recruitment of members of the family of β-arrestin scaffolding proteins to GPCRs can produce downstream activation of src, the MAP kinases as well as many other pathways that are associated with cell survival and differentiation. Indeed, as discussed above, the CXCR7 receptor may signal exclusively through this pathway (Rajagopal et al., 2010). The consequences of activating GPCRs in neurons have been extensively studied. It has been demonstrated that activation of heterotrimeric G protein can produce rapid effects on neuronal ion channels that result in changes in neuronal excitability and synaptic communication (Miller, 1998). In addition, activation of GPCRs can also produce long-term effects on the survival of neurons and other types of cells (e.g., Molina-Holgado et al., 2002; Yan, Lin, Irwin, & Paul, 1995; Vaudry et al., 2002). Evidence is accumulating that chemokines can produce both rapid and long-term effects on neurons through the direct activation of neuronally expressed chemokine receptors. For example, presynaptically localized GPCRs often function to reduce evoked transmitter release through the modulation of Ca and/or K channels, resulting in the subsequent inhibition of depolarization-induced Ca entry into nerve terminals (Miller, 1998). Experiments using heterologous expression systems have demonstrated that chemokine receptors can effectively signal to voltage-dependent Ca and K channels (Madani, Kozak, Kavanaugh, & Kabat, 1998; Oh et al., 2002). In keeping with these observations, activation of chemokine receptors expressed by neurons has been shown to alter Ca^{+2} influx as well as synaptic communication in the cerebellum (Ragozzino et al., 2002; Limatola et al., 2000; Ragozzino et al., 1998; Giovannelli et al., 1998), hippocampus (Meucci et al., 1998), and septum (Puma et al., 2001). Activation of CXCR4 receptors in cerebellar slices produces presynaptic inhibition of glutamate release at parallel fiber synapses (Ragozzino et al., 2002). In addition, activation of CXCR4, as well as several other types of chemokine receptors, has been shown to decrease excitatory transmission between hippocampal neurons in culture (Meucci et al., 1998) and activation of CXCR2 receptors can inhibit voltage-sensitive Ca^{+2} influx into acutely isolated septal neurons (Puma et al., 2001). Thus, as is the case with many other types of GPCRs, activation of presynaptically situated chemokine receptors may regulate transmitter release throughout the brain. Moreover, chemokines may also produce other types of electrophysiological effects resulting from activation of alternative signaling pathways. For example, activation of CXCR2 receptors in cerebellar slices produced Ca^{+2} mobilization, resulting in enhanced spontaneous transmitter release from granule and Purkinje cells (Ragozzino et al., 1998). In the peripheral nervous system, chemokine receptors have been shown to be expressed by sensory neurons, particularly small diameter nociceptors (Oh et al., 2001; Bolin et al., 1998). It was observed that activation of these receptors by chemokines

or gp120, the coat protein of HIV-1, produced powerful excitatory effects reminiscent of those produced by pain-producing agents such as bradykinin or capsaicin. Such effects could be of significance for our understanding of increased pain sensitivity associated with inflammation or with HIV-1 infection.

In addition to rapid regulation of neuronal excitability and synaptic transmission, there is also evidence that neuronal chemokine receptors can produce important effects on neuronal survival. Several reports have demonstrated the survival-promoting actions of chemokines on hippocampal and cerebellar granule neurons in culture (Meucci et al., 1998; Limatola et al., 2000; Araujo & Cotman, 1993; Meucci et al., 2000; Kaul et al., 1999; Tong et al., 2000), and it was observed that activation of neuronal chemokine receptors could inhibit neuronal death produced by a variety of pro-apoptotic stimuli. Furthermore, in keeping with the observation that different neurons express multiple types of chemokine receptors, several different types of chemokines seem to promote neuronal survival depending on the neuronal population under consideration. The signaling pathways through which chemokines enhance survival appear to involve activation of ERK and Akt (Meucci et al., 1998; Meucci et al., 2000; Lazarini et al., 2000; Xia et al., 2002; Xia et al., 2000), pathways that have frequently been linked to pro-survival responses. For example, it has been shown that the ability of fractalkine to enhance the survival of hippocampal neurons in culture depends on the activation of Akt (Meucci et al., 2000). However, it is interesting to note that in some reports SDF-1 actually produces neuronal death rather than increasing neuronal survival (Kaul & Lipton, 1999; Hesselgesser et al., 1998). Consistent with this, it has been shown that stimulation of CXCR4 receptors expressed by neuronal and non-neuronal cells can simultaneously produce activation of multiple members of the MAP kinase pathway, including those such as p38 that are associated with increased apoptosis (Kaul & Lipton, 1999; Vlahakis et al., 2002). Thus, the ultimate cellular response to activation of some chemokine receptors may depend on a balance between several different competing outputs—something that might change according to circumstances.

These data also raise questions about the circumstances under which the observed effects of chemokines might be physiologically relevant. For instance, what are the normal sources of chemokines that might activate these receptors and when is chemokine signaling normally utilized in the adult brain? Based on examination of the distribution of chemokine receptors and their potential ligands, it seems that, in addition to glia and microglia, neurons may also be the source of chemokines in some instances (e.g., Cowell & Silverstein, 2003; Xia et al., 2002; Harori, Nagai, Heisel, Ryu, & Kim, 2002; Schreiber et al., 2001; Meng, Oka, & Takashima, 1999). This can be illustrated by recent detailed reports on the distribution of SDF-1 and CXCR4 receptors in the brains of rodents and other species (Stumm et al., 2002; Banisadr et al., 2002; Cheng et al., 2000; Banisadr et al., 2000; Gleichmann et al., 2000; Tham et al., 2001; Van der Meer, Ulrich, Gonzalez-Scarano, & Lavi, 2000; Westmoreland et al., 2002), whereby SDF-1 is clearly expressed by the granule neurons of the dentate gyrus as well as neurons in the entorhinal cortex. In contrast, CXCR4 receptors are expressed by numerous neurons located in the lacunosum molecular layer of the hippocampus as well as in the hilar and molecular layers of the dentate gyrus. Based on what is known about the neuroanatomy of this part of the brain, synaptic contacts between SDF-1-expressing and CXCR4-expressing neurons are likely to occur. Whether SDF-1 is actually released from neurons and exactly what controls the secretion of chemokines in such cases is currently unknown. However, it has been reported that SDF-1 is tonically released from dentate granule neurons and that this downregulates the expression of CXCR4 receptors in this area of the brain (Kolodziej et al., 2008).

Neural stem cells in the subgranular zone of the dentate gyrus that participate in adult neurogenesis also express CXCR4 receptors (Bhattacharrya et al., 2008; Tran et al., 2007). SDF-1-expressing neurons and SDF-1-expressing endothelial cells in blood vessels are observed in close proximity to CXCR4-expressing neural stem cells, suggesting that SDF-1 might influence the development of these cells. It is known that the first synapses that contact developing neural stem cells are GABA mediated. The high Cl^- concentration found in neural stem cells means that activation of GABA-A receptors expressed by these cells results in an excitatory current due to the efflux of Cl^-. Prior to the establishment of *bona fide* synaptic connections, release of GABA from presynaptic neurons binds to high affinity GABA-A receptors to produce a tonic inward current. The presence of this current can be revealed by a GABA-A antagonist such as bicuculline, which blocks the tonic effects of GABA and therefore produces an outward current (Ge et al., 2007). Once GABAergic synapses are formed, GABA-mediated postsynaptic currents (PSCs) are also observed. When recording such GABA-mediated currents from DG neural stem cells, Bhattacharrya et al. (2008) observed that the effects of GABA were greatly enhanced by the addition of SDF-1. Like bicuculline, AMD3100 produced an outward current indicating that it contributed to the tonic inward current that can be observed in these cells. These data indicate that both GABA and SDF-1 are released in the DG and collaborate in producing excitatory inputs to neural stem cells. Thus, the neuroanatomical localization of SDF-1/CXCR4 together with the electrophysiological data indicate that, as in the original development of the DG discussed above, CXCR4 signaling is also important in the regulation of adult neurogenesis. However, the source of SDF-1 changes from the meninges (embryo/newborn) to DG interneurons (adult). Needless to say, the role of CXCR4 signaling we have demonstrated clearly indicates that adult neurogenesis may be a target for T-tropic HIV-1 strains in the brain.

In other circumstances, particularly in association with brain disease, it has been shown that chemokine synthesis and release can be upregulated by astrocytes or microglia (DeGroot & Woodroofe, 2001). Chemokines secreted from these cells might then act upon neuronal chemokine receptors and given the survival promoting effects of chemokines, it is possible that this might represent a survival strategy in the face of stressful stimuli.

CHEMOKINE-MEDIATED SIGNALING IN NEURONS

The effects produced by chemokines in the developing and adult nervous systems take place over vastly different time courses and presumably require the deployment of a wide range of signal transduction options. There are now several indications as to how this can be achieved. Interestingly, because of the versatility of chemokine receptors, it is possible that they can extend their signaling range through interactions with several other signaling systems. These signaling pathways may be blocked or inappropriately activated by HIV-1/gp120 leading to the resulting neuropathology.

As discussed above, all chemokine receptors are members of the extended family of GPCRs, and stimulation of these receptors will produce activated G protein subunits, which can then mediate different types of signaling. The vast majority of effects produced by chemokines in the hematopoietic/lymphopoietic system are blocked by pertussis toxin (PTX), indicating that chemokine receptors signal through activation of the Gi/o subfamily of Gprotein (Rossi & Zlotnik, 2000). In contrast, the PTX sensitivity of chemokine-mediated signaling in neurons is not always apparent (Gillard et al., 2001; Limatola et al., 2000; Xia et al., 2002). However, this may indicate that chemokine receptors in neurons sometimes signal through the non-PTX-sensitive Gz, which is highly expressed in neurons and is structurally related to Gi/o. Subsequently, activated G protein subunits may then mediate the rapid effects of chemokines on neuronal ion channels and synaptic communication. Furthermore, as with other GPCRs, chemokine receptors can also undergo cycles of desensitization, endocytosis, and resensitization in response to agonists (Cheng et al., 2000), and this phenomenon is associated with the binding of members of the arrestin family of scaffold proteins to the C terminal of the receptor (Perry & Lefkowitz, 2002). Binding of arrestins can then divert chemokine signaling into the MAP kinase pathway, something that might be important for the survival-promoting effects of chemokines, as discussed above. In addition, chemokine receptors may also exist as higher order structures, a phenomenon that appears to be common with GPCRs, and may even hetero-oligomerize with other closely related members of the chemokine receptor family (Rodriguez-Frade, Mellado, & Martinez, 2001; Issafras et al., 2002; Babcock, Farzan, & Sodroski, 2003). Indeed, one current model of chemokine receptor function suggests that receptor oligomerization is a *sine quae non* for all chemokine receptor signaling. It has also been suggested that chemokine receptors bind JAK kinases, which can then trans-phosphorylate tyrosine residues on chemokine receptor subunits following dimerization, in a manner similar to traditional cytokine receptors. As a result, these phosphotyrosines then act as docking sites for downstream signaling molecules including, in this case, a G protein (Rodriguez-Frade et al., 2001). In support of such an idea, a role for JAK signaling has been demonstrated in mediating certain effects of chemokines on leukocytes (Zhang et al., 2001). Presumably JAK-mediated signaling might also mediate some of the effects of chemokines in the nervous system.

What mechanisms might explain the different effects of chemokines observed during brain development? In both the cerebellum and dentate gyrus it has been shown that chemokines can produce proliferative as well as chemotactic effects (Klein et al., 2001; Lu et al., 2002; Bagri et al., 2002). However, it was observed that SDF-1 itself does not directly have proliferative effects on cerebellar granule cell progenitors, but rather it facilitates the effects of SHH (Klein et al., 2001). As it is well known that SHH signaling is inhibited by cAMP it has been suggested that inhibition of adenylate cyclase by SDF-1 could underlie the observed facilitation of SHH-induced proliferation. Subsequently, following an extended period of proliferation, postmitotic granule cell progenitors move to the inner aspect of the EGL and then migrate through the Purkinje cell layer to the IGL. The signals that terminate granule cell precursor proliferation and their SDF-1-mediated localization in the EGL are unknown, but since SDF-1 synthesis is maintained during this period, its influence must somehow be attenuated or overcome. According to one scenario, CXCR4 receptor expression by postmitotic granule cells is actually reduced (Reiss et al., 2002). However, two other interesting possibilities have also been suggested. One of these involves reverse signaling via ephrin-b, the ligand for the eph-b receptor. Both ephrin-b and its receptor are transmembrane-signaling molecules. Lu et al. (2001) demonstrated that expression of ephrin-b is upregulated in the EGL in the early postnatal period when granule cell migration is about to commence. Furthermore, these authors also showed that the cytoplasmic region of ephrin-b can bind a novel RGS (Regulator of Gprotein Signaling) protein, PDZ-RGS3, through interactions with its PDZ domain. Normally, RGS proteins act as GAP proteins that down-regulate GPCR signaling by accelerating the GTPase activity of the α-subunits of heterotrimeric G protein. In this case, it is possible that clustering or activation of ephrin-b by eph receptors would activate PDZ-RGS3, resulting in the termination of SDF-1-mediated signaling via CXCR4 receptors in the same cells. A further possibility is that GPCRs localized in the internal aspect of the EGL might antagonize SDF-1-mediated signaling. For example, it has been shown that receptors for pituitary adenylate cyclase activating protein (PACAP), a widely expressed neuropeptide, are localized postnatally in the EGL (Nicot et al., 2002). Activation of these receptors is linked to Gs as well as increases in cAMP. Given the known ability of cAMP to antagonize SHH-mediated signaling, these effects would oppose the faciliatory actions of SDF-1. Moreover, PACAP receptor activation also produces transactivation of TrkB neurotrophin receptors (Lee, Rajagopal, Kim, Chang, & Chao, 2002). Interestingly, TrkB receptors are not only localized on pre-migratory granule cells but it was observed that their activation by the neurotrophin BDNF may be a major chemotactic influence on granule cell migration to the IGL (Borghesani et al., 2002). Thus, PACAP receptor activation could simultaneously down-regulate SDF-1 signaling as it up-regulates BDNF signaling, two effects that would prime granule cell precursors for inward migration. In summary, therefore, there are a number of ways in which SDF-1/CXCR4 signaling might produce diverse effects on granule

cell development through interactions with other signaling pathways.

Further unique influences on chemokine-mediated signaling that may be important during development have recently been demonstrated. Several families of molecules that act as axon guidance cues have been defined, including molecules of the Slit and netrin families. Typically Slit, acting via its receptor roundabout (Robo), acts as an axonal repellent (Brose & Tessier-Lavigne, 2000). It has recently been shown that Slit/Robo are also expressed by leukocytes (Wu et al., 2001), whereby activation of Robo-opposed SDF-1-mediated leukocyte chemotaxis. Although this type of interaction has not yet been demonstrated in the nervous system, it seems likely that it would occur, since Slit/Robo and SDF-1/CXCR4 are certainly both expressed in similar regions of the developing nervous system. Furthermore, because the interaction between Slit and SDF-1 can be recapitulated in a heterologous expression system, this clearly indicates that the interaction between these two factors is not cell specific.

As discussed above, the recent identifications of a new receptor for SDF-1 named CXCR7 has suggested that SDF-1 signaling may have another dimension (Thelen & Thelen, 2008). However, it has been difficult to understand the signaling significance of SDF-1 binding to CXCR7. Reports have indicated that no detectable signaling occurs and that CXCR7 may just function as a "decoy" receptor or that CXCR7 may function through the formation of hetero-oligomers with CXCR4. However, SDF-1 does produce CXCR7 endocytosis and recent reports suggest that this may be coupled to activation of the β-arrestin signaling pathway. CXCR7 receptors are highly expressed by neurons and blood vessels in the developing and adult brains and so may play several important roles in mediating chemokine signaling in the nervous system.

HIV-1 ASSOCIATED NEUROPATHOLOGY

Considering the widespread expression of CXCR4 and other chemokine receptors in the nervous system, we should now consider how these are important in the neuropathogenesis of HAD. One important question is to define which population of cells in the brain is the most important in terms of their interactions with HIV-1/gp120. First we should consider the receptors that are expressed by neurons themselves. It is certainly clear that purified gp120 can be neurotoxic in vivo and in vitro (Corasaniti et al., 2001; Meucci & Miller, 1996). Interpretation of this data has not been entirely straightforward, however, as it is not clear whether gp120 can be directly toxic to neurons, or whether this can only occur in the context of other types of cells such as microglia and or astrocytes (Bezzi et al., 2001; Kaul et al., 2001). Another complication concerns the effects of the unique CXCR4 ligand SDF-1 on neurons. Here some reports suggest that SDF-1 has a survival-promoting effect on neurons, as do other chemokines (Meucci et al., 1998), whereas other reports suggest that SDF-1 has a uniquely pro-apoptotic effect (Hesselgesser et al., 1998). At any rate one possibility is that gp120 might interact directly with CXCR4 receptors expressed by neurons to produce neurotoxicity. One way this might happen is if gp120 acts to antagonize the survival-promoting effects of SDF-1 through competition for the same binding site. Alternatively, it appears that under some circumstances gp120 can not only bind to chemokine receptors but also elicit signaling through them (Davis et al., 1997). It is possible, therefore, that aberrant signaling elicited in this way might also be pro-apoptotic. As discussed above it has been demonstrated that activation of the CXCR4 receptor by SDF-1 may also result in pro- or anti-apoptotic signaling through p38 or ERK activation, respectively (Vlahakis et al., 2002). Thus, it is conceivable that the overall balance between these diverse outputs may determine the extent of gp120 or SDF-1-induced neuronal death or survival. In keeping with this possibility, blockers of different members of the MAP kinase pathway have been shown to ameliorate gp120-induced toxicity. For example, blockade of signaling via the JNK pathway inhibits gp120-induced neurotoxicity of cultured hippocampal neurons (Bodner et al., 2002). Inhibition of the enzyme MLK3, which is an upstream activator of JNK, blocks gp120-induced apoptosis of hippocampal neurons in culture, raising the possibility that this might be a therapeutic possibility in HAD. Activation of neuronal chemokine receptors aside from CXCR4 may also be important in HAD. Chemokines that activated these receptors have been widely reported to have anti-apoptotic/survival-promoting effects. Indeed, activation of receptors such as CX3CR1 with fractalkine can inhibit gp120-induced apoptosis (Meucci et al., 2000). The signaling pathways that mediate these effects include activation of the Akt pathway, which has been widely associated with anti-apoptotic actions.

Although the direct effects of gp120/chemokines on neurons may be of importance in deciphering the molecular basis of HAD, the contribution to gp120 neurotoxicity of chemokine receptors expressed by glia or microglia has also been considered (Bezzi et al., 2001), and one model suggests that activation of glial CXCR4 receptors by gp120 causes them to release glutamate which might then produce excitotoxic effects. Alternatively, it has been suggested that gp120 blocks the uptake of glutamate by neurons and/or glia, something that would also promote excitotoxicity (Vesce et al., 1997). Indeed, considering the fact that receptors such as CXCR4 are expressed on all types of brain cells, it is quite possible that several cellular/molecular mechanisms may contribute to HAD.

In this context one should consider the effects of HIV-1 on cells in the brain such as microglia, astrocytes, and endothelial cells that result in the upregulated expression of multiple inflammatory cytokines and chemokines that may then compromise the function of the brain through their actions. Thus it is becoming quite clear that HIV-1 infection produces the increased expression of numerous chemokines by these cells which can alter the permeability of the blood brain barrier (BBB) resulting in the influx of numerous leukocytes. As these cells also express chemokines and their receptors, including CXCR4/CCR5 receptors, they are a secondary source of HIV-1 as well as inflammatory cytokines of various types. For example, it is clear that HIV-1-infected microglia can up-regulate their expression of the chemokine MCP-1/CCL2. This

chemokine can increase the permeability of the BBB and attract different classes of leukocytes into the brain. The precise mechanism through which HIV-1 produces up-regulated chemokine synthesis by microglia and other cell types is not known. However, in addition to binding to chemokine receptors through their envelope protein gp120, it should also be recognized that other HIV-1-associated proteins and nucleic acid may also activate receptors that are responsible for these effects. One should consider, for example, Toll-like receptors (e.g., TLR7) which act as part of the brain's innate immune response and can be activated by HIV-1(Heil et al., 2004). Hence, a scenario for the effects of HIV-1 in the CNS may be as follows: HIV-1 enters the brain early in infection by a Trojan horse mechanism within leukocytes involved in immune surveillance. HIV-1 infects microglia (and possibly astrocytes) via binding to chemokine receptors expressed by these cells. Infected cells produce a variety of chemokines and cytokines and shed HIV-1-related proteins. These cytokines and proteins then have a number of secondary effects on the BBB and other structures in the brain leading to the influx of further infected leukocytes. Infiltrating leukocytes may then produce neurotoxic, anti-neurogenic, and other effects due to their elaboration of inflammatory chemokines and cytokines.

Currently, the cellular and molecular bases for HIV-1-induced syndromes in the PNS are also unknown. These syndromes constitute a group of painful neuropathies and related problems that can be produced by the virus or by antiretroviral drugs used to treat HIV-1 infection. It is known that pain-transmitting small sensory nociceptors do express chemokine receptors and that activation of these receptors can produce excitation through a variety of mechanisms including the transactivation of TRP channels expressed by nociceptors (Oh et al., 2001). As with neurons in the CNS, long-term exposure of sensory neurons to gp120 also produces neuronal apoptosis (unpublished observations). Thus, it is quite possible that gp120 might produce HIV-1-related allodynia and sensory neuropathies through direct interactions with chemokine receptors expressed by these neurons. In addition, however, the effects of HIV-1 in the peripheral nervous system may involve a scenario similar to that described above for the CNS. Thus, HIV-1 infection may trigger the up-regulated expression of chemokines and their receptors in the dorsal root ganglion (DRG), peripheral nerve, and spinal cord. These chemokines may then produce pro-algesic effects leading to chronic pain behaviors. In particular, HIV-1-related proteins such as gp120 or NRTIs can produce the up-regulation of chemokine expression by DRG neurons, satellite glial cells, and endoneurial fibroblasts in the peripheral nerve. In association with these effects, chemokine receptor expression is upregulated by DRG neurons, including nociceptors. As described above, the direct effects of chemokines on DRG nociceptors is to produce excitation. Hence, up-regulated chemokine signaling in these nerves is inherently pro-algesic. Hence, there appears to be two main ways in which chemokine signaling may contribute to HIV-1-related pain syndromes. The first of these is by direct binding of gp120 to CXCR4 receptors expressed by DRG nociceptors and the second is by the up-regulation of chemokine and chemokine receptor expression by cells in the DRG and peripheral nerve, which may also result in increased excitatory chemokine signaling in nociceptors.

REFERENCES

Altman, J. & Bayer, S. A. (1990a). Mosaic organization of the hippocampal neuroepithelium and the multiple germinal sources of dentate granule cells. *J Comp Neurol*, *301*, 325–342.

Altman, J. & Bayer, S. A. (1990b). Migration and distribution of two populations of hippocampal granule cell precursors during the perinatal and postnatal periods. *J Comp Neurol*, *301*, 365–381.

Arujo, D. M. & Cotman, C. (1993). Trophic effects of interleukin-4, -7, and–8 on hippocampal neuronal cultures: Potential involvement of glial-derived factors. *Brain Res*, *600*, 49–55.

Babcock, G. J., Farzan, M., & Sodroski, J. (2003). Ligand-independent dimerization of CXCR4, a principal HIV-1 coreceptor. *J Biol Chem*, *278*, 3378–3383.

Bagri, A., Gurney, T., He, X., Zou, Y. R., Littman, D. R., Tessier-Lavigne, M., et al. (2002). The chemokine SDF-1 regulates migration of dentate granule cells. *Development*, *129*, 4249–4260.

Bajetto, A., Bonavia, R., Barbero, S., & Schettini, G. (2002). Characterization of chemokines and their receptors in the central nervous system: Physiopathological implications. *J Neurochem*, *82*, 1311–1329.

Bajetto, A., Bonavia, R., Barbero, S., Florio, T., & Schettini, G. (2001). Chemokines and their receptors in the central nervous system. *Front Neuroendocrinol*, *22*, 147–184.

Banisadr, G., Dicou, E., Berbar, T., Rostène, W., Lombet, A., Haour, F. (2000). Characterization and visualization of [125I] stromal cell-derived factor-1alpha binding to CXCR4 receptors in rat brain and human neuroblastoma cells. *J Neuroimmunol*, *2*;110(1–2):151–60.

Banisadr, G., Fontanges, P., Haour, F., Kitabgi, P., Rostene, W. & Parsadaniantz, S. M. (2002). Neuroanatomical distribution of CXCR4 in adult rat brain and its localization in cholinergic and dopaminergic neurons. *Eur J Neurosci*, *16*, 1661–1671.

Berger, E. A., Murphy, P. M., & Farber, J. M. (1999). Chemokine receptors as HIV-1 receptors: Roles in viral entry, tropism, and disease. *Ann Rev Immunol*, *17*, 657–700.

Bezzi, P., Domercq, M., Brambilla, L., Galli, R., Schols, D., De Clercq, E., et al. (2001). CXCR4-activated astrocyte glutamate release via TNFalpha: Amplification by microglia triggers neurotoxicity. *Nat Neurosci*, *4*, 702–10.

Bhattacharyya, B. J., Banisadr, G., Jung, H., Ren, D., Cronshaw, D. G., Zou, Y., et al. (2008). The chemokine stromal cell-derived factor-1 regulates GABAergic inputs to neural progenitors in the postnatal dentate gyrus. *J Neurosci*, *28*, 6720–30.

Bleul, C., Schultze, J. L., & Springer, T. A. (1998). B lymphocyte chemotaxis regulated in association with microanatomic localization, differentiation state and B cell receptor engagement. *J Exp Med*, *187*, 753–762.

Bodner, A., Maroney, A. C., Finn, J. P., Ghadge, G., Roos, R., & Miller, R. J. (2002). Mixed lineage kinase 3 mediates gp120IIIB-induced neurotoxicity. *J Neurochem*, *82*, 1424–34.

Bolin, L. M., Murray, R., Lukacs, N. W., Strieter, R. M., Kunkel, S. L., Schall, T. J. et al. (1998). Primary sensory neurons migrate in response to the chemokine RANTES. *J Neuroimmunol*, *81*, 49–57.

Borghesani, P. R., Peyrin, J. M., Klein, R., Rubin, J., Carter, A. R., Schwartz, P. M., et al. (2002). BDNF stimulates migration of cerebellar granule cells. *Development*, *129*, 1435–42.

Borrell V. & Marín O. (2006). Meninges control tangential migration of hem-derived Cajal-Retzius cells via CXCL12/CXCR4 signaling. *Nat Neurosci*, *9*, 1284–93.

Braun, M., Wunderlin, M., Spieth, K., Knochel, W., Gierschik, P., & Moepps, B. (2002). Xenopus laevis Stromal cell-derived factor 1:

Conservation of structure and function during vertebrate development. *J Immunol*, *168*, 2340–7.
Brose, K. & Tessier-Lavigne, M. (2000). Slit proteins: Key regulators of axon guidance, axonal branching and cell migration. *Curr Opinion Neurobiol*, *10*, 95–102.
Cheng, Z. J., Zhao, J., Sun, Y., Hu, W., Wu, Y. L., Cen, B., et al. (2000). Beta-arrestin differentially regulates the chemokine receptor CXCR4-mediated signaling and receptor internalization, and this implicates multiple interaction sites between β-arrestin and CXCR4. *J Biol Chem*, *275*, 2479–85.
Corasaniti, M. T., Maccarrone, M., Nistico, R., Malorni, W., Rotiroti, D., & Bagetta, G. (2001). Exploitation of the HIV-1 coat glycoprotein, gp120, in neurodegenerative studies in vivo. *J Neurochem*, *79*, 1–8.
Cowell, R. M. & Silverstein, F. S. (2003). Developmental changes in the expression of chemokine receptor CCR1 in the rat cerebellum. *J Comp Neurol*, *457*, 7–23.
Davis, C. B., Dikic, I., Unutmaz, D., Hill, C. M., Arthos, J., Siani, M.A., et al. (1997). Signal transduction due to HIV-1 envelope interactions with chemokine receptors CXCR4 or CCR5. *J Exp Med*, *186*, 1793–8.
DeGroot, C. J. A. & Woodroofe, M. N. (2001). The role of chemokines and chemokine receptors in CNS inflammation. *Prog in Brain Res*, *132*, 533–544.
Doitsidou, M., Reichman-Fried, M., Stebler, J., Koprunner, M., Dorries, J., Meyer, D., et al. (2002). Guidance of primordial germ cell migration by the chemokine SDF-1. *Cell*, *111*, 647–659.
Gabuzda, D. & Wang, J. (2000). Chemokine receptors and mechanisms of cell death in HIV neuropathogenesis. *J Neurovirol*, *6* Suppl 1, S24–32.
Garden, G. A. (2002). Microglia in human immunodeficiency virus associated neurodegeneration. *Glia*, *40*, 240–251.
Ge, S., Pradhan, D. A., Ming, G. L., & Song, H. (2007). GABA sets the tempo for activity-dependent adult neurogenesis. *Trends Neurosci*, *30*, 1–8.
Gillard, S. E., Mastracci, R. M., & Miller, R. J. (2001). Expression of functional chemokine receptors by cerebellar neurons. *J Neuroimmunol*, *124*, 16–28.
Giovannelli, A., Limatola, C., Ragozzino, D., Mileo, A. M., Ruggieri, A., Ciotti, M. T., et al. (1998). CXC chemokines interleukin-8 (IL-8) and growth-related gene product alpha (GRO-α) modulate Purkinje neuron activity in mouse cerebellum. *J Neuroimmunol*, *92*, 122–32.
Gleichmann, M., Gillen, C., Czardybon, M., Bosse, F., Greiner-Petter, R., Auer, J. et al. (2000). Cloning and characterization of SDF-1γ, a novel SDF-1 chemokine transcript with developmentally regulated expression in the nervous system. *Eur J Neurosci*, *12*, 1857–1866.
Gorry, P. R., Bristol, G., Zack. J. A., Ritola, K., Swanstrom, R., Birch, C. J., et al. (2001). Macrophage tropism of human immunodeficiency virus type 1 isolates from brain and lymphoid tissues predicts neurotropism independent of coreceptor specificity. *J Virol*, *75*, 10073–89.
Gould, E. & Gross, C. G. (2002). Neurogenesis in adult mammals: Some progress and problems. *J Neurosci*, *22*, 619–23.
Harori, K., Nagai, A., Heisel, R., Ryu, J. K. & Kim, S. U. (2002). Fractalkine and fractalkine receptors in human neurons and glial cells. *J Neurosci Res*, *69*, 418–426.
Hartmann, D., Schulze, M., & Sievers, J. (1998). Meningeal cells stimulate and direct the migration of cerebellar external granule cells in vitro. *J Neurocytol*, *27*, 395–409.
Hartmann, D., De Strooper, B., & Saftig, P. (1999). Presenilin-1 deficiency leads to loss of Cajal-Retzius neurons and cortical dysplasia similar to human type 2 lissencephaly. *Current Biol*, *9*,719–727.
Heil, F., Hemmi, H., Hochrein, H., Ampenberger, F., Kirschning, C., Akira, S., et al. (2004). Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. *Science*, *303*, 1526–9.
Hesselgesser, J., Taub, D., Baskar, P., Greenberg, M., Hoxie, J., Kolson, D. L., et al. (1998). Neuronal apoptosis induced by HIV-1 gp120 and the chemokine SDF-1α is mediated by the chemokine receptor CXCR4. *Curr Biol*, *8*, 595–8.
Horuk, R., Martin, A. W., Wang, Z., Schweitzer, L., Gerassimides, A., Guo, H., et al. (1997). Expression of chemokine receptors by subsets of neurons in the central nervous system. *J Immunol*, *158*, 2882–2890.
Issafras, H., Angers, S., Bulenger, S., Blanpain, C., Parmentier, M., Labbe-Jullie, C., et al. (2002). Constitutive agonist-independent CCR5 oligomerization and antibody-mediated clustering occurring at physiological levels of receptors. *J Biol Chem*, *277*, 34666–73.
Jazin, E. E., Soderstrom, S., Ebendal, T., & Larhammer, D. (1997). Embryonic expression of the mRNA for the rat homologue of the fusin/CXCR4 HIV-1 co-receptor. *J Neuroimmunol*, *79*, 148–154.
Kaul, M. & Lipton, S. A. (1999). Chemokines and activated macrophages in HIV-1 and gp120-induced neuronal apoptosis. *Proc Natl Acad Sci*, *96*, 8212–6.
Kaul, M., Garden, G. A., & Lipton, S. A. (2001). Pathways to neuronal injury and apoptosis in HIV associated dementia. *Nature*, *410*, 988–994.
Klein, R. S., Rubin, J. B., Gibson, H. D., DeHaan, E. N., Alvarez-Hernandez, X., Segal, R. A. et al. (2001). SDF-1α induces chemotaxis and enhances sonic hedgehog induced proliferation of cerebellar granule cells. *Development*, *128*, 1971–1981.
Knaut, H., Werz, C., Gelser, R., & Nusslein-Volhard, C. (2002). A zebrafish homologue of the chemokine receptor CXCR4 is a germ-cell guidance receptor. *Nature*, published online Dec 18, 2002.
Kolodziej, A., Schulz, S., Guyon, A., Wu, D. F., Pfeiffer, M., Odemis, V., et al. (2008). Tonic activation of CXC chemokine receptor 4 in immature granule cells supports neurogenesis in the adult dentate gyrus. *J Neurosci*, *28*, 4488–500.
Kolson, D.L. & Gonzalez-Scarano, F. (2001). HIV-associated neuropathies: Role of HIV-1, CMV, and other viruses. *J Peripher Nerv Syst*, *6*, 2–7.
Kury, P., Greiner-Petter, R., Cornely, C., Jurgens, T., & Muller, H. W. (2002). Mammalian Achaete Scute homolog 2 is expressed in the adult sciatic nerve and regulates the expression of Krox 24, Mob-1, CXCR4 and p57kip2 in Schwann cells. *J Neurosci*, *22*, 7586–7595.
Lavi, E., Strizki, J. M., Ulrich, A. M., Zhang, W., Fu, L., Wang, Q., et al. (1997). CXCR4 (Fusin), a co-receptor for the type I human immunodeficiency virus (HIV-1), is expressed in the human brain in a variety of cell types, including microglia and neurons. *Am J Pathol*, *151*, 1035–1042.
Lazarini, F., Casanova, P., Tham, T. N., De Clercq, E., Arenzana-Seisdedos, F., Baleux, F. et al. (2000). Differential signaling of the chemokine receptor CXCR4 by stromal cell-derived factor 1 and the HIV glycoprotein in rat neurons and astrocytes. *Eur J Neurosci*, *12*, 117–25.
Lee, F. S., Rajagopal, R., Kim, A. H., Chang, P. C., & Chao, M. V. (2002). Activation of Trk neurotrophin receptor signaling by pituitary adenylate cyclase-activating polypeptides. *J Biol Chem*, *277*, 9096–102.
Li, G., Adesnik, H., Li, J., Long, J., Nicoll, R. A., Rubenstein, J. L., et al. (2008). Regional distribution of cortical interneurons and development of inhibitory tone are regulated by Cxcl12/Cxcr4 signaling. *J Neurosci*, *28*, 1085–1098.
Limatola, C., Giovannelli, A., Maggi, L., Ragozzino, D., Castellani, L., Ciotti, M. T., et al. (2000). SDF-1α-mediated modulation of synaptic transmission in rat cerebellum. *Eur J Neurosci*, *12*, 2497–504.
Limatola, C., Ciotti, M. T., Mercanti, D., Vacca, F., Ragozzino, D., Giovannelli, A., et al. (2000). The chemokine growth-related gene product β protects rat cerebellar granule cells from apoptotic cell death through α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptors. *Proc Natl Acad Sci USA*, *97*, 6197–201.
Lopez-Bendito, G., Sanchez-Alcaniz, J. A., Pla, R., Borrell, V., Pico, E., Valdeolmillos, M., et al. (2008). Chemokine signaling controls intracortical migration and final distribution of GABAergic interneurons. *J Neurosci*, *28*, 1613–1624.
Lu, Q., Sun, E. E., Klein, R. S. & Flanagan, J. G. (2001). Ephrin-B reverse signaling is mediated by a novel PDZ-RGS protein and selectively inhibits G protein coupled chemoattraction. *Cell*, *105*, 69–79.
Lu, M., Grove, E. A., & Miller, R. J. (2002). Abnormal development of the hippocampal dentate gyrus in mice lacking the CXCR4 chemokine receptor. *Proc Natl Acad Sci*, *99*, 7090–7095.
Luan, J., Furuta, Y., Du, J., & Richmond, A. (2001). Developmental expression of two CXC chemokines, MIP-2 and KC, and their receptors. *Cytokine*, *14*, 253–63.
Ma, Q., Jones, D., Borghesani, P. R., Segal, R. A., Nagasawa, T, Kishimoto, T., et al. (1998). Impaired B lymphopoiesis, myelopoiesis, and derailed

cerebellar neuronal migration in CXCR4 and SDF-1 deficient mice. *Proc Natl Acad Sci*, *95*, 9448–9453.

Ma, Q., Jones, D., & Springer, T. A. (1999). The chemokine receptor CXCR4 is required for the retention of B lineage and granulocytic precursors within the bone marrow microenvironment. *Immunity*, *10*, 463–471.

Madani, N., Kozak, S. L., Kavanaugh, M. P., & Kabat, D. (1998). gp120 envelope glycoproteins of human immunodeficiency viruses competitively antagonize signaling by coreceptors CXCR4 and CCR5. *Proc Nat'l Acad Sci USA*, *95*, 8005–8010.

Martin-Garcia, J., Kolson, D. L., & Gozalez-Scarano, F. (2002). Chemokine receptors in the brain: Their role in HIV infection and pathogenesis. *AIDS*, *16*, 1709–1730.

McGrath, K. E., Koniski, A. D., Maltby, K. M., McGann, J. K., & Palis, J. (1999). Embryonic expression and function of the chemokine SDF-1 and its receptor, CXCR4. *Dev Biol*, *213*, 442–56.

Meng, S. Z., Oka, A., & Takashima, S. (1999). Development expression of monocyte chemoattractant protein–1 in the human cerebellum and brainstem. *Brain Dev*, *21*, 30–35.

Meucci, O. & Miller, R. J. (1996). gp120-induced neurotoxicity in hippocampal pyramidal neuron cultures: Protective action of TGF-β1. *J Neurosci*, *16*, 4080–8.

Meucci, O., Fatatis, A., Simen, A., Bushell, T. J., Gray, P. W., & Miller, R. J. (1998). Chemokines regulate hippocampal neuronal signaling and gp120 neurotoxicity. *Proc Natl Acad Sci*, *95*, 14500–14505.

Meucci, O., Fatatis, A., Simen, A. A., & Miller, R. J. (2000). Expression of CX3CR1 chemokine receptors on neurons and their role in neuronal survival. *Proc Natl Acad Sci*, *97*, 8075–80.

Miller, R. J. (1998). Presynaptic receptors. *Ann Rev of Pharmacol and Toxicol*, *38*, 201–227.

Moepps, B., Frodl, R., Rodewald, H. R., Bagglioni, M., & Gierschik, P. (1997). Two murine homologues of the human chemokine receptor CXCR4 mediating stromal cell derived factor–1-alpha activation of Gi2 are differentially expressed in vivo. *Eur J Immunol*, *27*, 2102–2112.

Moepps, B., Braun, M., Knopfle, K., Dillinger, K., Knochel, W., & Gierschik, P. (2000). Characterization of a Xenopus laevis CXC chemokine receptor 4: Implications for hematopoietic cell development in the vertebrate embryo. *Eur J Immunol*, *30*, 2924–34.

Molina-Holgado, E., Vela, J. M., Arevalo-Martin, A., Almazan, G., Molina-Holgado, F., Borrell, J. et al. (2002). Cannabinoids promote oligodendrocyte progenitor survival: Involvement of cannabinoid receptors and phosphatidylinositol-3 kinase/Akt signaling. *J. Neurosci*, *22*, 9742–53.

Murphy, P. M., Baggiolini, M., Charo, I. F., Hebert, C. A., Horuk, R., Matsushima, K., et al. (2000). International union of pharmacology. XXII. Nomenclature: Chemokine receptors. *Pharmacol. Review*, *52*, 145–76.

Murphy, P. M. (2002). International Union of Pharmacology. Update on chemokine receptor nomenclature. *Pharmacol. Review*, *54*, 227–9.

Nagasawa, T., Hirota, S., Tachibana, K., Takakura, N., Nishikawa, S., Kitamura, Y., et al. (1996). Defects of B-cell lymphopoiesis and bone marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. *Nature*, *382*, 635–8.

Nath, A, (2002). Human immunodeficiency virus (HIV) proteins in the neuropathogenesis of HIV dementia. *J Infect Dis*, *186* (suppl2), S193–198.

Nicot, A., Lelievre, V., Tam, J., Waschek, J. A., & DiCicco-Bloom, E. (2002). Pituitary adenylate cyclase activating polypeptide and sonic hedgehog interact to control cerebellar granule precursor cell proliferation. *J Neurosci*, *22*, 9244–9254.

Oh, S. B., Tran, P. B., Gillard, S. E., Hurley, R. W. Hammond, D. L., & Miller, R. J. (2001). Chemokines and gp120 produce pain hypersensitivity by directly exciting polymodal nociceptors. *J Neurosci*, *21*, 5027–5035.

Oh, S. B., Endoh, T., Simen, A. A., Ren, D., & Miller, R. J. (2002). Regulation of calcium currents by chemokines and their receptors. *J Neuroimmunol*, *123*, 66–75.

Paredes, M. F., Li, G., Berger, O., Baraban, S. C., & Pleasure, S. J. (2006). Stromalderived factor-1 (CXCL12) regulates laminar position of Cajal-Retzius cells in normal and dysplastic brains. *J Neurosci*, *26*, 9404–9412.

Perry, S. J. & Lefkowitz, R. J. (2002). Arresting developments in heptahelical receptor signaling and regulation. *Trends Cell Biol*, *12*, 130–8.

Proudfoot, A. E. I. (2002). Chemokine receptors: Multifaceted therapeutic targets. *Nature Reviews Immunol*, *2*, 106–115.

Proudfoot, A. E. I., Shaw, J. P., Power, C. A., & Wells, T. N. C. (2002). Chemokines. In R. M. Ransohoff, K. Suzuki, A. E. I. Proudfoot, W. F. Hickey, & J. K. Harrison, (Ed.). *Universes in delicate balance: Chemokines and the nervous system* (p. 65–85). Philadelphia; Elsevier.

Puma, C., Danik, M., Quirion, R., Ramon, F., & Williams, S. (2001). The chemokine interleukin-8 acutely reduces Ca(2+) currents in identified cholinergic septal neurons expressing CXCR1 and CXCR2 receptor mRNAs. *J Neurochem*, *78*, 960–71.

Rajagopal, S., Kim, J., Ahn, S., Craig, S., Lam, C. M., Gerard, N. P., et al. (2010). Beta-arrestin- but not G protein-mediated signaling by the "decoy" receptor CXCR7. *Proc Natl Acad Sci USA*, *107*, 628–32.

Ragozzino, D., Giovannelli, A., Mileo, A. M., Limatola, C., Santoni, A., & Eusebi, F. (1998). Modulation of the neurotransmitter release in rat cerebellar neurons by GRO -β. *Neuroreport*, *9*, 3601–6.

Ragozzino, D., Renzi, M., Giovannelli, A., & Eusebi, F. (2002). Stimulation of chemokine CXC receptor 4 induces synaptic depression of evoked parallel fibers inputs onto Purkinje neurons in mouse cerebellum. *Neuroimmunol*, *127*, 30–6.

Reiss, K., Mentlein, R., Sievers, J., & Hartmann, D. (2002). Stromal cell derived factor 1 is secreted by meningeal cells and acts as chemotactic factor on neuronal stem cells of the cerebellar external granule layer. *Neurosci*, *115*, 295–305.

Robinson, S., Tani, M., Streiter, R. M., Ransohoff, R. M., & Miller, R. H. (1998). The chemokine growth related oncogene–α promotes spinal cord precursor proliferation. *J Neurosci*, *18*, 10457–10463.

Rodriguez-Frade, J. M., Mellado, M., & Martinez, A. C. (2001). Chemokine receptor dimerization: Two are better than one. *Trends in Immunol*, *22*, 612–7.

Rossi, D. & Zlotnik, A. (2000). The biology of chemokines and their receptors. *Ann Rev Immunol*, *18*, 217–242.

Schreiber, R. C., Krivacic, K., Kirby, B., Vaccariello, S. A., Wei, T., Ransohoff, R. M., Zigmond, R. E. (2001). Monocyte chemoattractant protein (MCP)-1 is rapidly expressed by sympathetic ganglion neurons following axonal injury. *Neuroreport*, *5*;12(3):601–6.

Schwartz, G. N. & Farber, J. M. (2002). Development and function of the hematopoietic-lymphopoietic system. In R. M. Ransohoff, K. Suzuki, A. E. I. Proudfoot, W. F. Hickey, & J. K. Harrison, (Ed.). *Universes in delicate balance: Chemokines and the nervous system* (p. 65–85). Philadelphia; Elsevier.

Schönemeier, B., Kolodziej, A., Schulz, S., Jacobs, S., Hoellt, V., & Stumm, R. (2008). Regional and cellular localization of the CXCl12/SDF-1 chemokine receptor CXCR7 in the developing and adult rat brain. *J Comp Neurol*, *510*, 207–20.

Sievers, J., Hartmann, D., Pehlemann, F. W., & Berry, M. (1992). Development of astroglial cells in the proliferative matrices, the granule cell layer and the hippocampal fissure of the hamster dentate gyrus. *J Comp Neurol*, *320*, 1–32.

Sierro, F., Biben, C., Martínez-Muñoz, L., Mellado, M., Ransohoff, R. M., Li, M., et al. (2007). Disrupted cardiac development but normal hematopoiesis in mice deficient in the second CXCL12/SDF-1 receptor, CXCR7. *Proc Natl Acad Sci USA*, *104*, 14759–64.

Stumm, R. K., Rummel, J., Junker, V., Culmsee, C., Pfeiffer, M., Krieglstein, J., et al. (2002). A dual role for the SDF-1/CXCR4 chemokine receptor system in adult brain: Isoform selective regulation of SDF-1 expression modulates CXCR4 dependent neuronal plasticity and cerebral leukocyte recruitment after focal ischemia *J Neurosci*, *22*, 5865–5878.

Stumm, R. K., Zhou, C., Ara, T., Lazarini, F., Dubois-Dalcq, M., Nagasawa, T., et al. (2003). CXCR4 regulates interneuron migration in the developing neocortex. *J Neurosci*, *23*, 5123–5130.

Szekanecz, Z. & Koch, A. E. (2001). Chemokines and angiogenesis. *Current Opinion Rheumatol*, *13*, 202–8.

Tachibana, K., Hirota, S., Iizasa, H., Yoshida, H., Kawabata, K., Kataoka, Y., et al. (1998). The chemokine receptor CXCR4 is essential for vascularization of the gastrointestinal tract. *Nature*, *393*, 591–4.

Tham, T. N., Lazarini, F., Francheschini, I. A., Lachapelle, F., Amara, A., & Dubois-Dalcq, M. (2001). Developmental pattern of expression of the alpha chemokine stromal cell derived factor 1 in the rat central nervous system. *Eur J Neurosci*, *13*, 845–856.

Thelen, M. & Thelen, S. (2008). CXCR7, CXCR4, and CXCL12: An eccentric trio? *J Neuroimmunol*, *198*, 9–13.

Tiveron, M. C., Rossel, M., Moepps, B., Zhang, Y. L., Seidenfaden, R., Favor, J., et al. (2006). Molecular interaction between projection neuron precursors and invading interneurons via stromal-derived factor 1 (CXCL12)/CXCR4 signaling in the cortical subventricular zone/intermediate zone. *J Neurosci*, *26*, 13273–13278.

Toggas, S. M., Masliah, E., Rockenstein, E. M., Rall, G. F., Abraham, C. R., & Mucke L. (1994). Central nervous system damage produced by expression of the HIV-1 coat protein gp120 in transgenic mice. *Nature*, *367*, 188–93.

Tong, N., Perry, S. W., Zhang, Q., James, H. J., Guo, H., Brooks, A., et al. (2000). Neuronal fractalkine expression in HIV-1 encephalitis: Roles for macrophage recruitment and neuroprotection in the central nervous system. *J Immunol*, *164*, 1333–9.

Tran, P. B., Ren, D., Chenn, A., & Miller, R. J. (2007). Chemokine receptor expression by neural progenitor cells in neurogenic regions of mouse brain. *J Comp Neurol*, *500*, 1007–33.

Tsai, H. H., Frost, E., To, V., Robinson, S., French-Constant, C., Geertman, R., et al. (2002). The chemokine receptor CXCR2 controls positioning of oligodendrocyte precursors in developing spinal cord by arresting their migration. *Cell*, *110*, 373–383.

Van der Meer, P., Ulrich, A. M., Gonzalez-Scarano, F., & Lavi, E. (2000). Immunohistochemical analysis of CCR2, CCR3, CCR5 and CXCR4 in the human brain: Potential mechanisms for HIV dementia. *Exp and Mol Pathol*, *69*,192–201.

Vaudry, D., Pamantung, T. F., Basille, M., Rousselle, C., Fournier, A. Vaudry, H., et al. (2002). PACAP protects cerebellar granule neurons against oxidative stress induced apoptosis. *Eur J Neurosci*, *15*, 1451–60.

Vlahakis, S. R., Villasis-Keever, A., Gomez, T., Vanegas, M., Vlahakis, N. and Paya, C. V. (2002). Gprotein coupled chemokine receptors induce both survival and apoptotic signaling pathways. *J Immunol*, *169*, 5546–5554.

Vesce, S., Bezzi, P., Rossi, D., Meldolesi, J., & Volterra, A. (1997). HIV-1 gp120 glycoprotein affects the astrocyte control of extracellular glutamate by both inhibiting the uptake and stimulating the release of the amino acid. *FEBS Lett*, *411*, 107–9.

Westmoreland, S. V., Alvarez, X., deBakker, C., Aye, P., Wilson, M. L., Williams, K. C., et al. (2002). Developmental expression of CCR5 and CXCR4 in the rhesus macaque brain. *J Neuroimmunol*, *122*, 146–158.

Williams, K. C. & Hickey, W. F. (2002). Central nervous system damage, monocytes and macrophages,and neurological disorders in AIDS. *Ann Rev Neurosci*, 2537–562.

Wu, Q., Miller, R. H., Ransohoff, R. M., Robinson, S., & Nishayama, A. (2000). Elevated levels of the chemokine GRO-1 correlate with elevated oligodendrocyte progenitor proliferation in the jimpy mutant. *J Neurosci*, *20*, 2609–2617.

Wu, J. Y, Feng, L., Park, H. T., Havlioglu, N., Wen, L., Tang, H., et al. (2001). The neuronal repellent Slit inhibits leukocyte chemotaxis induced by chemotactic factors. *Nature*, *410*, 948–952.

Xia, M. & Hyman, B. T. (2002). GROalpha/KC, a chemokine receptor CXCR2 ligand, can be a potent trigger for neuronal ERK1/2 and PI-3 kinase pathways and for tau hyperphosphorylation-a role in Alzheimer's disease? *J Neuroimmunol*, *122*, 55–64.

Xia, M. Q., Bacskai, B. J., Knowles, R. B., Qin, S. X., & Hyman, B. T. (2000). Expression of the chemokine receptor CXCR3 on neurons and the elevated expression of its ligand IP-10 in reactive astrocytes: In vitro ERK1/2 activation and role in Alzheimer's disease. *J Neuroimmunol*, *108*, 227–35.

Xiang, Y., Li, Y., Zhang, Z., Cui, K., Wang, S., Yuan, X., et al. (2002). Nerve growth cone guidance mediated by Gprotein coupled receptors. *Nature Neurosci*, *5*, 843–848.

Yan, G. M., Lin, S. Z., Irwin, R. P., & Paul, S. M. (1995). Activation of muscarinic cholinergic receptors blocks apoptosis of cultured cerebellar granule neurons. *Mol Pharmacol*, *47*, 248–57.

Zhang, X. F., Wang, J. F., Matczak, E., Proper, J. A., & Groopman, J. E. (2001). Janus kinase 2 is involved in stromal cell-derived factor-1alpha-induced tyrosine phosphorylation of focal adhesion proteins and migration of hematopoietic progenitor cells. *Blood*, *97*, 3342–8.

Zhu, Y., Yu, T., Zhang, X. C., Nagasawa, T., Wu, J. Y., & Rao, Y. (2002). Role of the chemokine SDF-1 as the meningeal attractant for embryonic cerebellar neurons. *Nature Neurosci*, *5*, 719–720.

Zou, Y. R., Kottmann, A. H., Kuroda, M., Taniuchi, I., & Littman, D. R. (1998). Function of chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. *Nature*, *393*, 595–599.

1.4

VIRAL AND HOST GENETIC FACTORS

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Neurological disorders caused by HIV-1 infection are common and diverse. In the present chapter we highlight both host and viral genetic determinants of HIV-induced neuropathogenesis in the central and peripheral nervous systems. The key properties of neuropathogenesis including neuroinvasion, neurotropism, neurovirulence and neurosusceptibility are used as guidelines to the discussion.

INTRODUCTION

Human immunodeficiency virus type 1 (HIV-1) causes a broad spectrum of neurological disorders that constitute a substantial source of morbidity and mortality. HIV-1 infects cells within the central nervous system (CNS) soon after primary infection (Davis et al., 1992; Sasseville & Lackner, 1997; Poli et al., 1990) although not all individuals with HIV/AIDS develop clinically detectable neurological disease (Power & Johnson, 2001). This raises the question of why some individuals develop clinically apparent neurological disease while others do not.

In this chapter, we will discuss both host and viral genetic factors that contribute to HIV neuropathogenesis. HIV neuropathogenesis can be subdivided into stages including *neuroinvasion* (entry of the virus into nervous system tissues and cells without multiplication of the virus), *neurotropism* (infection of cells of the nervous system), and *neurovirulence* (ability to cause neurological dysfunction). In the case of the CNS, neurovirulence has been applied chiefly to impaired neurocognitive performance including HIV-associated dementia (HAD) and its often antecedent disorder, minor neurocognitive disorder (MND); more recent studies group these two conditions under the neuropsychological term, HIV-associated neurocognitive disorders (HAND). In the peripheral nervous system (PNS), the occurrence of distal sensory polyneuropathy (DSP) is the chief neurological disorder assumed to be caused directly by HIV infection with or without exacerbation by specific antiretroviral drugs. We will outline various viral and host factors which contribute to each stage of neuropathogenesis. It can be difficult to separate viral from host pathogenic factors as they interact, but ultimately neural cell loss or dysfunction, presumably caused by a combination of toxic viral proteins and host inflammatory molecules, are usually the convergent end points (Rumbaugh & Nath, 2006).

Other non-genetic host factors also affect an individual's *neurosusceptibility* (Patrick, Johnston, & Power, 2002), including age; immune status; availability and compliance with medications; concurrent infections including hepatitis C; illicit drug use; and nutritional status (reviewed in Jayadev & Garden, 2009); however, we will not discuss these latter factors in this chapter. There is also increasing evidence that drug selection may contribute to the development of neurological disease (Cysique & Brew, 2009); this latter issue requires further study. Herein, we focus on the genetic determinants of the host and virus which contribute to the development of neurological disease.

HOST DETERMINANTS OF HIV-1 NEUROPATHOGENESIS

Neuropathogenesis is influenced by several host factors which both increase and decrease neurosusceptibility. We will discuss how host genetic differences have been demonstrated to contribute to neuroinvasion, neurotropism, and neurovirulence. In addition, we will briefly review how gender and ethnicity might contribute to genetic differences observed. Finally, we will examine host genetic factors that contribute to the response to and side effects of antiretroviral drugs, as they relate to neurological disease and the development of immune reconstitution inflammatory syndrome (IRIS) and opportunistic infections of the nervous system. Important host genes and genetic polymorphisms in HIV neuropathogenesis are summarized in table 1.4.1.

NEUROINVASION

There are five possible mechanisms by which HIV might enter the brain (first four reviewed in Buckner et al., 2006). The first mechanism involves infected macrophages and lymphocytes entering the CNS with subsequent spread of virus to resident cells. This process involves leukocyte recruitment and adhesion, with subsequent migration across the blood-brain barrier, and has been termed the *Trojan horse* mechanism (Peluso, Haase, Stowring, Edwards, & Ventura, 1985). These processes are mediated by adhesion molecules found on leukocytes and the endothelial cells of the blood-brain barrier. There is evidence of upregulation of adhesion molecules in patients with HIV infection and associated neurological disease (Heidenreich, Arendt, Jander, Jablonowski,

& Stoll, 1994; Nottet et al., 1996). There are also reports of increased expression of monocyte chemoattractants expressed in the HIV-infected brain (reviewed in Dunfee et al., 2006); however, the precise mechanisms by which they ultimately contribute to the development of neurological disease have yet to be elucidated.

The second potential mechanism by which HIV enters the CNS involves direct infection of the cells of the blood-brain barrier by cell-free HIV (Buckner et al., 2006). Although endothelial cells do not express the primary HIV-1 receptor, CD4, it appears that HIV interacts with endothelial cell surface proteoglycans through gp120 (Bobardt et al., 2004; Argyris et al., 2003), although this mechanism has not been convincingly demonstrated in vivo to date. The third mechanism involves endocytosis of the HIV virion by endothelial cells and/or astrocytic foot processes (Buckner et al., 2006). A fourth mechanism involves cell-free HIV entering the brain directly by passing through a disrupted blood-brain barrier. There are many studies supporting disruption of the blood-brain barrier during HIV infection (reviewed in Toborek et al., 2005), lending credibility to the notion that circulating free virus might access the brain via a more permeable blood-brain barrier. Finally, HIV infection of the choroid plexus might also serve as a portal of entry for the virus into the brain (Falangola, Hanly, Galvao-Castro, & Petito, 1995; Petito et al., 1999). Again, it remains unclear which, if any, host genetic factors contribute to these mechanisms.

NEUROTROPISM

In terms of host factors, neurotropism is determined by individual cell types in the CNS permissive to viral entry and replication as well as expression of individual receptor molecules that mediate cellular entry by the virus. The cells productively infected by HIV-1 in the CNS are of macrophage and microglial (myeloid) lineage. Astrocytes are also infected by HIV-1, resulting in the expression of viral proteins with minimal replication of the viral genome (Saito et al., 1994; Gorry et al., 1999; Messam & Major, 2000; Neumann et al., 1995; Churchill et al., 2009). Although there is significant neuronal loss and injury, neurons per se are not infected to any extent, as determined by viral genome and antigen detection. There is no compelling evidence for in vivo infection of oligodendrocytes or brain endothelial cells (reviewed in Gonzalez-Scarano & Martin-Garcia 2005).

The HIV-1 envelope glycoprotein is responsible for viral binding and entry into the cell. Apart from using CD4 as the primary receptor, HIV-1 also requires chemokine receptors such as CCR5, CXCR4, CCR2b, and CCR3 to act as co-receptors (reviewed in Berger et al., 1999; Choe et al., 1996; Doranz et al., 1996). Cells of macrophage and microglial lineage express CCR5 and CCR3, together with CD4 (He et al., 1997; Bagasra et al., 1996; Nuovo, Gallery, MacConnell, & Braum, 1994). Microglia and invading macrophages are considered the principal reservoir for active viral replication in the brain (Clements & Zink, 1996; Narayan, Joag, & Stephens, 1995). Astrocytes express both CXCR4 and CCR5 but not CD4, limiting infection in these cell types (Saito et al., 1994; Gorry et al., 1999; Messam & Major, 2000; Neumann et al., 1995).

Several chemokine receptor polymorphisms have been described that correlate with differences in HIV-1 disease progression and survival prognosis (reviewed in Piacentini, Biasin, Fenizia, & Clerici, 2009). The most familiar of these is the Δ32 variant of the CCR5 co-receptor. Homozygotes for this allele exhibit a significantly diminished susceptibility to HIV-1 infection (Samson et al., 1996; Liu et al., 1996). Heterozygous individuals demonstrate delayed progression of disease (Dean et al., 1996; Meyer et al., 1997; Stewart et al., 1997). Heterozygotes have also been noted to be less represented among patients with neurological disease (Boven, van der Bruggen, van Asbeck, Marx, & Nottet, 1999; van Rij et al., 1999, Singh et al., 2003). Allelic variants in the CCR5 promoter region as well as other co-receptor variants have also been described and are associated with variable progression (reviewed in Piacentini et al., 2009). There is some suggestion that the CCR5–59353 variant found in the promoter region may confer protection in terms of neurological impairment (Singh et al., 2003).

In addition to selective infection of specific cell types, there also appear to be specific regions within the brain that are differentially permissive to infection; white matter and subcortical grey matter are apparently more permissive to infection, perhaps due to the increased abundance of myeloid cells in these regions. It has also been reported that viral envelope sequences cluster together by brain region based on phylogenetic analyses (Chang et al., 1998; Shapshak et al., 1999). Based on pathological studies, the basal ganglia and hippocampus appear to be preferentially affected in terms of neuronal loss (Masliah, Ge, Achim, Hansen, & Wiley, 1992; Reyes et al., 1991) with proportionally more gp41 immunoreactivity detected in these same regions (Kure et al., 1991). Other brain regions including cerebral cortex and brainstem are affected to varying degrees (Everall et al., 1999; Wiley et al., 1991), which may reflect the relative abundance of susceptible resident cells including perivascular macrophages and microglia in a given anatomical site.

NEUROVIRULENCE

There are multiple host characteristics correlated with the occurrence and severity of HIV-associated neurological disease. These include specific host niche genes and variants thereof. Socio-demographic aspects and co-morbidities, although not necessarily shown to be causative, likely also modulate the severity of HIV-1 neurovirulence.

Host Inflammatory Response

Inflammation and immune activation are pivotal contributors to HIV neurovirulence (reviewed in Gonzalez-Scarano & Martin-Garcia 2005). In fact, activated macrophages and multinucleated giant cells are among the as the most robust pathological correlates of HIV neurocognitive impairment (Glass, Fedor, Wesselingh, & McArthur, 1995). Inflammatory indices associated with HIV neurological disease include

pro-inflammatory cytokines and chemokines, such as interleukin-1β (Williams & Hickey, 2002; Gartner, 2000; Tyor et al., 1992), tumor necrosis factor-α (Sippy, Hofman, Wallach, & Hinton, 1995; Achim, Heyes, & Wiley, 1993; Wesselingh et al., 1993; Gelbard et al., 1994), interferon-α (Sas et al., 2009), normal T-cell expressed and secreted (RANTES)/CCL5 (Kelder et al., 1998), and CCL2 (monocyte chemoattractant protein) (Monteiro de Almeida, 2006). Many of these important mediators of inflammation are upregulated in the brains or cerebrospinal fluid (CSF) of individuals with HIV-associated dementia. Proteases are another group of molecules contributing to HIV neurovirulence, as exemplified by the conversion of the chemokine CXCL12 to a highly neurotoxic protein through its proteolytic cleavage by matrix metalloproteinase (MMP)-2 (Zhang et al., 2003; Vergote et al., 2006). However, very recent data from our group indicate host-encoded microRNAs are selectively suppressed in the brains of HIV/AIDS patients (Noorbakhsh et al., 2010). This mechanism and many of the above, however, have not been definitively associated with individual genetic polymorphisms. Nonetheless, genetic polymorphisms resulting in gene induction or upregulation have been clearly documented for the chemokines CCL2 (Gonzalez et al., 2002), CCL5–403A, -28G (Guerini et al., 2008; Kelder et al., 1998; Letendre, Lanier, & McCutchan, 1999), CXCL12–3'A (Winkler et al., 1998; Singh et al., 2003), and the cytokine, tumor necrosis factor (TNF)-α (Quasney et al., 2001, Pemberton, Stone, Price, van Bockxmeer, & Brew, 2008); these will be discussed in further detail below. Most of the host genes described above are principally induced in monocyte/macrophage cells, and perhaps, astrocytes, but the contributions of different T lymphocyte subsets to neurovirulence remain largely unexplored. However, it is during HIV infection that different populations of T cells infiltrate the brain and dorsal root ganglia and in some instances exert pathogenic effects (Wesselingh et al., 1993; Zhu et al., 2005).

TUMOR NECROSIS FACTOR-A

TNF-α is upregulated in the brains and CSF of patients with HIV-associated dementia, and increased TNF-α mRNA levels in HAD patients is correlated with dementia severity (Achim et al., 1993; Wesselingh et al., 1993; Gelbard et al., 1994; Tyor et al., 1992). The genetic polymorphism TNF-α-308A has been observed more frequently among HAD patients (Quasney et al., 2001). Located in the promoter region, this polymorphism causes increased TNF-α production. TNF-α can cause direct neuronal damage and induces quinolinic acid production (Pemberton et al., 1997), which likewise causes neuronal damage and has been found in higher concentrations in patients with HAD (Achim et al., 1993).

MONOCYTE CHEMOATTRACTANT PROTEIN-1/CCL2

Increased CCL2 expression has been correlated with an increased risk of accelerated systemic disease progression and the development of HAD. The 2578G variant is associated with increased CCL2 production, presumably resulting in increased infiltration of monocytes (Gonzales et al., 2002). Interestingly, homozygotes for the 2578G allele have a decreased risk of acquiring HIV; however, following infection with HIV-1, accelerated disease progression occurs as described above (Gonzales et al., 2002).

NORMAL T-CELL EXPRESSED AND SECRETED (RANTES)/CCL5–403A, -28G

The CCL5–28G mutation has been shown to increase CCL5 expression in HIV-infected individuals, which reduces CD4+ T lymphocyte depletion rates, thus delaying the progression of disease (Liu et al., 1999). Despite this effect, neurocognitive impairment has been associated with higher levels of CCL5 (Letendre et al., 1999). It has been speculated that CCL5 may stimulate HIV-replication in the CSF (Letendre et al., 1999). Furthermore, an increased rate of the CCL5–403 G/A polymorphism has been documented in patients with "non-determined leukoencephalopathy" compared to HIV seropositive controls (see below for details) (Guerini et al., 2008).

CXCL12/STROMAL CELL-DERIVED FACTOR-1

The chemokine CXCL12/SDF-1 is the cognate ligand for CXCR4, and has been reported to exhibit a genetic polymorphism which contributes to disease progression (Winkler et al., 1998, van Rij et al., 1998). The polymorphism CXCL12–3'A has been shown to both delay and accelerate the progression to AIDS (Winkler et al., 1998, van Rij et al., 1998). In addition, it has also been shown to increase perinatal transmission of HIV-1 (John et al., 2000). In a meta-analysis, however, no significant difference between homozygotes, heterozygotes, and wild-type was noted (Ioannidis et al., 2003). In a subsequent study, CXCL12–3'A was found to be associated with more rapid disease progression, and increased development of neurocognitive impairment in children (Singh et al., 2003).

APOLIPOPROTEIN (APO) E

ApoE functions in cholesterol transport (Mahley & Rall Jr., 2000), and is produced in the CNS by glia, macrophages, and neurons (Boyles, Pitas, Wilson, Mahley, & Taylor, 1985; Pitas, Boyles, Lee, Hui, & Weisgraber, 1987; Han et al., 1994). The role of ApoE in the pathogenesis of HIV-associated dementia remains unclear; studies report conflicting results. It has been reported that HIV-infected patients carrying an ApoE-ε4 allele have a higher frequency of neurological disease (Corder et al., 1998). HAD patients with the ApoE-ε4 allele have also been shown to have dysregulated sterol and lipid metabolism (Cutler et al., 2004). Other studies, however, have found no statistically significant difference between the presence of any ApoE alleles and HIV dementia (Dunlop et al., 1997; Pemberton et al., 2008; Diaz-Arrastia, Gong, Kelly, & Gelman, 2004).

High Throughput Analyses of Host Genes

With the advent of high throughput technologies, including genomics and proteomics, several host genes have been highlighted in HIV neuropathogenesis using brain tissues and cerebrospinal fluid. A seminal study of brain transcriptomics in HIV/AIDS patients with and without HAD revealed substantial dysregulation of ion channel expression that was, in some cases, directly linked to glutamate receptors (Gelman et al., 2004). Similarly, transcriptomic analyses of HIV-1 *tat*-transfected cells, using a brain-derived Tat sequence from a person with HAD, disclosed induction of an enzyme mediating heparan sulphate synthesis, HS3ST3B1, which was also found to be increased in the brains of patients with HAD (Boven et al., 2007). Similarly, transcriptomic analyses of astrocytes transduced by HIV-1 revealed upregulation of interferon-mediated antiviral responses (OAS1, IFIT1), intercellular contacts (SH3, glia-derived nexin), cell homing/adhesion (matrix metalloproteinases), and cell-cell signaling (neuropilin 1 and 2) (Borjabad, Brooks, & Volsky, 2010). Proteomic studies have reported multiple results including increased heat shock protein 70, heme oxygenase-1, and inducible nitric oxide synthase in HIV *tat*-transfected cells (Pocernich, Sultana, Mohmmad-Abdul, Nath, & Butterfield, 2005). Similarly, diverse findings have been reported for proteomic analyses including monocytes derived from patients with HIV infection (Luo et al., 2003). Very recently, we reported that microRNA profiling in brains from persons with and without HIV infection revealed that microRNAs targeting caspase 6 were markedly suppressed in HIV-infected brains (Noorbakhsh et al., 2010). Other high throughput technologies including deep sequencing will reveal new aspects of gene expression and regulation contributing to neurovirulence in HIV infection but will require analyses based on substantial bioinformatics capacity and application of a systems biology approach (Noorbakhsh et al., 2009).

Gender

Gender differences in HIV-1 seropositive patients have been described in association with drug abuse, with a more frequent history of drug abuse in females compared to males (Mason et al., 1998; Metsch et al., 1998); socioeconomic status and education, with lower levels noted in females (Ickovics & Rodin, 1992; Stern, Silva, Chaisson, & Evans, 1996); psychiatric co-morbidities, with greater psychiatric disease described in female patients (Melnick et al., 1994); as well as mortality rates, with higher rates described in females (Melnick et al., 1994). Studies examining differences in neuropsychological profiles, however, have shown conflicting results. Several studies have identified differences in neuropsychological performance (Failde-Garrido, Alvarez, & Simon-Lopez, 2008), while others have not (Tozzi et al., 2007; Pereda et al., 2000; Rabkin et al., 2000). Failde-Garrido et al. (2008) demonstrated different impairment patterns between genders, with greater deficits in visual memory, attention, psychomotor speed, and abstract reasoning noted in men while women displayed greater deficits in attention, psychomotor speed, and verbal memory.

In another study investigating a variety of neurologic syndromes in HIV-seropositive patients, Lopez et al. did not find differences in the presence of these neurological syndromes in females compared to males (Lopez et al., 1999). Similarly, a prospective study found no difference in the development of neurological symptoms and signs over time in females compared to males (Robertson et al., 2004).

Ethnicity

Many of the aforementioned genetic polymorphisms associated with varying degrees of neurological disease in HIV are found at varying frequencies worldwide (Voevodin, Samilchuk, & Dashti, 1998; Su et al., 1999; Martinson et al., 1997; Su et al., 2000; Martinson et al., 2000). For example, the CCR5-Δ32 allele is frequently found in European populations but is rare in the Middle East, Asia, and Africa (Voevodin et al., 1998; Martinson et al., 1997). Numerous studies looking at specific allele frequencies such as CCR5-Δ32, CCL2–2518GG, and TNFα-308 in particular ethnic groups demonstrate variable frequencies of genetic polymorphisms (Pemberton et al., 2008; Parczewski et al., 2009; Vilades et al., 2007).

HLA Subtypes

Multiple studies have demonstrated an association between HLA subtypes and susceptibility to HIV-1 infection and immune control of the virus (de Sorrentino et al., 2000; Fabio et al., 1990; Goulder & Watkins, 2008; MacDonald et al., 2000; Telenti, 2005). Genetic polymorphisms in the MHC region may account for as much as 15% of the variability seen in indices of immune control such as viral load (Fellay et al., 2007). It is reasonable to assume that improved immune control might lead to a reduced incidence of neurological disease in these patients. Interestingly, it has been found that HLA-DQB*0402 and DRB1*08 alleles are associated with a higher likelihood of developing toxoplasmic encephalitis in the setting of HIV (de Sorrentino et al., 2000). In addition, our clinical observations imply that HIV seropositive individuals of aboriginal (North American Indian) origin do not develop neurological disease as often as Caucasians or other ethnic groups (Power and Gill, unpublished); this may reflect differences in HLA background, as reported for the same ethnic groups who develop the neuromyelitis phenotype of multiple sclerosis (Mirsattari et al., 2001).

Response to Antiretroviral Therapy

Host genetic polymorphisms have been suggested as contributors to the efficacy of selected antiretrovirals in addition to their side-effects. In terms of efficacy, genetic differences in drug transport, metabolism, and clearance have all been described (reviewed in Phillips & Mallal, 2008). An intensively

Table 1.4.1 IMPORTANT HOST GENES AND GENETIC POLYMORPHISMS IN HIV NEUROPATHOGENESIS

GENETIC POLYMORPHISM	EFFECT	REFERENCES
	Neurotropism	
Δ32 variant of the CCR5 co-receptor	Diminished neurological disease	Boven et al., 1999; van Rij et al., 1999, Singh et al., 2003
CCR5–59353 variant (in promoter region)	Protection from neurological impairment	Singh et al., 2003
	Neurovirulence	
TNF-α-308A	Observed more frequently among HAD patients	Quasney et al., 2001
2578G variant of CCL2	Correlated with the development of HAD	Gonzales et al., 2002
CCL5–28G mutation	Associated with neurocognitive impairment	Letendre et al., 1999
CCL5–403 G/A polymorphism	Non-determined leukoencephalopathy	Guerini et al., 2008
CXCL12–3'A polymorphism	Increased development of neurocognitive impairment in children	Singh et al., 2003
	Other	
ApoE-ε4 allele	Conflicting results	Corder et al., 1998; Dunlop et al., 1997; Pemberton et al., 2008; Diaz-Arrastia et al., 2004
HLA-DQB*0402 and DRB1*08 alleles	Higher likelihood of developing toxoplasmic encephalitis in the setting of HIV	de Sorrentino et al., 2000
Mitochondrial 4917G polymorphism of haplotype T	Higher incidence of peripheral neuropathy in NRTI-exposed patients	Hulgan et al., 2005; Canter et al., 2008
TNF-α-1031*2	Higher incidence of peripheral neuropathy in NRTI-exposed patients	Cherry et al., 2008
IL-12B (3_ UTR)*2	Lower incidence of peripheral neuropathy in NRTI-exposed patients	Cherry et al., 2008
Polymorphism in the gene encoding for the hepatic enzyme CYP2B6	Greater incidence of neurological side-effects with Efavirenz	Haas et al., 2004
HLA-B44 and HLA-A2, B44 DR4	More common in patients who experience neuroIRIS in the form of CMV retinitis and/or encephalomyelitis	Price et al., 2001

studied example of this phenomenon is the P-glycoprotein drug transporter in which genetic polymorphisms are known to affect the bioavailability of protease inhibitors and may also influence viral replication (reviewed in Owen, Chandler, & Back, 2005). In addition, many of the genetic polymorphisms mentioned previously in this chapter have also been studied in patients receiving antiretroviral therapy (Hendrickson et al., 2008). In particular, it has been observed that the polymorphisms CCR5-Δ32, CX3CR1-V249I, and CCL5 haplotype H1 all decreased time to viral suppression, whereas the CCR5 P1 haplotype, CXCL12–3'A, and two CCCL5 haplotypes were associated with delayed viral suppression (Hendrickson et al., 2008). It is conceivable these polymorphisms might also lead to variable outcomes in the development of neurological disease.

Multiple studies have demonstrated how genetic polymorphisms contribute to the development of neurological side effects associated with antiretroviral therapies. Nucleoside reverse transcriptase inhibitors (NRTIs) were a frequent cause of distal sensory polyneuropathy in HIV-seropositive patients although recently these drugs have been used less frequently as a result of this off-target effect. The putative mechanism involves mitochondrial injury resulting in peripheral neuropathy (Cui, Locatelli, Xie, & Sommadossi, 1997; Dalakas, Semino-Mora, & Leon-Monzon, 2001, Zhu et al., 2007). One group has demonstrated that the mitochondrial haplotype T is associated with a higher incidence of peripheral neuropathy in NRTI-exposed patients (Hulgan et al., 2005). This same group has demonstrated that it is the mitochondrial 4917G polymorphism of haplotype T which is associated with peripheral neuropathy (Canter et al., 2008). In addition, it has been demonstrated that specific genetic polymorphisms in inflammatory cytokines are also associated with increased and decreased incidence of peripheral neuropathy, suggesting a role for inflammation in the pathophysiology of NRTI-associated peripheral neuropathy (Cherry et al., 2008). Specifically, TNF-α-1031*2 was positively associated with the development of neuropathy while IL-12B (3_ UTR)*2 appeared to be protective.

Efavirenz, a non-nucleoside reverse transcriptase inhibitor, is commonly associated with neurological side effects including dizziness, concentration difficulties, somnolence or difficulty sleeping, and vividly abnormal dreams (Marzolini et al., 2001). A polymorphism in the gene encoding for the hepatic enzyme CYP2B6 has been associated with a greater incidence of neurological side effects (Haas et al., 2004), and this genotype has also been associated with a higher plasma level of the drug suggesting that the increased side effects represent a dose-response effect. Interestingly, in this study, all of the patients developed tolerance to these side effects over time.

Differences in the development of hyperlipidemia and hypertriglyceridemia have also been described among HIV seropositive patients receiving protease inhibitors (Chang et al., 2009; Tarr et al., 2005; Fauvel et al., 2001; Foulkes et al., 2006). Dyslipidemia contributes to cardiovascular disease and increased risk of cardiovascular disease in patients with HIV (reviewed in Anuurad, Semrad, & Berglund, 2009).

Immune Reconstitution Inflammatory Syndrome

There is evidence that genetic factors might participate in the development of neurological immune reconstitution inflammatory syndrome (neuroIRIS). One study has shown that HLA-B44 and HLA-A2, B44 DR4 are more common in patients who experience neuroIRIS in the form of CMV retinitis and/or encephalomyelitis (Price, Keane, Stone, Cheong, & French, 2001). This same group has demonstrated that IRIS appears to be associated with polymorphisms in the TNF-α, IL-12B, and IL-6 genes (Price et al., 2002). Another study examined the frequency of genetic polymorphisms observed in the CCR5, CCL5, CCR2, and CXL12 genes among patients with HIV-related progressive multifocal leukoencephalopathy (PML) or an entity termed "non-determined leukoencephalopathy" (Guerini et al., 2008). Guerini et al.'s term, "non-determined leukoencephalopathy," describes a PML-like leukoencephalopathy, which occurs after initiation of antiretroviral therapy in the absence of detectable JC virus in the CSF, and resembles, in many ways, neuroIRIS. In the latter study, the investigators reported no differences in the distribution of the mutations of CCR5, CCR2, or CXCL in the populations studied; however, they did observe a statistically significant increased rate of the CCL5–403 G/A polymorphism in the non-determined leukoencephalopathy group compared with HIV seropositive controls with neurological disease.

VIRUS-ENCODED DETERMINANTS OF HIV-1 NEUROPATHOGENESIS

In addition to differences in host factors, neuropathogenesis is also affected by a variety of viral factors which contribute to neuroinvasion, neurotropism, and neurovirulence. We will discuss how different viral proteins and genetic polymorphisms thereof contribute to differences in these aspects of neuropathogenesis. We will also address these issues in direct relation to clade differences and in the relative occurrence of shared genetic differences between clades.

NEUROINVASION AND NEUROTROPISM

Several mechanisms of neuroinvasion have been highlighted above although the principal route of viral entry into the CNS remains unclear. For example, a low CD4 T cell nadir, indicative of marked immunosuppression in the peripheral circulation at the time of HIV-1 seroconversion, confers a greater risk of developing subsequent neurological disease. By inference, HIV-1 strains, which replicate efficiently in blood as a quasi-species by causing early immunosuppression, are more likely to enter the nervous system and eventually cause disease, as suggested by studies of simian immunodeficiency virus (SIV) infections (van Marle & Power, 2005). What viral factors determine the relative success in terms of brain entry remain obscure to date; although, it is evident most viral strains entering the CNS are macrophage-tropic while both macrophage- and dual-tropic strains are present in the PNS (Jones et al., 2005). The brain and peripheral nerve microenvironments are distinct from the peripheral circulation in terms of immunological surveillance, cytokine milieu, and target cell characteristics. This uniqueness in the CNS environment can contribute to the variation of viral genotypic characteristics. Analysis of viral isolates from different brain regions of HIV-infected patients revealed the predominance of CCR5 co-receptor usage for entry and macrophage tropism (Albright et al., 1999; Gorry et al., 2001; Ohagen et al., 2003; Power et al., 1998). The different viral genotypes residing in diverse areas of the brain combined with their differential tropism and co-receptor use suggest that neurotropic variants exist that may be governing the neurological manifestations of HIV disease in infected patients (Smit et al., 2001). In this section, the role of different viral proteins and their genetic variations related to neurotropism will be discussed (Table 1.4.2).

Viral Differences in Receptor Usage

As previously discussed, in the nervous system, productive HIV infection is limited to macrophages and microglia. The CD4 molecule expressed by microglial cells binds to the viral envelope glycoprotein, gp120, and initiates viral entry. This binding induces a conformational change and allows subsequent interaction with a chemokine receptor, usually CCR5 or CXCR4, leading to fusion of viral and target cell membrane (Deng et al., 1996; Dragic et al., 1996; Wu et al., 1996). The viral strains that are macrophage-tropic (R5) tend to use the CCR5 co-receptor while the CXCR4 co-receptor is used principally by the lymphotropic (X4) virus strains. There are some viral strains that are dual-tropic (X4R5) and infect both the macrophages and lymphocytes. In fact, dual-tropic strains have been described in both the peripheral and central nervous systems (Choe et al., 1996). Several viruses also engage different sets of co-receptors, including CCR3 and CCR2b (Choe et al., 1996). CCR5 is chiefly used as the co-receptor in the CNS, and macrophage tropism is essential for CNS HIV infection (Albright et al., 1999; Gorry et al., 2001; Ohagen et al., 2003;

Power et al., 1998). Macrophages and microglia express CCR5 and CCR3 together with lower levels of CD4 compared with CD4+ T cells (Bannert, Schenten, Craig, & Sodroski, 2000). Several brain-derived HIV strains have been identified in patients with HAD that replicate efficiently in macrophages and microglia yet have reduced dependence on CD4 and CCR5 receptors (Gorry et al., 2002). Reduced CD4 viral dependence on CD4 has been associated with increased macrophage tropism (Thomas et al., 2007).

Env

The *env* gene of the HIV genome encodes the viral envelope protein gp160, which is subsequently cleaved into two proteins: gp120 and gp41. During HIV infection, the envelope protein gp120 binds to the CD4 receptor on susceptible cells resulting in viral entry (Wyatt & Sodroski, 1998). The protein gp120 is detectable in the autopsied brains of HIV-infected patients (Jones, Bell, & Nath, 2000). It can disrupt blood-brain barrier integrity and enhance monocyte migration, thereby enhancing viral entry into the brain (Kanmogne et al., 2007). Mutations in gp120, especially in the hypervariable V3, V4, and V5 domains, are distinct in the brain compared to other tissues (Dunfee et al., 2007; Shimizu, Shimizu, Takeuchi, & Hoshino, 1994; Gartner et al., 1997). Analysis of V3 region sequences of the *env* gene from the brains of HIV-infected patients with and without dementia showed predominant CCR5 use. Given that macrophages are the chief cells infected in the CNS, the prevalence of CCR5 use in brain-derived HIV strains from patients with and without dementia may have important clinical implications (Shah et al., 2006). Phylogenetic analyses of full length viral sequences isolated from blood plasma and CSF of infected individuals showed clustering of sequences by compartment (Pond et al., 2008). Mutations in positions 5, 9, 13, and 19 of the V3 loop have been demonstrated as signatures for brain-derived HIV strains. Particularly, proline at position 13 is the most significant marker for compartmentalized HIV strain (in contrast to histidine in HIV strains found in the periphery) (Power et al., 1994; Pillai et al., 2006), even though this substitution is not thought to influence macrophage tropism (Thomas et al., 2007). Moreover, the V4 and V5 regions appear to be important for neurotropism (Gartner et al., 1997). On the other hand, a variant in the CD4-binding site of HIV gp120, Asn 283 (N283), has been identified at high frequency in the brain from patients with HAD. N283 increases gp120's affinity for CD4, enhancing the capacity of the HIV envelope to use low levels of CD4 and increasing viral replication in macrophages and microglia (Dunfee et al., 2006). The envelope from a neurovirulent SIV strain interacts with CCR5 in a CD4-independent manner similar to that of the neurotropic HIV-1 strain (Bonavia, Bullock, Gisselman, Margulies, & Clements, 2005). Additionally, there is significant sequence diversity in the V1-V2 loop among demented and nondemented groups in brain-derived *env* sequences showing distinct phylogenetic clustering (Power et al., 1994; Power et al., 1998).

In the peripheral nervous system, analysis of the C2V3 nucleotide sequences of the *env* gene from patients with and without sensory neuropathy showed no distinct clustering. The charge and amino acid composition of the V3 loop isolated from the peripheral nervous system suggests some CCR5 dependence as opposed to seemingly exclusive CCR5 dependence as observed in the CNS (Jones et al., 2005).

Nef

Nef is a 27KDa accessory HIV-1 protein synthesized early in the viral cycle. It interacts with various host proteins and plays a key role in CD4 downregulation and viral infectivity (reviewed in Foster & Garcia, 2008). Although it is not essential for viral infection and replication, it has been observed that *nef*-deleted strains of HIV have lower infectivity (Messmer, Ignatius, Santisteban, Steinman, & Pope, 2000). In the CNS, *nef* mRNA and protein has been detected in the astrocytes of brain autopsies of HIV-infected individuals (Saito et al., 1994; Tornatore, Chandra, Berger, & Major, 1994). In vitro infection of microglia cells infected with *nef*-expressing viral strains induced higher p24 levels compared with *nef*-null strains, indicating its role in infecting the macrophage (Si et al., 2002). *Nef* also increases blood-brain barrier permeability (Sporer et al., 2000) and recruits leukocytes into the CNS (Koedel et al., 1999), perhaps resulting in increased viral entry. Sequence analysis of *nef* reveals that the gene is more conserved in the brains of patients with dementia, possibly as a consequence of positive selection pressure(s) (van Marle et al., 2004).

LTR

The long terminal repeats (LTRs) within the HIV genome are important for viral replication, playing an important role in viral integration and acting as promoters/enhancers. The LTR contains three regions: U5, R, and U3; the U3 region of the promoter is comprised of the promoter, enhancer, and modulatory regions. The LTR relies on various viral and host transcription factors for its activity. These host factors include NFκB, Sp, ATF/CREB, and members of the C/EBP (Kilareski, Shah, Nonnemacher, & Wigdahl, 2009). Of interest, Nef/LTR-deleted SIV strains fail to infect the CNS because it has diminished neurotropism or inefficient viral entry (Thompson et al., 2003). The LTR is essential for HIV infection in the CNS; diversity in its sequence has been reported within and between different regions of the brain and the majority of these variations is located in the region upstream from the NF-kβ sites (Kilareski et al., 2009).

LTR has three C/EBP binding sites upstream of the transcriptional start site that play a crucial role in viral transcription in macrophage-monocyte cells. Brain-derived LTRs possess a 6G configuration (where T is substituted by G at position 6) at C/EBP site I. This mutation is present mainly in the mid-frontal gyrus of the brains of patients with dementia and is associated with a high affinity for the C/EBP factor resulting in a higher rate of viral replication. Conversely, a mutation (4C) in the C/EBP site II, found predominantly in the cerebellum of patients with HAD, is associated with lower rates of viral replication (Ross et al., 2001; Burdo,

Gartner, Mauger, & Wigdahl, 2004). The high affinity of C/EBP binding may be associated with the maintenance and pathogenesis of HIV-1 in the brain, while the low affinity binding may be required for sustaining a reservoir for HIV infection (Kilareski et al., 2009).

Tat

Tat is a basic protein of 104 amino acids encoded by HIV-1 that binds to the transactivation region (TAR) segment of the LTR and regulates transcription. It is the first protein produced during viral replication and is involved in the assembly of the pre-initiation complex during viral transcription (Brady & Kashanchi, 2005). Tat activity is limited in the undifferentiated monocyte as there is a lack of cyclinT1, a molecule through which Tat exerts its actions on viral replication. However, differentiation of monocytes into macrophages increases cyclinT1 expression in the cell and results in greater Tat activity (Yu, Wang, Shaw, Qin, & Rice, 2006). Cooperative interaction can occur among Tat, Vpr, and CyclinT1, leading to upregulated transcription of the viral genome (Sawaya, Khalili, Gordon, Taube, & Amini, 2000). Distinct functional and sequence variation is observed in Tat between brain and periphery. Comparison of matched brain- and spleen-derived *tat* sequences indicates that similarity among brain-derived clones was greater than that between the brain- and spleen-derived clones, perhaps stemming from different selective pressure between the organs (Mayne et al., 1998). Tat-mediated LTR transactivation in astrocytes is unique, and involves complex interplay between viral and cellular transcription factors (Coyle-Rink et al., 2002). In certain CNS-derived cells, Tat is capable of activating HIV-1 in a TAR-independent pathway (Taylor et al., 1992). *Tat* clones derived from patients with HAD also show different functional properties compared to those derived from nondemented HIV-infected patients; analyses of brain-derived *tat* sequences showed distinct clustering between HAD and nondemented subjects (Bratanich et al., 1998). For example, LTR transactivation is decreased while the pro-apoptotic gene, PDCD7, is upregulated by *tat* cloned from individuals with HAD (Boven et al., 2007). Remarkably, brain-derived *tat* sequences are heterogeneous in regions which influence viral replication and intracellular transport (Bratanich et al., 1998).

Pol

HIV-1 *pol* encodes a polyprotein of 1447 amino acids which is cleaved into several functional proteins including reverse transcriptase (RT), protease, RNAse H, and integrase. Like the other viral proteins discussed before, distinct sequence variation for the *pol* region is found in the CNS. Different patterns of RT and protease mutations develop in systemic infection compared to the CNS (Caragounis et al., 2008). However, unlike *tat* and *env*, phylogenetic clustering or distinct polymorphisms were not observed within brain-derived RT sequences from HIV/AIDS patients with or without HAD (Bratanich et al., 1998). Many of the mutations in RT sequences are within the active sites of the enzyme, possibly leading to positive selection pressure (Huang, Alter, & Wooley, 2002). These findings are complemented by previous observations that drug-resistant mutations found in the brain and CSF differ from those detected in the periphery (Cunningham et al., 2000; Venturi et al., 2000).

NEUROVIRULENCE

Several experimental approaches have been utilized to delineate the contributions of different HIV-1 genes and regions to neurovirulence including in vitro and in vivo models. Furthermore, clinically-derived HIV-1 sequences, largely derived from the brain, have also been informative. Recombinant viral proteins can be applied, or vectors containing the viral gene of interest can be transfected into monocytoid (macrophage/microglia) or astrocytic cells. However, it should be kept in mind that the effects of the proteins vary depending on the mode of delivery and give rise

Table 1.4.2 **ROLE OF VIRAL PROTEINS IN NEUROTROPISM**

Env	• **Disrupts BBB and mediates viral entry into the brain** • **Brain-derived *envs* have mutations in the V3, V4, and V5 that are associated with increased macrophage tropismIncreases blood-brain barrier permeability** • **Increases viral infectivity in macrophages and microglia**	• **Kanmogne et al., 2007** • **Dunfee et al., 2007; Shimizu et al., 1994; Shah et al., 2006**
Nef	• **Increases blood-brain barrier permeability** • **Increases viral infectivity in macrophages and microglia**	• **Sporer et al., 2000** • **Si et al., 2002**
LTR	• **Essential for viral infection of CNS** • **Diversity in LTR sequence within and between different regions of the brain is associated with effective viral infection**	• **Thompson et al., 2003** • **Kilareski et al., 2009**
Pol	• **Distinct sequence variations in the RT sequence are found in the CNS, many of the which occur within the active sites of the enzyme leading to positive selection pressure**	• **Huang et al., 2002**
Tat	• **Exhibits distinct functional and sequence variation between brain and periphery perhaps stemming from different selective pressure between the organs** • **Mediates LTR transactivation in astrocytes in an unique manner involving complex interplay between viral and cellular transcription factors** • **Capable of activating HIV-1 in a TAR-independent pathway**	• **Mayne et al., 1998** • **Coyle-Rink et al., 2002** • **Taylor et al., 1992**

to contradictory results. Similarly, HIV-1 gp120 has been transgenically expressed in mice using a GFAP promoter that limits its expression largely to the CNS (Toggas et al., 1994) and PNS (Keswani, Jack, Zhou, & Hoke, 2006). A *vpr* transgenic animal expresses Vpr only in monocytoid cell lines (Jones et al. 2007) while transgenic animals for *nef*, *tat*, and LTR express the transgene in T cells (Brady, Pennington, Miles, & Dzierzak, 1993; Lindemann et al., 1994; Brady et al. 1995). This section summarizes the role of different viral proteins in neurovirulence based on the *ex vivo* and in vivo data (Table 1.4.3).

Envelope Proteins

Gp120 mediates its neurotoxic effects by direct action on the neurons or indirectly by activating macrophages, microglia, and astrocytes (Lipton, Sucher, Kaiser, & Dreyer, 1991; Lipton, 1992; Lannuzel, Lledo, Lamghitnia, Vincent, & Tardieu, 1995). Gp120 might elicit oxidative stress in neurons and astrocytes through calcium signal dysregulation, although neurons are more susceptible to injury (Haughey & Mattson, 2002; Mattson, Haughey, & Nath, 2005). An anti-oxidant enzyme, Mn-superoxide dismutase, is reported to be down-regulated in neurons and upregulated in astrocytes by gp120, rendering differential effects on astrocytes and neurons (Saha & Pahan, 2007). The interaction of gp120 with the chemokine receptors CXCR4 and CCR5 activates intracellular pathways leading to neuronal apoptosis (Kaul et al., 2005). This action is mediated by a number of downstream molecules including RNA-activated protein kinase (Alirezaei et al., 2007), p38, and mitogen-activated protein kinase (Singh et al., 2005). Gp120 can also induce host molecules, including MMPs (Russo et al., 2007), IL-1β (Cheung et al., 2008), and TNF-α (Buriani et al., 1999).

Gp120 induces excitotoxicity by suppressing the expression of the glutamate transporter gene, EAAT2 (Wang et al., 2003), or by activating NMDA receptors. The V3 region within gp120 binds to the NMDA receptor at the glycine binding site and stimulates glutamate release (Pattarini et al., 1998). However, NMDA receptor antagonists have not been able to reverse gp120-mediated neurotoxicity (Alessia & Italo, 2004). Dopaminergic neurons also appear to be vulnerable to gp120-mediated injury (Bennett, Rusyniak, & Hollingsworth, 1995). Gp120 can act synergistically with some drugs of abuse including methamphetamine and cocaine that also damage the dopaminergic system (Nath et al., 2000).

Gp41 has been readily detected in the autopsied brains of patients (Kure et al., 1990). It induces NOS2 production, resulting in neurotoxicity mediated by the N-terminal domain of the protein (Adamson et al., 1996; Adamson, Kopnisky, Dawson, & Dawson, 1999). Gp41 might also be involved in increasing excitotoxicity by eliciting the release of glutamate and noradrenaline (Wang & White, 2000).

HIV-1 gp120 also participates in the pathogenesis of peripheral neuropathy. It can exert axonal toxicity directly resulting in "dying back" of axons and nerve damage (reviewed in Cornblath & Hoke, 2006). Gp120 is also involved in eliciting peripheral pain responses (Milligan et al., 2000; Herzberg & Sagen, 2001), and perineural HIV-1 gp120 exposure induces a persistent mechanical hypersensitivity (Wallace et al., 2007). Gp120 may also act through its interaction with the CCR2 or CXCR4 receptors on Schwann cells, leading to the release of toxic cytokines and subsequent neuronal injury (Bhangoo, Ripsch, Buchanan, Miller, & White, 2009).

Transgenic mice expressing gp120 under control of the GFAP promoter, permitting expression in astrocytes, exhibit features which are reminiscent of HIV-associated dementia, including neuronal injury and glial activation. Moreover, these same animals show neurobehavioral deficits. Likewise, transgenic expression of g120 in the PNS is limited to Schwann cells without an apparent neurobehavioral or neuropathological phenotype. However, if these animals are exposed to neurotoxic antiretroviral drugs, they exhibit features of peripheral neuropathy (Toggas et al., 1994; Keswani et al., 2006).

Importantly, many of the studies described above use gp120 genes or proteins derived from representative HIV-1 sequences, including viruses from patients with HIV-associated dementia. However, there are multiple studies indicating that HIV-1 gp120, particularly from brain-derived sequences, contains mutations highly associated with the development of dementia (Power et al., 1998). Several of these mutations have been shown to influence in vitro neurovirulence but none have been expressed in vivo in transgenic mice or in other in vivo models (Bonavia et al., 2005).

Vpr

HIV-1 Vpr is a 96 amino acid (14KDa) accessory viral protein synthesized late in the viral life cycle (Schwartz et al., 1991) and it is essential for infection of macrophages and monocytes. Vpr is a soluble protein that is released by infected cells although the conditions governing its release remain unclear despite its potent extracellular actions. Vpr plays an important role in different cellular functions, including cell cycle arrest in the G_2 phase, induction of apoptosis, and nuclear import of viral DNA into macrophages and other nondividing cells (Ayyavoo et al., 1997). It is detectable in CSF and brain tissue from HIV-infected subjects (Levy, Refaeli, MacGregor, & Weiner, 1994; Jones et al., 2007). Vpr can bind to the LTR sequence of HIV-1 and contributes to viral production. A substantial proportion of patients with HAD exhibits a particular sequence at the C/EBP binding site I in the LTR derived from the brain allowing Vpr to bind with high affinity to the C/EBP binding site, thereby influencing viral replication (Burdo, Gartner, Mauger, & Wigdahl, 2004). Vpr expression in microglia induces chemoattractant CCL5, which is essential for viral infection (Si et al., 2002). Application of recombinant Vpr (rVpr) as well as intracellular Vpr expression induces neuronal apoptosis through caspase-9 activation (Patel, Mukhtar, & Pomerantz, 2000; Patel, Mukhtar, Harley, Kulkosky, & Pomerantz, 2002; Cheng et al., 2007; Jones et al., 2007). rVpr also inhibits axonal outgrowth in neurons through induction of mitochondrial dysfunction; however, point mutations of arginine residues at sites 73, 77, or 80 can render soluble rVpr incapable of causing axonal damage (Kitayama et al., 2008). Of interest, a similar point mutation (R77Q) in

blood-derived HIV-1 stains has been associated with long-term nonprogressive HIV infection (Lum et al., 2003; Mologni et al., 2006). Vpr induces H_2O_2 and mitochondrial ROS (reactive oxygen species) production in the microglia, underscoring its role in generating oxidative stress in the brain (Rom et al., 2009; Deshmane et al., 2009); although, neuroinflammation is not a feature of Vpr's pathogenic actions unlike gp120 and Tat. HIV-1 Vpr has also been proposed to form ion channels within the plasma membrane (Piller, Ewart, Premkumar, Cox, & Gage, 1996; Piller, Ewart, Jans, Gage, & Cox, 1999). This proposed channel-forming property has been attributed to the N-terminal of the peptide, and the channels are thought to be open at negative potentials. Vpr can also alter membrane properties by inducing rapid changes in neuronal membrane currents by inhibiting voltage-dependent potassium channels (Jones et al., 2007).

Recent findings suggest that Vpr can exert its actions directly on astrocytes and microglia. Exposure to Vpr diminishes expression of astrocyte-specific markers and causes caspase-6 mediated apoptosis (Noorbakhsh et al., 2010). Vpr also drives the expression of HIF-1α in microglia cells, which can lead to oxidative stress (Deshmane et al., 2009). Induction of calcium signal by Vpr has been observed in astrocytes and neurons (Rom et al., 2009; Noorbakhsh et al., 2010), and can cause dysregulation of intracellular signaling pathways. Alteration or dysfunction of astrocytes and glial cells, in turn, can cause neuronal damage.

Studies from our group indicate that Vpr is expressed in the PNS of HIV-infected persons and indeed, a transgenic mouse expressing Vpr in the PNS exhibits mechanical allodynia with evidence of dorsal root neuronal injury (Acharjee et al., 2009).

Nef

Nef appears to play an important albeit undefined role in HIV neuropathogenesis. Nef has an N-terminal myristoylation site which is co-translationally modified (Hammes, Dixon, Malim, Cullen, & Greene, 1989) and associates with the plasma membrane (Bentham, Mazaleyrat, & Harris, 2006). Recent studies also indicate that Nef may be secreted (Campbell et al., 2008); antibodies to Nef have been detected in the serum in a large proportion of HIV-infected individuals, suggesting Nef is secreted extracellularly (Ameisen et al., 1989; Cheingsong-Popov et al., 1990). Nevertheless, the presence of extracellular Nef remains controversial. Recombinant Nef (rNef) is cytotoxic to neurons and glial cell culture (Trillo-Pazos, McFarlane-Abdulla, Campbell, Pilkington, & Everall, 2000). HIV-1 Nef might contribute directly to neuropathogenesis by causing astrocyte death together with indirect neuronal death through the cytotoxic actions of induced IP-10 on neurons (van Marle et al., 2004). Grafting of Nef-transduced macrophages into the rat hippocampus induced monocyte/macrophage recruitment, expression of TNF-alpha, astrogliosis, and behavior changes in rats (Mordelet et al., 2004). Nef is also shown to induce quinolinic acid production in macrophages (Smith et al., 2001). Quinolinic acid is a neurotoxic molecule, and indeed, it is elevated in the brains of HIV-demented individuals (Heyes et al., 1991). Nef has sequence and structural similarity to a neurotoxic scorpion peptide and also shares some of its functional properties (Werner et al., 1991). Nef induces a range of host genes including MMP-9, MCP-1, IL-6, TNF-α, IFN-γ, and CCL5 in the CNS which can influence neuronal viability (Koedel et al., 1999; Sporer et al., 2000; Si et al., 2002). Transgenic expression of *nef* under the control of the LTR did not exhibit neuropathogenic effects despite its immunosuppressive phenotype (Hanna et al., 2009).

Tat

Tat is a secreted viral protein with diverse effects on neural cells in the CNS (Li et al., 2009). Tat causes neuronal damage by acting directly on neurons or indirectly by activating glial cells. Tat-induced neuronal injury occurs through multiple cellular pathways; Tat induces neuronal apoptosis through mitochondrial dysfunction and caspase activation (Kruman, Nath, & Mattson, 1998). Several types of neurons in the dentate gyrus, striatum, and the CA3 region of the hippocampus are more vulnerable to Tat (Rappaport et al., 1999; Maragos et al., 2003; Li et al., 2009). In vivo and in vitro studies have shown that Tat can affect dopaminergic neuron function (Zauli et al., 2000; Ferris, Frederick-Duus, Fadel, Mactutus, & Booze, 2009; Zhu et al., 2009). Tat induces cytosolic calcium in neuronal cells using extracellular sources and IP_3 sensitive intracellular stores of the cell (Haughey, Holden, Nath, & Geiger, 1999). Tat also activates ryanodine receptors in the endoplasmic reticulum, inducing an unfolded protein response and mitochondrial hyperpolarization (Norman et al., 2008). Mitochondrial hyperpolarization leads to generation of ROS, activation of caspases, and ultimately, apoptosis (Kruman et al., 1998; Singh et al., 2004). Amino acids 31–61 of Tat are thought to be the active region that is involved in electrophysiological changes, specifically, by depolarizing the membrane (Magnuson et al., 1995; Nath et al., 1996; Cheng et al., 1997), increasing the whole-cell inward current and decreasing membrane resistance (Brailoiu, Brailoiu, Chang, & Dun, 2008). Tat interacts with neurons through at least two receptors, heparan sulfate and lipoprotein receptor associate protein (LRP) (Liu et al., 2000). Heparan sulfate helps Tat to localize to the cell membrane while LRP helps to internalize it through endocytosis. Tat can also bind to the NR1 subunit of the NMDA receptor (Li et al., 2008). Tat's interaction with NMDA receptors is yet another way by which it exerts its effects. Tat induces calcium entry through NMDA receptors and causes synaptic loss in neuronal cultures (Kim et al., 2008). LRP, an NMDA receptor and the post-synaptic density protein-95 (PSD-95) form a complex and Tat stimulates this complex leading to neuronal injury (Eugenin et al., 2007). Tat might also cause synaptic dysfunction by upregulating mir-128, which is a neuron-encoded microRNA involved in regulating SNAP25 expression. Tat can also act synergistically with other toxic molecules, like gp120, (Bansal et al., 2000; Nath et al., 2000a) and substances of abuse (Nath et al. 2002), resulting in exacerbation of neuronal dysfunction.

In addition to direct toxicity, Tat acts on the glial cells causing them to release host toxic molecules. Tat stimulates production of a multitude of host cytokines and chemokines, including TNF-α, CCL2, CCL5, CXCL10, and SDF-1 (Li et al., 2009). MMPs (Johnston et al., 2001), NOS2 (Polazzi, Levi, & Minghetti, 1999), and quinolic acid (Smith et al., 2001) expression are induced by Tat, all of which can result in neurotoxicity. Sequence variation in Tat is linked to differences in neurotoxic potential. For example, clade B Tat is more neurotoxic compared with clade C Tat, and alteration in a dicysteine motif within the neurotoxic region of clade B Tat is associated with this difference (Mishra, Vetrivel, Siddappa, Ranga, & Seth, 2008).

Virus-encoded microRNAs

MicroRNAs (miRNA) are small RNA molecules encoded from the genomic (host or viral) DNA in the same manner as RNA. The hairpin secondary structure in the RNA is recognized and cleaved sequentially by the action of two enzymes, Dicer and Drosha, giving rise to an miRNA which is a duplex of two RNA strands approximately 22 nucleotides long (Yeung, Bennasser, Le, & Jeang, 2005). The microRNA binds to the 3' UTR with imperfect complementarity and functions as a transcription repressor. There is growing evidence that many viruses express miRNA and regulate their replication and host response (Klase et al., 2007; Klase et al., 2009). Recent studies show that HIV also encodes microRNAs through processing of the HIV-1 TAR element by the Dicer enzyme (Klase et al., 2007). This viral miRNA is detectable in infected cells and appears to contribute to viral latency and alters cellular function (Klase et al., 2007; Klase et al., 2009). Further research may elucidate the role of miRNA and differential Dicer enzyme expression in HIV replication in the CNS.

Table 1.4.3 **ROLE OF VIRAL PROTEINS IN NEUROVIRULENCE**

GENE	FUNCTION	REFERENCE
Env	• Elicit oxidative stress in neurons and astrocytes • Induce neuronal apoptosis • Cause glutamate excitotoxicity by suppressing the expression of glutamate transporter gene, EAAT2 or by activating NMDA receptors • Induces painful peripheral neuropathy	• Haughey et al., 2002; Mattson, 2005 • Kaul et al., 2005 • Pattarini et al., 1998; Wang et al., 2003 • Reviewed in Cornblath & Hoke, 2006; Wallace, 2007; Bhangoo et al., 2009
Nef	• *nef* sequence is more conserved in the brains of patients with dementia, possibly as a consequence of positive selection pressure(s) • Causes astrogliosis and astrocyte death • Mediates behavior changes in rats • Induces quinolinic acid production in macrophages • Activates host genes including MMP-9, MCP-1, IL-6, TNF-α, IFN-γ, and CCL5 in the CNS which can influence neuronal viability	• van Marle et al., 2004 • Mordelet et al., 2004 • Smith et al., 2001 • Koedel et al., 1999; Sporer et al., 2000; Si et al., 2002
Vpr	• Expression in microglia induces chemoattractant CCL5, which is essential for viral infection • Expression induces neuronal apoptosis through caspase-9 activation • Inhibits axonal outgrowth in neurons through induction of mitochondrial dysfunction • Induces H_2O_2 and mitochondrial ROS production in the microglia, underscoring its role in generating oxidative stress in the brain • Alters neuronal membrane properties • Causes astrogliosis and causes caspase-6-mediated apoptosis • Can cause peripheral neuropathy	• Si et al., 2002 • Patel et al., 2000; Patel et al., 2002; Cheng et al., 2007; Jones et al., 2007 • Kitayama et al., 2008 • Rom et al., 2009; Deshmane et al., 2009 • Piller et al., 1996; Piller et al., 1999; Jones et al., 2007 • Noorbakhsh et al., 2010 • Acharjee et al., 2009
Tat	• Induces neuronal apoptosis through mitochondrial dysfunction and caspase activation • Causes cytosolic calcium influx in neuronal cells • Induces unfolded protein response, mitochondrial hyperpolarization, generation of ROS, activation of caspases, and ultimately, apoptosis • Alters neuronal membrane properties by inducing membrane depolarization, increasing whole-cell inward current, and decreasing membrane resistance • Affects dopaminergic neuron function • Activates NMDA receptor and causes neuronal injury • Causes synaptic dysfunction by upregulating mir-128 which is a neuron-encoded microRNA involved in regulating SNAP25 expression • Acts synergistically with other toxic molecules, like gp120, and substances of abuse resulting in exacerbation of neuronal dysfunction • Stimulates production of a multitude of host toxic molecules including TNF-α, CCL2, CCL5, CXCL10, and SDF-1, MMP, iNOS, and quinolic acid which can result in neurotoxicity	• Kruman et al., 1998 • Haughey et al., 1999 • Norman et al., 2008; Kruman et al., 1998; Singh et al., 2004 • Magnuson et al., 1995; Nath et al., 1996; Cheng et al., 1997; Brailoiu et al., 2008 • Zauli et al., 2000; Ferris et al., 2009; Zhu et al., 2009. • Li et al., 2008; Kim et al., 2008 • Eletto et al., 2008 • Bansal et al., 2000; Nath et al., 2000a; Nath et al., 2002 • Li et al., 2009, Johnston et al., 2001, Polazzi et al., 1999, Smith et al., 2001

HIV-1 CLADE-RELATED NEUROPATHOGENESIS

There are four genetic groups of HIV-1virus recognized worldwide: group M (major), group O (outlier), group N (new, non-M, non-O) (Hemelaar, Gouws, Ghys, & Osmanov, 2006), and group P (Plantier et al., 2009). Group M accounts for the vast majority of cases and is subdivided into nine major subtypes or clades (Hemelaar et al., 2006). Clade C accounts for 50% of cases worldwide, followed by clades A (12%), B (10%), G (6%), and D (3%) (Hemelaar et al., 2006). Clade B is the subtype most frequently encountered in North America and Europe (Hemelaar et al., 2006) and is therefore the clade which has been the most studied in the clinic. Prevalence of subtypes differs markedly throughout the world with the largest variability in clade prevalence in Africa (Hemelaar et al., 2006). The classification system was initially based on the *env* sequence but applies to all regions of the viral genome (Hemelaar et al., 2006). Of interest, we reported that molecular diversity in brain-derived HIV-1 A and D clade *env* sequences displayed greater evolutionary distance than B clade brain-derived viruses (Zhang et al., 2001). Similarly, molecular diversity between matched brain and spleen *env* clones was clade-dependent and concentrated in the hypervariable V4 region (Zhang et al., 2001).

Whether HIV clade contributes to pathogenesis, progression to AIDS, mortality, drug resistance, or neuropathogenenesis remains a topic of ongoing debate and research (Hemelaar et al., 2006; Liner, Hall, & Robertson, 2007). There is evidence supporting differences in disease progression comparing different subtypes in isolated populations (Kiwanuka et al., 2008; Kaleebu et al., 2002; Vasan et al., 2006). There have also been several studies highlighting possible differences in neurological complications comparing different subtypes. This topic was reviewed by Liner and colleagues (Liner et al., 2007). Previously, it was believed that the prevalence of HIV-associated dementia was rare in clade C-seropositive patients (Teja, Talasila, & Vemu, 2005; Wadia et al., 2001). This notion is also supported by the observation that the pathological changes seen within brains from HIV-1 clade C-infected patients are different from those described in clade B patients with the striking absence of multifocal microglial nodules and multinucleated giant cells (Mahadevan et al., 2007). More recently however, Gupta and colleagues found a prevalence of 60.5% of mild to moderate cognitive impairment among a group of HIV-1 clade C-seropositive adults from South India, which is similar to reports of prevalence in clade B infection. No cases of severe cognitive impairment were identified in this cohort (Gupta et al., 2007). In a prospective study completed in South Africa, Modi and colleagues reported a 38% prevalence of HAD, similar to that reported in clade B infection (Modi, Hari, Modi, & Mochan, 2007). Significant differences in the frequency of HAD comparing HIV-1 clade D and clade A have also been reported (Sacktor et al., 2009). It appears as though conflicting evidence regarding prevalence of various neurological complications among HIV-1 clades exists and further study is therefore warranted.

Possible mechanisms have also been explored for viral subtype differences in disease progression. One postulated mechanism relates to subtype differences in viral tropism related to differences in the use of chemokine receptors by different virus subtypes. CCR5 is the co-receptor used by non-syncytium-inducing variants for viral entry and CXCR4 is the co-receptor most commonly used by syncytium-inducing variants (Peeters et al., 1999; Kaleebu et al., 2007; Zhang et al., 1996; Tscherning et al., 1998). Strains utilizing the CXCR4 co-receptor replicate more quickly which is correlated with faster disease progression (Peeters et al., 1999; Tscherning et al., 1998). Many HIV-1 strains also change co-receptor usage as the disease progresses with higher frequency of co-receptor CXCR4 use later in the disease during the rapidly progressing end-stage (Kaleebu et al., 2007). There also appears to be an earlier shift to CXCR4 co-receptor use from CCR5 co-receptor use in clade D compared to clade A, which has been postulated as a reason for increased systemic pathogenicity seen in clade D (Kaleebu et al., 2007). In the CNS, however, macrophage tropism and use of CCR5 as co-receptor for viral entry appear to be important prerequisites for infection (Power et al., 1998; Reddy et al., 1996; Gorry et al., 2001; Albright et al., 1999; Chan et al., 1999). CXCR4-dependent viruses and dual tropic viruses are infrequently found in the CNS (Reddy et al., 1996; Gorry et al., 2001), and although these differences in co-receptor use may prove to be important when considering differences in disease progression, it seems unlikely that these differences explain the variation seen in neurological disease among subtypes.

One possible mechanism for subtype difference relates to the Tat protein and excitotoxicity. HIV-infected macrophages can cause neuronal injury through the extracellular release of Tat protein (Magnuson et al., 1995). Tat activates the NMDA receptor (Song, Nath, Geiger, Moore, & Hochman, 2003), resulting in excitotoxicity. It appears as though this activation occurs through the direct binding of Tat protein to the NMDA receptor and the resulting neurotoxic response is clade specific (Li et al., 2008; Mishra, Vetrivel, Siddappa, Ranga, & Seth, 2008). When comparing clades B and C, it appears as though there is an equal amount of Tat protein released; however, the Tat released from clade C results in significantly lower toxicity (Li et al., 2008; Mishra et al., 2008). It is thought that the Cys31Ser mutation in clade C Tat may be critical for activation of the NMDA receptor with resultant excitotoxicity without affecting the binding of Tat to the NMDA receptor (Li et al., 2008).

Differences between clades B and C have also been demonstrated using the SCID mouse HIV encephalitis model (Rao et al., 2008). Clade B infected mice develop more severe neurobehavioral deficits as compared to clade C infected mice with similar brain viral loads. Mice infected with clade C, however, showed decreased pathology and reduced monocyte chemotaxis (Rao et al., 2008). It appears as though chemotaxis is induced by macrophages through the release of Tat (Rao et al., 2008), which also results in the release of CCL2 by astrocytes and monocytes (Conant et al., 1998; Weiss et al., 1999; D'Aversa, Yu, & Berman, 2004). It appears as though

there is reduced release of CCL2 chemokine with infection by clade C virus, resulting in reduced monocyte chemotaxis.

Another possible mechanism involves differences in the V3 loop of the envelope glycoprotein, gp120, between subtypes, which also may affect neurovirulence (Liner et al., 2007; De Jong et al., 1992; Zhong et al., 1995). Clade D strains appear to have a more variable pattern of the V3 loop as compared to clade C, and in particular, this variability has been shown in HIV entry into the CD4 cell (Hwang, Boyle, Lyerly, & Cullen, 1991; Zhang et al., 2001). Similar types of viral envelope diversity have also been shown to affect the progression of neurological disease in other retroviruses (Johnston et al., 2000; Mankowski et al., 1997). The V3 region of the envelope has been shown to influence the release of neurotoxins from macrophages and has been postulated to be directly neurotoxic (Liner et al., 2007; Kaul et al., 1999; Kong et al., 1996; Power et al., 1998; Yeung, Pulliam, & Lau, 1995; Zhao, Kim, Morgello, & Lee, 2001).

FUTURE PERSPECTIVES

Most of the studies described herein have been performed using the models of HIV-infected brain cells or using brain tissues from patients with or without HAD from HIV-1 clade B-infected persons. The full spectrum of non-clade B virus-related neurological disease remains unknown. With increasing migration, there is growing viral subtype diversity globally, which may impact on care and provides increasing impetus for the further study of all viral subtypes (Krentz et al., 2009). Similarly, the contributions of viral and host genetic diversity to the occurrence of HIV-associated neurological disease and/or neurodevelopment in children remains unclear to date.

In addition, genome-wide scans of different populations infected by HIV will likely highlight new susceptibility loci or mutations for HIV-related neurological disorders. Comprehensive molecular studies of peripheral neuropathy are lacking and it is plausible individual differences in the development of HIV DSP and other peripheral nervous system manifestations of HIV infection may also be, in part, affected by viral and host genetic differences. Moreover, the impact of antiretroviral therapy and ensuing drug resistance mutations on the neurological disease phenotypes and response to treatments remains to be defined. The increasing use of advanced technologies such as pyrosequencing as well as verifiable clinical and epidemiological data are imperative for the ongoing understanding of HIV/AIDS neuropathogenesis, permitting the development of new diagnostic and therapeutic options for this broad group of disabling neurological disorders.

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REFERENCES

Acharjee, S., Zhu, Y., Noorbakhsh, F., Stemkowski, P., Olechowski, C., Cohen, E., et al. (2009). Human immunodeficiency virus-1 vpr expression in the peripheral nervous system: Evidence for neuronal injury and neuropathic pain. *Abstract SfN 2009*, *561*.13/DD11

Achim, C. L., Heyes, M. P., & Wiley, C. A. (1993). Quantitation of human immunodeficiency virus, immune activation factors, and quinolinic acid in AIDS brains. *Journal of Clinical Investigation*, *91*(6), 2769–2775.

Adamson, D. C., Kopnisky, K. L., Dawson, T. M., & Dawson, V. L. (1999). Mechanisms and structural determinants of HIV-1 coat protein, gp41-induced neurotoxicity. *J Neurosci*, *19*(1), 64–71.

Adamson, D., Wildemann, B., Sasaki, M., Glass, J., McArthur, J., Christov, V., et al. (1996). Immunologic NO synthase: Elevation in severe AIDS dementia and induction by HIV-1 gp41. *Science*, *274*(5294), 1917–21; 1917.

Albright, A. V., Shieh, J. T., Itoh, T., Lee, B., Pleasure, D., O'Connor, M. J., et al. (1999). Microglia express CCR5, CXCR4, and CCR3, but of these, CCR5 is the principal coreceptor for human immunodeficiency virus type 1 dementia isolates. *Journal of Virology*, *73*(1), 205–213.

Alessia, B. & Italo, M. (2004). The chemokine receptor CXCR4 and not the N-methyl-D-aspartate receptor mediates gp120 neurotoxicity in cerebellar granule cells. *Journal of Neuroscience Research*, *75*(1), 75–82.

Alirezaei, M., Watry, D. D., Flynn, C. F., Kiosses, W. B., Masliah, E., Williams, B. R. G., et al. (2007). Human immunodeficiency virus-1/Surface glycoprotein 120 induces apoptosis through RNA-activated protein kinase signaling in neurons. *J Neurosci*, *27*(41), 11047–11055.

Ameisen, J., Guy, B., Chamaret, S., Loche, M., Mouton, Y., Neyrinck, J., et al. (1989). Antibodies to the nef protein and to nef peptides in HIV-1â€"Infected seronegative individuals. *AIDS Research and Human Retroviruses*, *5*(3), 279–291.

Anuurad, E., Semrad, A., & Berglund, L. (2009). Human immunodeficiency virus and highly active antiretroviral therapy-associated metabolic disorders and risk factors for cardiovascular disease. *Metabolic Syndrome & Related Disorders*, *7*(5), 401–410.

Argyris, E. G., Acheampong, E., Nunnari, G., Mukhtar, M., Williams, K. J., & Pomerantz, R. J. (2003). Human immunodeficiency virus type 1 enters primary human brain microvascular endothelial cells by a mechanism involving cell surface proteoglycans independent of lipid rafts. *Journal of Virology*, *77*(22), 12140–12151.

Ayyavoo, V., Mahalingam, S., Rafaeli, Y., Kudchodkar, S., Chang, D., Nagashunmugam, T., et al. (1997). HIV-1 viral protein R (vpr) regulates viral replication and cellular proliferation in T cells and monocytoid cells in vitro. *J Leukoc Biol*, *62*(1), 93–99.

Bagasra, O., Lavi, E., Bobroski, L., Khalili, K., Pestaner, J. P., Tawadros, R., et al. (1996). Cellular reservoirs of HIV-1 in the central nervous system of infected individuals: Identification by the combination of in situ polymerase chain reaction and immunohistochemistry. *AIDS*, *10*(6), 573–585.

Bannert, N., Schenten, D., Craig, S., & Sodroski, J. (2000). The level of CD4 expression limits infection of primary rhesus monkey macrophages by a T-tropic simian immunodeficiency virus and macrophagetropic human immunodeficiency viruses. *Journal of Virology*, *74*(23), 10984–10993.

Bansal, A. K., Mactutus, C. F., Nath, A., Maragos, W., Hauser, K. F., & Booze, R. M. (2000). Neurotoxicity of HIV-1 proteins gp120 and tat in the rat striatum. *Brain Research*, *879*(1–2), 42–49.

Bennett, B. A., Rusyniak, D. E., & Hollingsworth, C. K. (1995). HIV-1 gp120-induced neurotoxicity to midbrain dopamine cultures. *Brain Research*, *705*(1–2), 168–176.

Bentham, M., Mazaleyrat, S., & Harris, M. (2006). Role of myristoylation and N-terminal basic residues in membrane association of the human immunodeficiency virus type 1 nef protein. *J Gen Virol*, *87*(3), 563–571.

Berger, E. A., Murphy, P. M., & Farber, J. M. (1999). Chemokine receptors as HIV-1 coreceptors: Roles in viral entry, tropism, and disease. *Annual Review of Immunology*, *17*, 657–700.

Bhangoo, S., Ripsch, M., Buchanan, D., Miller, R., & White, F. (2009). Increased chemokine signaling in a model of HIV1-associated peripheral neuropathy. *Molecular Pain*, *5*(1), 48.

Bobardt, M. D., Salmon, P., Wang, L., Esko, J. D., Gabuzda, D., Fiala, M., et al. (2004). Contribution of proteoglycans to human immunodeficiency virus type 1 brain invasion. *Journal of Virology*, *78*(12), 6567–6584.

Bonavia, A., Bullock, B. T., Gisselman, K. M., Margulies, B. J., & Clements, J. E. (2005). A single amino acid change and truncated TM are sufficient for simian immunodeficiency virus to enter cells using CCR5 in a CD4-independent pathway. *Virology*, *341*(1), 12–23.

Borjabad, A., Brooks, A. I., & Volsky, D. J. (2010). Gene expression profiles of HIV-1-infected glia and brain: Toward better understanding of the role of astrocytes in HIV-1-associated neurocognitive disorders. *Journal of Neuroimmune Pharmacology: The Official Journal of the Society on NeuroImmune Pharmacology*, *5*(1), 44–62.

Boven, L. A., van der Bruggen, T., van Asbeck, B. S., Marx, J. J., & Nottet, H. S. (1999). Potential role of CCR5 polymorphism in the development of AIDS dementia complex. *FEMS Immunology & Medical Microbiology*, *26*(3–4), 243–247.

Boven, L. A., Noorbakhsh, F., Bouma, G., van der Zee, R., Vargas, D. L., Pardo, C., et al. (2007). Brain-derived human immunodeficiency virus-1 tat exerts differential effects on LTR transactivation and neuroimmune activation. *Journal of Neurovirology*, *13*(2), 173–184.

Boyles, J. K., Pitas, R. E., Wilson, E., Mahley, R. W., & Taylor, J. M. (1985). Apolipoprotein E associated with astrocytic glia of the central nervous system and with nonmyelinating glia of the peripheral nervous system. *Journal of Clinical Investigation*, *76*(4), 1501–1513.

Brady, H. J., Abraham, D. J., Pennington, D. J., Miles, C. G., Jenkins, S., & Dzierzak, E. A. (1995). Altered cytokine expression in T lymphocytes from human immunodeficiency virus tat transgenic mice. *Journal of Virology*, *69*(12), 7622–7629.

Brady, H. J., Pennington, D. J., Miles, C. G., & Dzierzak, E. A. (1993). CD4 cell surface downregulation in HIV-1 nef transgenic mice is a consequence of intracellular sequestration. *EMBO Journal*, *12*(13), 4923–4932.

Brady, J. & Kashanchi, F. (2005). Tat gets the "green" light on transcription initiation. *Retrovirology*, *2*(1), 69.

Brailoiu, G. C., Brailoiu, E., Chang, J. K., & Dun, N. J. (2008). Excitatory effects of human immunodeficiency virus 1 tat on cultured rat cerebral cortical neurons. *Neuroscience*, *151*(3), 701–710.

Bratanich, A., Liu, C., McArthur, J., Fudyk, T., Glass, J., Mittoo, S., et al. (1998). Brain-derived HIV-1 tat sequences from AIDS patients with dementia show increased molecular heterogeneity. *J Neurovirol*, *4*, 387–393.

Buckner, C. M., Luers, A. J., Calderon, T. M., Eugenin, E. A., & Berman, J. W. (2006). Neuroimmunity and the blood-brain barrier: Molecular regulation of leukocyte transmigration and viral entry into the nervous system with a focus on neuroAIDS. *Journal of Neuroimmune Pharmacology: The Official Journal of the Society on NeuroImmune Pharmacology*, *1*(2), 160–181.

Burdo, T. H., Gartner, S., Mauger, D., & Wigdahl, B. (2004). Region-specific distribution of human immunodeficiency virus type 1 long terminal repeats containing specific configurations of CCAAT/enhancer-binding protein site II in brains derived from demented and nondemented patients. *Journal of Neurovirology*, *10*(Suppl 1), 7–14.

Burdo, T. H., Nonnemacher, M., Irish, B. P., Choi, C. H., Krebs, F. C., Gartner, S., et al. (2004). High-affinity interaction between HIV-1 vpr and specific sequences that span the C/EBP and adjacent NF-kappaB sites within the HIV-1 LTR correlate with HIV-1-associated dementia. *DNA Cell Biol*, *23*, 261–269.

Buriani, A., Petrelli, L., Facci, L., Romano, P., Dal Tosso, R., Leon, A., et al. (1999). Human immunodeficiency virus type 1 envelope glycoprotein gp120 induces tumor necrosis factor-alpha in astrocytes. *J NeuroAIDS*, *2*(2), 1–13.

Campbell, T., Khan, M., Huang, M., Bond, V., & Powell, M. (2008). HIV-1 nef protein is secreted into vesicles that can fuse with target cells and virions. *Ethn Dis*, *18*(2 Suppl 2), 14–9.

Canter, J. A., Haas, D. W., Kallianpur, A. R., Ritchie, M. D., Robbins, G. K., Shafer, R. W., et al. (2008). The mitochondrial pharmacogenomics of haplogroup T: MTND2*LHON4917G and antiretroviral therapy-associated peripheral neuropathy. *Pharmacogenomics Journal*, *8*(1), 71–77.

Caragounis, E. C., Gisslén, M., Lindh, M., Nordborg, C., Westergren, S., Hagberg, L., et al. (2008). Comparison of HIV-1 *pol* and *env* sequences of blood, CSF, brain and spleen isolates collected ante-mortem and post-mortem. *Acta Neurologica Scandinavica*, *117*(2), 108–116.

Carlum, S., Elisabeth, B., Francescopaolo Di, C., Hee Jung, C., & Monique, S. (2007). HIV-1 gp120 as well as alcohol affect blood–brain barrier permeability and stress fiber formation: Involvement of reactive oxygen species. *Alcoholism: Clinical and Experimental Research*, *31*(1), 130–137.

Chan, S. Y., Speck, R. F., Power, C., Gaffen, S. L., Chesebro, B., & Goldsmith, M. A. (1999). V3 recombinants indicate a central role for CCR5 as a coreceptor in tissue infection by human immunodeficiency virus type 1. *Journal of Virology*, *73*(3), 2350–2358.

Chang, J., Jozwiak, R., Wang, B., Ng, T., Ge, Y. C., Bolton, W., et al. (1998). Unique HIV type 1 V3 region sequences derived from six different regions of brain: Region-specific evolution within host-determined quasispecies. *AIDS Research & Human Retroviruses*, *14*(1), 25–30.

Chang, S. Y., Ko, W. S., Kao, J. T., Chang, L. Y., Sun, H. Y., Chen, M. Y., et al. (2009). Association of single-nucleotide polymorphism 3 and c.553G>T of APOA5 with hypertriglyceridemia after treatmennt with highly active antiretroviral therapy containing protease inhibitors in HIV-infected individuals in Taiwan. *Clinical Infectious Diseases*, *48*(6), 832–835.

Cheingsong-Popov, R., Panagiotidi, C., Ali, M., Bowcock, S., Watkins, P., Aronstam, A., et al. (1990). Antibodies to HIV-1 nef(p27): Prevalence, significance, and relationship to seroconversion. *AIDS Research and Human Retroviruses*, *6*(9), 1099–1105.

Cheng, J., Nath, A., Knudsen, B., Hochman, S., Geiger, J. D., Ma, M., et al. (1997). Neuronal excitatory properties of human immunodeficiency virus type 1 tat protein. *Neuroscience*, *82*(1), 97–106.

Cheng, X., Mukhtar, M., Acheampong, E. A., Srinivasan, A., Rafi, M., Pomerantz, R. J., et al. (2007). HIV-1 vpr potently induces programmed cell death in the CNS in vivo. *DNA & Cell Biology*, *26*(2), 116–131.

Cherry, C. L., Rosenow, A., Affandi, J. S., McArthur, J. C., Wesselingh, S. L., & Price, P. (2008). Cytokine genotype suggests a role for inflammation in nucleoside analog-associated sensory neuropathy (NRTI-SN) and predicts an individual's NRTI-SN risk. *AIDS Research & Human Retroviruses*, *24*(2), 117–123.

Cheung, R., Ravyn, V., Wang, L., Ptasznik, A., & Collman, R. G. (2008). Signaling mechanism of HIV-1 gp120 and virion-induced IL-1{beta} release in primary human macrophages. *J Immunol*, *180*(10), 6675–6684.

Choe, H., Farzan, M., Sun, Y., Sullivan, N., Rollins, B., Ponath, P. D., et al. (1996). The beta-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. *Cell*, *85*(7), 1135–1148.

Churchill, M. J., Wesselingh, S. L., Cowley, D., Pardo, C. A., McArthur, J. C., Brew, B. J., et al. (2009). Extensive astrocyte infection is prominent in human immunodeficiency virus-associated dementia. *Annals of Neurology*, *66*(2), 253–258.

Clements, J. E. & Zink, M. C. (1996). Molecular biology and pathogenesis of animal lentivirus infections. *Clinical Microbiology Reviews*, *9*(1), 100–117.

Conant, K., Garzino-Demo, A., Nath, A., McArthur, J. C., Halliday, W., Power, C., et al. (1998). Induction of monocyte chemoattractant protein-1 in HIV-1 tat-stimulated astrocytes and elevation in AIDS dementia. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(6), 3117–3121.

Corder, E. H., Robertson, K., Lannfelt, L., Bogdanovic, N., Eggertsen, G., Wilkins, J., et al. (1998). HIV-infected subjects with the E4 allele for APOE have excess dementia and peripheral neuropathy. *Nature Medicine*, *4*(10), 1182–1184.

Cornblath, D. R. & Hoke, A. (2006). Recent advances in HIV neuropathy. *Current Opinion in Neurology*, *19*(5), 446–450.

Coyle-Rink, J., Sweet, T. M., Abraham, S., Sawaya, B. E., Batuman, O., Khalili, K., et al. (2002). Interaction between TGF[beta] signaling

proteins and C/EBP controls basal and tat-mediated transcription of HIV-1 LTR in astrocytes. *Virology*, *299*(2), 240–247.

Cui, L., Locatelli, L., Xie, M. Y., & Sommadossi, J. P. (1997). Effect of nucleoside analogs on neurite regeneration and mitochondrial DNA synthesis in PC-12 cells. *Journal of Pharmacology & Experimental Therapeutics*, *280*(3), 1228–1234.

Cunningham, P. H., Smith, D. G., Satchell, C., Cooper, D. A., & Brew, B. (2000). Evidence for independent development of resistance to HIV-1 reverse transcriptase inhibitors in the cerebrospinal fluid. *AIDS*, *14*(13), 1949–1954.

Cutler, R. G., Haughey, N. J., Tammara, A., McArthur, J. C., Nath, A., Reid, R., et al. (2004). Dysregulation of sphingolipid and sterol metabolism by ApoE4 in HIV dementia. *Neurology*, *63*(4), 626–630.

Cysique, L. A. & Brew, B. J. (2009). Neuropsychological functioning and antiretroviral treatment in HIV/AIDS: A review. *Neuropsychology Review*, *19*(2), 169–185.

Dalakas, M. C., Semino-Mora, C., & Leon-Monzon, M. (2001). Mitochondrial alterations with mitochondrial DNA depletion in the nerves of AIDS patients with peripheral neuropathy induced by 2'3'-dideoxycytidine (ddC). *Laboratory Investigation*, *81*(11), 1537–1544.

D'Aversa, T. G., Yu, K. O., & Berman, J. W. (2004). Expression of chemokines by human fetal microglia after treatment with the human immunodeficiency virus type 1 protein tat. *Journal of Neurovirology*, *10*(2), 86–97.

Davis, L. E., Hjelle, B. L., Miller, V. E., Palmer, D. L., Llewellyn, A. L., Merlin, T. L., et al. (1992). Early viral brain invasion in iatrogenic human immunodeficiency virus infection. *Neurology*, *42*(9), 1736–1739.

De Jong, J. J., De Ronde, A., Keulen, W., Tersmette, M., & Goudsmit, J. (1992). Minimal requirements for the human immunodeficiency virus type 1 V3 domain to support the syncytium-inducing phenotype: Analysis by single amino acid substitution. *Journal of Virology*, *66*(11), 6777–6780.

de Sorrentino, A. H., Marinic, K., Motta, P., Sorrentino, A., Lopez, R., & Illiovich, E. (2000). HLA class I alleles associated with susceptibility or resistance to human immunodeficiency virus type 1 infection among a population in Chaco Province, Argentina. *Journal of Infectious Diseases*, *182*(5), 1523–1526.

Dean, M., Carrington, M., Winkler, C., Huttley, G. A., Smith, M. W., Allikmets, R., et al. (1996). Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. hemophilia growth and development study, multicenter AIDS cohort study, multicenter hemophilia cohort study, San Francisco city cohort, ALIVE study. *Science*, *273*(5283), 1856–1862.

Deng, H. K., Liu, R., Ellmeier, W., Choe, S., Unutmaz, D., Burkhart, M., et al. (1996). Identification of a major co-receptor for primary isolates of HIV-1. *Nature*, *381*, 661.

Deshmane, S. L., Mukerjee, R., Fan, S., Del Valle, L., Michiels, C., Sweet, T., et al. (2009). Activation of the oxidative stress pathway by HIV-1 vpr leads to induction of hypoxia-inducible factor 1Î± expression. *Journal of Biological Chemistry*, *284*(17), 11364–11373.

Diaz-Arrastia, R., Gong, Y., Kelly, C. J., & Gelman, B. B. (2004). Host genetic polymorphisms in human immunodeficiency virus-related neurologic disease. *Journal of Neurovirology*, *10*(Suppl 1), 67–73.

Doranz, B. J., Rucker, J., Yi, Y., Smyth, R. J., Samson, M., Peiper, S. C., et al. (1996). A dual-tropic primary HIV-1 isolate that uses fusin and the beta-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. *Cell*, *85*(7), 1149–1158.

Dragic, T., Litwin, V., Allaway, G. P., Martin, S. R., Huang, Y. X., Nagashima, K. A., et al. (1996). HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5. *Nature*, *381*, 667.

Dunfee, R., Thomas, E. R., Gorry, P. R., Wang, J., Ancuta, P., & Gabuzda, D. (2006). Mechanisms of HIV-1 neurotropism. *Current HIV Research*, *4*(3), 267–278.

Dunfee, R. L., Thomas, E. R., Wang, J., Kunstman, K., Wolinsky, S. M., & Gabuzda, D. (2007). Loss of the N-linked glycosylation site at position 386 in the HIV envelope V4 region enhances macrophage tropism and is associated with dementia. *Virology*, *367*(1), 222–234.

Dunfee, R. L., Thomas, E. R., Gorry, P. R., Wang, J., Taylor, J., Kunstman, K., et al. (2006). The HIV env variant N283 enhances macrophage tropism and is associated with brain infection and dementia. *Proceedings of the National Academy of Sciences*, *103*(41), 15160–15165.

Dunlop, O., Goplen, A. K., Liestol, K., Myrvang, B., Rootwelt, H., Christophersen, B., et al. (1997). HIV dementia and apolipoprotein E. *Acta Neurologica Scandinavica*, *95*(5), 315–318.

Eletto, D., Russo, G., Passiatore, G., Del Valle, L., Giordano, A., Khalili, K., et al. (2008). Inhibition of SNAP25 expression by HIV-1 tat involves the activity of mir-128a. *Journal of Cellular Physiology*, *216*(3), 764–770.

Eugenin, E. A., King, J. E., Nath, A., Calderon, T. M., Zukin, R. S., Bennett, M. V. L., et al. (2007). HIV-tat induces formation of an LRPâ€"PSD-95â€"NMDARâ€"nNOS complex that promotes apoptosis in neurons and astrocytes. *Proceedings of the National Academy of Sciences*, *104*(9), 3438–3443.

Everall, I. P., Heaton, R. K., Marcotte, T. D., Ellis, R. J., McCutchan, J. A., Atkinson, J. H., et al. (1999). Cortical synaptic density is reduced in mild to moderate human immunodeficiency virus neurocognitive disorder. HNRC group. HIV Neurobehavioral Research Center. *Brain Pathology*, *9*(2), 209–217.

Fabio, G., Smeraldi, R. S., Gringeri, A., Marchini, M., Bonara, P., & Mannucci, P. M. (1990). Susceptibility to HIV infection and AIDS in Italian haemophiliacs is HLA associated. *British Journal of Haematology*, *75*(4), 531–536.

Failde-Garrido, J. M., Alvarez, M. R., & Simon-Lopez, M. A. (2008). Neuropsychological impairment and gender differences in HIV-1 infection. *Psychiatry & Clinical Neurosciences*, *62*(5), 494–502.

Falangola, M. F., Hanly, A., Galvao-Castro, B., & Petito, C. K. (1995). HIV infection of human choroid plexus: A possible mechanism of viral entry into the CNS. *Journal of Neuropathology & Experimental Neurology*, *54*(4), 497–503.

Fauvel, J., Bonnet, E., Ruidavets, J. B., Ferrieres, J., Toffoletti, A., Massip, P., et al. (2001). An interaction between apo C-III variants and protease inhibitors contributes to high triglyceride/low HDL levels in treated HIV patients. *AIDS*, *15*(18), 2397–2406.

Fellay, J., Shianna, K. V., Ge, D., Colombo, S., Ledergerber, B., Weale, M., et al. (2007). A whole-genome association study of major determinants for host control of HIV-1. *Science*, *317*(5840), 944–947.

Ferris, M., Frederick-Duus, D., Fadel, J., Mactutus, C., & Booze, R. (2009). In vivo microdialysis in awake, freely moving rats demonstrates HIV-1 tat-induced alterations in dopamine transmission. *Synapse*, *63*, 181–185.

Foster, J., & Garcia, J. V. (2008). HIV-1 nef: At the crossroads. *Retrovirology*, *5*(1), 84.

Foulkes, A. S., Wohl, D. A., Frank, I., Puleo, E., Restine, S., Wolfe, M. L., et al. (2006). Associations among race/ethnicity, ApoC-III genotypes, and lipids in HIV-1-infected individuals on antiretroviral therapy. *PLoS Medicine/Public Library of Science*, *3*(3), e52.

Gartner, S. (2000). HIV infection and dementia. *Science*, *287*(5453), 602–604.

Gartner, S., McDonald, R., Hunter, E., Bouwman, F., Liu, Y., & Popovic, M. (1997). Gp120 sequence variation in brain and in T-lymphocyte human immunodeficiency virus type 1 primary isolates. *J Hum Virol*, *1*(1), 3–18.

Gelbard, H. A., Nottet, H. S., Swindells, S., Jett, M., Dzenko, K. A., Genis, P., et al. (1994). Platelet-activating factor: A candidate human immunodeficiency virus type 1-induced neurotoxin. *Journal of Virology*, *68*(7), 4628–4635.

Gelman, B. B., Soukup, V. M., Schuenke, K. W., Keherly, M. J., Holzer, C.,3rd, Richey, F. J., et al. (2004). Acquired neuronal channelopathies in HIV-associated dementia. *Journal of Neuroimmunology*, *157*(1–2), 111–119.

Glass, J. D., Fedor, H., Wesselingh, S. L., & McArthur, J. C. (1995). Immunocytochemical quantitation of human immunodeficiency virus in the brain: Correlations with dementia. *Annals of Neurology*, *38*(5), 755–762.

Gonzalez, E., Rovin, B. H., Sen, L., Cooke, G., Dhanda, R., Mummidi, S., et al. (2002). HIV-1 infection and AIDS dementia are influenced by a

mutant MCP-1 allele linked to increased monocyte infiltration of tissues and MCP-1 levels. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(21), 13795–13800.

Gonzalez-Scarano, F., & Martin-Garcia, J. (2005). The neuropathogenesis of AIDS. *Nature Reviews Immunology*, *5*(1), 69–81.

Gorry, P. R., Bristol, G., Zack, J. A., Ritola, K., Swanstrom, R., Birch, C. J., et al. (2001). Macrophage tropism of human immunodeficiency virus type 1 isolates from brain and lymphoid tissues predicts neurotropism independent of coreceptor specificity. *Journal of Virology*, *75*(21), 10073–10089.

Gorry, P. R., Howard, J. L., Churchill, M. J., Anderson, J. L., Cunningham, A., Adrian, D., et al. (1999). Diminished production of human immunodeficiency virus type 1 in astrocytes results from inefficient translation of gag, env, and nef mRNAs despite efficient expression of tat and rev. *Journal of Virology*, *73*(1), 352–361.

Gorry, P. R., Taylor, J., Holm, G. H., Mehle, A., Morgan, T., Cayabyab, M., et al. (2002). Increased CCR5 affinity and reduced CCR5/CD4 dependence of a neurovirulent primary human immunodeficiency virus type 1 isolate. *Journal of Virology*, *76*(12), 6277–6292.

Goulder, P. J., & Watkins, D. I. (2008). Impact of MHC class I diversity on immune control of immunodeficiency virus replication. *Nature Reviews Immunology*, *8*(8), 619–630.

Guerini, F. R., Delbue, S., Zanzottera, M., Agliardi, C., Saresella, M., Mancuso, R., et al. (2008). Analysis of CCR5, CCR2, SDF1 and RANTES gene polymorphisms in subjects with HIV-related PML and not determined leukoencephalopathy. *Biomedicine & Pharmacotherapy*, *62*(1), 26–30.

Gupta, J. D., Satishchandra, P., Gopukumar, K., Wilkie, F., Waldrop-Valverde, D., Ellis, R., et al. (2007). Neuropsychological deficits in human immunodeficiency virus type 1 clade C-seropositive adults from south India. *Journal of Neurovirology*, *13*(3), 195–202.

Haas, D. W., Ribaudo, H. J., Kim, R. B., Tierney, C., Wilkinson, G. R., Gulick, R. M., et al. (2004). Pharmacogenetics of efavirenz and central nervous system side effects: An adult AIDS clinical trials group study. *AIDS*, *18*(18), 2391–2400.

Habegger de Sorrentino, A., Lopez, R., Motta, P., Marinic, K., Sorrentino, A., Iliovich, E., et al. (2005). HLA class II involvement in HIV-associated toxoplasmic encephalitis development. *Clinical Immunology*, *115*(2), 133–137.

Hammes, S. R., Dixon, E. P., Malim, M. H., Cullen, B. R., & Greene, W. C. (1989). Nef protein of human immunodeficiency virus type 1: Evidence against its role as a transcriptional inhibitor. *Proceedings of the National Academy of Sciences of the United States of America*, *86*(23), 9549–9553.

Han, S. H., Einstein, G., Weisgraber, K. H., Strittmatter, W. J., Saunders, A. M., Pericak-Vance, M., et al. (1994). Apolipoprotein E is localized to the cytoplasm of human cortical neurons: A light and electron microscopic study. *Journal of Neuropathology & Experimental Neurology*, *53*(5), 535–544.

Hanna, Z., Priceputu, E., Chrobak, P., Hu, C., Dugas, V., Goupil, M., et al. (2009). Selective expression of human immunodeficiency virus nef in specific immune cell populations of transgenic mice is associated with distinct AIDS-like phenotypes. *Journal of Virology*, *83*(19), 9743–9758.

Haughey, N. J., Holden, C. P., Nath, A., & Geiger, J. D. (1999). Involvement of inositol 1,4,5-trisphosphate-regulated stores of intracellular calcium in calcium dysregulation and neuron cell death caused by HIV-1 protein tat. *Journal of Neurochemistry*, *73*(4), 1363–1374.

Haughey, N. J., & Mattson, M. P. (2002). Calcium dysregulation and neuronal apoptosis by the HIV-1 proteins tat and gp120. *Journal of Acquired Immune Deficiency Syndromes: JAIDS*, *31*(Suppl 2), S55–61.

He, J., Chen, Y., Farzan, M., Choe, H., Ohagen, A., Gartner, S., et al. (1997). CCR3 and CCR5 are co-receptors for HIV-1 infection of microglia. *Nature*, *385*(6617), 645–649.

Heidenreich, F., Arendt, G., Jander, S., Jablonowski, H., & Stoll, G. (1994). Serum and cerebrospinal fluid levels of soluble intercellular adhesion molecule 1 (sICAM-1) in patients with HIV-1 associated neurological diseases. *Journal of Neuroimmunology*, *52*(2), 117–126.

Hemelaar, J., Gouws, E., Ghys, P. D., & Osmanov, S. (2006). Global and regional distribution of HIV-1 genetic subtypes and recombinants in 2004. *AIDS*, *20*(16), W13–23.

Hendrickson, S. L., Jacobson, L. P., Nelson, G. W., Phair, J. P., Lautenberger, J., Johnson, R. C., et al. (2008). Host genetic influences on highly active antiretroviral therapy efficacy and AIDS-free survival. *Journal of Acquired Immune Deficiency Syndromes: JAIDS*, *48*(3), 263–271.

Herzberg, U. & Sagen, J. (2001). Peripheral nerve exposure to HIV viral envelope protein gp120 induces neuropathic pain and spinal gliosis. *Journal of Neuroimmunology*, *116*(1), 29–39.

Heyes, M., Brew, B., Martin, A., Price, R., Salazar, A., Sidtis, J., et al. (1991). Quinolinic acid in cerebrospinal fluid and serum in HIV-1 infection: Relationship to clinical and neurological status. *Annals of Neurology*, *29*(2), 202–9; 202.

Huang, K. J., Alter, G. M., & Wooley, D. P. (2002). The reverse transcriptase sequence of human immunodeficiency virus type 1 is under positive evolutionary selection within the central nervous system. *Journal of Neurovirology*, *8*(4), 281–294.

Hulgan, T., Haas, D. W., Haines, J. L., Ritchie, M. D., Robbins, G. K., Shafer, R. W., et al. (2005). Mitochondrial haplogroups and peripheral neuropathy during antiretroviral therapy: An adult AIDS clinical trials group study. *AIDS*, *19*(13), 1341–1349.

Hwang, S. S., Boyle, T. J., Lyerly, H. K., & Cullen, B. R. (1991). Identification of the envelope V3 loop as the primary determinant of cell tropism in HIV-1. *Science*, *253*(5015), 71–74.

Ickovics, J. R., & Rodin, J. (1992). Women and AIDS in the United States: Epidemiology, natural history, and mediating mechanisms. *Health Psychology*, *11*(1), 1–16.

Ioannidis, J. P., Contopoulos-Ioannidis, D. G., Rosenberg, P. S., Goedert, J. J., De Rossi, A., Espanol, T., et al. (2003). Effects of CCR5-delta32 and CCR2–64I alleles on disease progression of perinatally HIV-1-infected children: An international meta-analysis. *AIDS*, *17*(11), 1631–1638.

Jayadev, S. & Garden, G. A. (2009). Host and viral factors influencing the pathogenesis of HIV-associated neurocognitive disorders. *Journal of Neuroimmune Pharmacology: The Official Journal of the Society on NeuroImmune Pharmacology*, *4*(2), 175–189.

John, G. C., Rousseau, C., Dong, T., Rowland-Jones, S., Nduati, R., Mbori-Ngacha, D., et al. (2000). Maternal SDF1 3'A polymorphism is associated with increased perinatal human immunodeficiency virus type 1 transmission. *Journal of Virology*, *74*(12), 5736–5739.

Johnston, J. B., Jiang, Y., van Marle, G., Mayne, M. B., Ni, W., Holden, J., et al. (2000). Lentivirus infection in the brain induces matrix metalloproteinase expression: Role of envelope diversity. *Journal of Virology*, *74*(16), 7211–7220.

Johnston, J., Zhang, K., Silva, C., Shalinsky, D., Conant, K., Ni, W., et al. (2001). HIV-1 tat neurotoxicity is prevented by matrix metalloproteinase inhibitors. *Annals of Neurology*, *49*(2), 230–241.

Jones, G. J., Barsby, N. L., Cohen, E. A., Holden, J., Harris, K., Dickie, P., et al. (2007). HIV-1 vpr causes neuronal apoptosis and in vivo neurodegeneration. *J Neurosci*, *27*(14), 3703–3711.

Jones, G., Zhu, Y., Silva, C., Tsutsui, S., Pardo, C. A., Keppler, O. T., et al. (2005). Peripheral nerve-derived HIV-1 is predominantly CCR5-dependent and causes neuronal degeneration and neuroinflammation. *Virology*, *334*(2), 178–193.

Jones, M. V., Bell, J. E., & Nath, A. (2000). Immunolocalization of HIV envelope gp120 in HIV encephalitis with dementia. *AIDS*, *14*(17), 2709–2713.

Kaleebu, P., French, N., Mahe, C., Yirrell, D., Watera, C., Lyagoba, F., et al. (2002). Effect of human immunodeficiency virus (HIV) type 1 envelope subtypes A and D on disease progression in a large cohort of HIV-1-positive persons in Uganda. *Journal of Infectious Diseases*, *185*(9), 1244–1250.

Kaleebu, P., Nankya, I. L., Yirrell, D. L., Shafer, L. A., Kyosiimire-Lugemwa, J., Lule, D. B., et al. (2007). Relation between chemokine receptor use, disease stage, and HIV-1 subtypes A and D: Results from a rural Ugandan cohort. *Journal of Acquired Immune Deficiency Syndromes: JAIDS*, *45*(1), 28–33.

Kanmogne, G. D., Schall, K., Leibhart, J., Knipe, B., Gendelman, H. E., & Persidsky, Y. (2007). HIV-1 gp120 compromises blood-brain barrier integrity and enhances monocyte migration across blood-brain barrier: Implication for viral neuropathogenesis. *Journal of Cerebral Blood Flow & Metabolism*, *27*(1), 123–134.

Kaul, M. & Lipton, S. A. (1999). Chemokines and activated macrophages in HIV gp120-induced neuronal apoptosis. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(14), 8212–8216.

Kaul, M., Zheng, J., Okamoto, S., Gendelman, H. E., & Lipton, S. A. (2005). HIV-1 infection and AIDS: Consequences for the central nervous system. *Cell Death & Differentiation*, *12*(Suppl 1), 878–892.

Kelder, W., McArthur, J. C., Nance-Sproson, T., McClernon, D., & Griffin, D. E. (1998). Beta-chemokines MCP-1 and RANTES are selectively increased in cerebrospinal fluid of patients with human immunodeficiency virus-associated dementia. *Annals of Neurology*, *44*(5), 831–835.

Keswani, S. C., Jack, C., Zhou, C., & Hoke, A. (2006). Establishment of a rodent model of HIV-associated sensory neuropathy. *Journal of Neuroscience*, *26*(40), 10299–10304.

Kilareski, E., Shah, S., Nonnemacher, M., & Wigdahl, B. (2009). Regulation of HIV-1 transcription in cells of the monocyte-macrophage lineage. *Retrovirology*, *6*(1), 118.

Kim, H. J., Martemyanov, K. A., & Thayer, S. A. (2008). Human immunodeficiency virus protein tat induces synapse loss via a reversible process that is distinct from cell death. *J. Neurosci.*, *28*(48), 12604–12613.

Kitayama, H., Miura, Y., Ando, Y., Hoshino, S., Ishizaka, Y., & Koyanagi, Y. (2008). Human immunodeficiency virus type 1 vpr inhibits axonal outgrowth through induction of mitochondrial dysfunction. *J. Virol.*, *82*(5), 2528–2542.

Kiwanuka, N., Laeyendecker, O., Robb, M., Kigozi, G., Arroyo, M., McCutchan, F., et al. (2008). Effect of human immunodeficiency virus type 1 (HIV-1) subtype on disease progression in persons from Rakai, Uganda, with incident HIV-1 infection. *Journal of Infectious Diseases*, *197*(5), 707–713.

Klase, Z., Kale, P., Winograd, R., Gupta, M. V., Heydarian, M., Berro, R., et al. (2007). HIV-1 TAR element is processed by dicer to yield a viral micro-RNA involved in chromatin remodeling of the viral LTR. *BMC Mol Biol*, *8*, 63.

Klase, Z., Winograd, R., Davis, J., Carpio, L., Hildreth, R., Heydarian, M., et al. (2009). HIV-1 TAR miRNA protects against apoptosis by altering cellular gene expression. *Retrovirology*, *6*(1), 18.

Koedel, U., Kohleisen, B., Sporer, B., Lahrtz, F., Ovod, V., Fontana, A., et al. (1999). HIV type 1 nef protein is a viral factor for leukocyte recruitment into the central nervous system. *J Immunol*, *163*(3), 1237–1245.

Kong, L. Y., Wilson, B. C., McMillian, M. K., Bing, G., Hudson, P. M., & Hong, J. S. (1996). The effects of the HIV-1 envelope protein gp120 on the production of nitric oxide and proinflammatory cytokines in mixed glial cell cultures. *Cellular Immunology*, *172*(1), 77–83.

Krentz, H., & Gill, M. J. (2009). The five-year impact of an evolving global epidemic, changing migration patterns, and policy changes in a regional canadian HIV population. *Health Policy*, *90*(2–3), 296–302.

Kruman, I., Nath, A., & Mattson, M. (1998). HIV protein tat induces apoptosis by a mechanism involving mitochondrial calcium overload and caspase activation. *Exp Neurol*, *154*, 276–288.

Kure, K., Llena, J. F., Lyman, W. D., Soeiro, R., Weidenheim, K. M., Hirano, A., et al. (1991). Human immunodeficiency virus-1 infection of the nervous system: An autopsy study of 268 adult, pediatric, and fetal brains. *Human Pathology*, *22*(7), 700–710.

Kure, K., Lyman, W. D., Weidenheim, K. M., & Dickson, D. W. (1990). Cellular localization of an HIV-1 antigen in subacute AIDS encephalitis using an improved double-labeling immunohistochemical method. *Am J Pathol*, *136*(5), 1085–1092.

Lannuzel, A., Lledo, P., Lamghitnia, H. O., Vincent, J., & Tardieu, M. (1995). HIV-1 envelope proteins gp120 and gp160 potentiate NMDA-induced [Ca2+]i increase, alter [Ca2+]i homeostasis and induce neurotoxicity in human embryonic neurons. *European Journal of Neuroscience*, *7*(11), 2285–93; 2285.

Letendre, S. L., Lanier, E. R., & McCutchan, J. A. (1999). Cerebrospinal fluid beta chemokine concentrations in neurocognitively impaired individuals infected with human immunodeficiency virus type 1. *Journal of Infectious Diseases*, *180*(2), 310–319.

Levy, D. N., Refaeli, Y., MacGregor, R. R., & Weiner, D. B. (1994). Serum vpr regulates productive infection and latency of human immunodeficiency virus type 1. *Proc Natl Acad Sci U S A*, *91*, 10873–10877.

Li, W., Huang, Y., Reid, R., Steiner, J., Malpica-Llanos, T., Darden, T. A., et al. (2008). NMDA receptor activation by HIV-tat protein is clade dependent. *Journal of Neuroscience*, *28*(47), 12190–12198.

Li, W., Li, G., Steiner, J., & Nath, A. (2009). Role of tat protein in HIV neuropathogenesis. *Neurotoxicity Research*, *16*(3), 205–220.

Lindemann, D., Wilhelm, R., Renard, P., Althage, A., Zinkernagel, R., & Mous, J. (1994). Severe immunodeficiency associated with a human immunodeficiency virus 1 NEF/3'-long terminal repeat transgene. *Journal of Experimental Medicine*, *179*(3), 797–807.

Liner, K. J., 2nd, Hall, C. D., & Robertson, K. R. (2007). Impact of human immunodeficiency virus (HIV) subtypes on HIV-associated neurological disease. *Journal of Neurovirology*, *13*(4), 291–304.

Lipton, S. A. (1992). Requirement for macrophages in neuronal injury induced by HIV envelope protein gp120. *NeuroReport*, *3*, 913–915.

Lipton, S. A., Sucher, N. J., Kaiser, P. K., & Dreyer, E. B. (1991). Synergistic effects of HIV coat protein and NMDA receptor-mediated neurotoxicity. *Neuron*, *7*(1), 111–118.

Liu, H., Chao, D., Nakayama, E. E., Taguchi, H., Goto, M., Xin, X., et al. (1999). Polymorphism in RANTES chemokine promoter affects HIV-1 disease progression. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(8), 4581–4585.

Liu, R., Paxton, W. A., Choe, S., Ceradini, D., Martin, S. R., Horuk, R., et al. (1996). Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell*, *86*(3), 367–377.

Liu, Y., Jones, M., Hingtgen, C., Bu, G., Laribee, N., Tanzi, R., et al. (2000). Uptake of HIV-1 tat proteinmediated by low density lipoprotein receptor-related protein disrupts the neuronal metabolic balance of the receptor ligands. *Nature Medicine*, *6*, 1380–1387.

Lopez, O. L., Wess, J., Sanchez, J., Dew, M. A., & Becker, J. T. (1999). Neurological characteristics of HIV-infected men and women seeking primary medical care. *European Journal of Neurology*, *6*(2), 205–209.

Lum, J. J., Cohen, O. J., Nie, Z., Weaver, J. G., Gomez, T. S., Yao, X. J., et al. (2003). Vpr R77Q is associated with long-term nonprogressive HIV infection and impaired induction of apoptosis. *J Clin Invest*, *111*, 1547–1554.

Luo, X., Carlson, K. A., Wojna, V., Mayo, R., Biskup, T. M., Stoner, J., et al. (2003). Macrophage proteomic fingerprinting predicts HIV-1-associated cognitive impairment. *Neurology*, *60*(12), 1931–1937.

MacDonald, K. S., Fowke, K. R., Kimani, J., Dunand, V. A., Nagelkerke, N. J., Ball, T. B., et al. (2000). Influence of HLA supertypes on susceptibility and resistance to human immunodeficiency virus type 1 infection. *Journal of Infectious Diseases*, *181*(5), 1581–1589.

Magnuson, D., Knudsen, B., Geiger, J., Brownstone, R., & Nath, A. (1995). Human immunodeficiency virus type 1 tat activates non-N-methyl-D-aspartate excitatory amino acid receptors and causes neurotoxicity. *Annals of Neurology*, *37*(3), 373–80.

Mahadevan, A., Shankar, S. K., Satishchandra, P., Ranga, U., Chickabasaviah, Y. T., Santosh, V., et al. (2007). Characterization of human immunodeficiency virus (HIV)-infected cells in infiltrates associated with CNS opportunistic infections in patients with HIV clade C infection. *Journal of Neuropathology & Experimental Neurology*, *66*(9), 799–808.

Mahley, R. W. & Rall, S. C., Jr. (2000). Apolipoprotein E: Far more than a lipid transport protein. *Annual Review of Genomics & Human Genetics*, *1*, 507–537.

Mankowski, J. L., Flaherty, M. T., Spelman, J. P., Hauer, D. A., Didier, P. J., Amedee, A. M., et al. (1997). Pathogenesis of simian immunodeficiency virus encephalitis: Viral determinants of neurovirulence. *Journal of Virology*, *71*(8), 6055–6060.

Maragos, W. F., Tillman, P., Jones, M., Bruce-Keller, A. J., Roth, S., Bell, J. E., et al. (2003). Neuronal injury in hippocampus with human

immunodeficiency virus transactivating protein, tat. *Neuroscience*, *117*(1), 43–53.
Martinson, J. J., Chapman, N. H., Rees, D. C., Liu, Y. T., & Clegg, J. B. (1997). Global distribution of the CCR5 gene 32-basepair deletion. *Nature Genetics*, *16*(1), 100–103.
Martinson, J. J., Hong, L., Karanicolas, R., Moore, J. P., & Kostrikis, L. G. (2000). Global distribution of the CCR2–64I/CCR5–59653T HIV-1 disease-protective haplotype. *AIDS*, *14*(5), 483–489.
Marzolini, C., Telenti, A., Decosterd, L. A., Greub, G., Biollaz, J., & Buclin, T. (2001). Efavirenz plasma levels can predict treatment failure and central nervous system side effects in HIV-1-infected patients. *AIDS*, *15*(1), 71–75.
Masliah, E., Ge, N., Achim, C. L., Hansen, L. A., & Wiley, C. A. (1992). Selective neuronal vulnerability in HIV encephalitis. *Journal of Neuropathology & Experimental Neurology*, *51*(6), 585–593.
Mason, K. I., Campbell, A., Hawkins, P., Madhere, S., Johnson, K., & Takushi-Chinen, R. (1998). Neuropsychological functioning in HIV-positive African-American women with a history of drug use. *Journal of the National Medical Association*, *90*(11), 665–674.
Mattson, M. P., Haughey, N. J., & Nath, A. (2005). Cell death in HIV dementia. *Cell Death & Differentiation*, *12*(Suppl 1), 893–904.
Mayne, M., Bratanich, A. C., Chen, P., Rana, F., Nath, A., & Power, C. (1998). HIV-1 tat molecular diversity and induction of TNF-α: Implications for HIV-induced neurological disease. *Neuroimmunomodulation*, *5*, 184–192.
Melnick, S. L., Sherer, R., Louis, T. A., Hillman, D., Rodriguez, E. M., Lackman, C., et al. (1994). Survival and disease progression according to gender of patients with HIV infection. The Terry Beirn Community Programs for Clinical Research on AIDS. *JAMA*, *272*(24), 1915–1921.
Messam, C. A. & Major, E. O. (2000). Stages of restricted HIV-1 infection in astrocyte cultures derived from human fetal brain tissue. *Journal of Neurovirology*, *6*(Suppl 1), S90–4.
Messmer, D., Ignatius, R., Santisteban, C., Steinman, R. M., & Pope, M. (2000). The decreased replicative capacity of simian immunodeficiency virus SIVmac239Delta nef is manifest in cultures of immature dendritic cells and T cells. *J. Virol.*, *74*(5), 2406–2413.
Metsch, L. R., McCoy, C. B., McCoy, H. V., Shultz, J., Inciardi, J., Wolfe, H., et al. (1998). Social influences: Living arrangements of drug using women at risk for HIV infection. *Women & Health*, 27(1–2), 123–136.
Meyer, L., Magierowska, M., Hubert, J. B., Rouzioux, C., Deveau, C., Sanson, F., et al. (1997). Early protective effect of CCR-5 delta 32 heterozygosity on HIV-1 disease progression: Relationship with viral load. The SEROCO study group. *AIDS*, *11*(11), F73–8.
Milligan, E. D., Mehmert, K. K., Hinde, J. L., Harvey, L. O., Martin, D., Tracey, K. J., et al. (2000). Thermal hyperalgesia and mechanical allodynia produced by intrathecal administration of the human immunodeficiency virus-1 (HIV-1) envelope glycoprotein, gp120. *Brain Research*, *861*(1), 105–116.
Mirsattari, S. M., Johnston, J. B., McKenna, R., Del Bigio, M. R., Orr, P., Ross, R. T., et al. (2001). Aboriginals with multiple sclerosis: HLA types and predominance of neuromyelitis optica. *Neurology*, *56*(3), 317–323.
Mishra, M., Vetrivel, S., Siddappa, N., Ranga, U., & Seth, P. (2008). Clade-specific differences in neurotoxicity of human immunodeficiency virus-1 B and C tat of human neurons: Significance of dicysteine C30C31 motif. *Annals of Neurology*, *63*(3), 366–376.
Modi, G., Hari, K., Modi, M., & Mochan, A. (2007). The frequency and profile of neurology in black South African HIV-infected (clade C) patients—a hospital-based prospective audit. *Journal of the Neurological Sciences*, *254*(1–2), 60–64.
Mologni, D., Citterio, P., Menzaghi, B., Poma, B. Z., Riva, C., Broggini, V., et al. (2006). Vpr and HIV-1 disease progression: R77Q mutation is associated with long-term control of HIV-1 infection in different groups of patients. *AIDS*, *20*(4), 567–574.
Monteiro de Almeida, S., Letendre, S., Zimmerman, J., Kolakowski, S., Lazzaretto, D., McCutchan, J. A., et al. (2006). Relationship of CSF leukocytosis to compartmentalized changes in MCP-1/CCL2 in the CSF of HIV-infected patients undergoing interruption of antiretroviral therapy. *Journal of Neuroimmunology*, *179*(1–2), 180–185.
Mordelet, E., Kissa, K., Cressant, A., Gray, F., Ozden, S., Vidal, C., et al. (2004). Histopathological and cognitive defects induced by nef in the brain. *FASEB J.*, *18*(15), 1851–1861.
Na, H., Acharjee, S., Jones, G., Vivithanaporn, P., Noorbakhsh, F., et al. (2011). Interactions between human immunodeficiency virus (HIV)-1 Vpr expression and innate immunity influence neurovirulence. *Retrovirology, 8*, 44–61.
Narayan, O., Joag, S. V., & Stephens, E. B. (1995). Selected models of HIV-induced neurological disease. *Current Topics in Microbiology & Immunology*, *202*, 151–166.
Nath, A., Haughey, N., Jones, M., Anderson, C., Bell, J., & Geiger, J. (2000). Synergistic neurotoxicity by human immunodeficiency virus proteins tat and gp120: Protection by memantine. *Annals of Neurology*, *47*, 186–194.
Nath, A., Psooy, K., Martin, C., Knudsen, B., Magnuson, D. S., Haughey, N., et al. (1996). Identification of a human immunodeficiency virus type 1 tat epitope that is neuroexcitatory and neurotoxic. *J. Virol.*, *70*(3), 1475–1480.
Nath, A., Hauser, K. F., Wojna, V., Booze, R. M., Maragos, W., Prendergast, M., et al. (2002). Molecular basis for interactions of HIV and drugs of abuse. *JAIDS: Journal of Acquired Immune Deficiency Syndromes*, *31 Supplement*(2), S62–S69.
Nath, A., Anderson, C., Jones, M., Maragos, W., Booze, R., Mactutus, C., et al. (2000). Neurotoxicity and dysfunction of dopaminergic systems associated with AIDS dementia. *J Psychopharmacol*, *14*(3), 222–227.
Neumann, M., Felber, B. K., Kleinschmidt, A., Froese, B., Erfle, V., Pavlakis, G. N., et al. (1995). Restriction of human immunodeficiency virus type 1 production in a human astrocytoma cell line is associated with a cellular block in rev function. *Journal of Virology*, *69*(4), 2159–2167.
Noorbakhsh, F., Overall, C. M., & Power, C. (2009). Deciphering complex mechanisms in neurodegenerative diseases: The advent of systems biology. *Trends in Neurosciences*, *32*(2), 88–100.
Noorbakhsh, F., Ramachandran, R., Barsby, N., Ellestad, K. K., LeBlanc, A., Dickie, P., et al. (2010). MicroRNA profiling reveals new aspects of HIV neurodegeneration: Caspase-6 regulates astrocyte survival. *FASEB J*, fj. 09–147819.
Norman, J. P., Perry, S. W., Reynolds, H. M., Kiebala, M., De Mesy Bentley, K. L., Trejo, M., et al. (2008). HIV-1 tat activates neuronal ryanodine receptors with rapid induction of the unfolded protein response and mitochondrial hyperpolarization. *PLoS ONE*, *3*(11), e3731.
Nottet, H. S., Persidsky, Y., Sasseville, V. G., Nukuna, A. N., Bock, P., Zhai, Q. H., et al. (1996). Mechanisms for the transendothelial migration of HIV-1-infected monocytes into brain. *Journal of Immunology*, *156*(3), 1284–1295.
Nuovo, G. J., Gallery, F., MacConnell, P., & Braun, A. (1994). In situ detection of polymerase chain reaction-amplified HIV-1 nucleic acids and tumor necrosis factor-alpha RNA in the central nervous system. *American Journal of Pathology*, *144*(4), 659–666.
Ohagen, A., Devitt, A., Kunstman, K. J., Gorry, P. R., Rose, P. P., Korber, B., et al. (2003). Genetic and functional analysis of full-length human immunodeficiency virus type 1 env genes derived from brain and blood of patients with AIDS. *Journal of Virology*, *77*(22), 12336–12345.
Owen, A., Chandler, B., & Back, D. J. (2005). The implications of P-glycoprotein in HIV: Friend or foe? *Fundamental & Clinical Pharmacology*, *19*(3), 283–296.
Parczewski, M., Leszczyszyn-Pynka, M., Kaczmarczyk, M., Adler, G., Binczak-Kuleta, A., Loniewska, B., et al. (2009). Sequence variants of chemokine receptor genes and susceptibility to HIV-1 infection. *Journal of Applied Genetics*, *50*(2), 159–166.
Patel, C. A., Mukhtar, M., Harley, S., Kulkosky, J., & Pomerantz, R. J. (2002). Lentiviral expression of HIV-1 vpr induces apoptosis in human neurons. *J Neurovirol*, *8*, 86–99.
Patel, C. A., Mukhtar, M., & Pomerantz, R. J. (2000). Human immunodeficiency virus type 1 vpr induces apoptosis in human neuronal cells. *J Virol*, *74*, 9717–9726.

Patrick, M. K., Johnston, J. B., & Power, C. (2002). Lentiviral neuropathogenesis: Comparative neuroinvasion, neurotropism, neurovirulence, and host neurosusceptibility. *Journal of Virology*, *76*(16), 7923–7931.

Pattarini, R., Pittaluga, A., & Raiteri, M. (1998). The human immunodeficiency virus-1 envelope protein gp120 binds through its V3 sequence to the glycine site of N-methyl—aspartate receptors mediating noradrenaline release in the hippocampus. *Neuroscience*, *87*(1), 147–157.

Peeters, M., Vincent, R., Perret, J. L., Lasky, M., Patrel, D., Liegeois, F., et al. (1999). Evidence for differences in MT2 cell tropism according to genetic subtypes of HIV-1: Syncytium-inducing variants seem rare among subtype C HIV-1 viruses. *Journal of Acquired Immune Deficiency Syndromes & Human Retrovirology*, *20*(2), 115–121.

Peluso, R., Haase, A., Stowring, L., Edwards, M., & Ventura, P. (1985). A Trojan horse mechanism for the spread of visna virus in monocytes. *Virology*, *147*(1), 231–236.

Pemberton, L. A., Kerr, S. J., Smythe, G., & Brew, B. J. (1997). Quinolinic acid production by macrophages stimulated with IFN-gamma, TNF-alpha, and IFN-alpha. *Journal of Interferon & Cytokine Research*, *17*(10), 589–595.

Pemberton, L. A., Stone, E., Price, P., van Bockxmeer, F., & Brew, B. J. (2008). The relationship between ApoE, TNFA, IL1a, IL1b and IL12b genes and HIV-1-associated dementia. *HIV Medicine*, *9*(8), 677–680.

Pereda, M., Ayuso-Mateos, J. L., Gomez Del Barrio, A., Echevarria, S., Farinas, M. C., Garcia Palomo, D., et al. (2000). Factors associated with neuropsychological performance in HIV-seropositive subjects without AIDS. *Psychological Medicine*, *30*(1), 205–217.

Petito, C. K., Chen, H., Mastri, A. R., Torres-Munoz, J., Roberts, B., & Wood, C. (1999). HIV infection of choroid plexus in AIDS and asymptomatic HIV-infected patients suggests that the choroid plexus may be a reservoir of productive infection. *Journal of Neurovirology*, *5*(6), 670–677.

Phillips, E. J., & Mallal, S. A. (2008). Pharmacogenetics and the potential for the individualization of antiretroviral therapy. *Current Opinion in Infectious Diseases*, *21*(1), 16–24.

Piacentini, L., Biasin, M., Fenizia, C., & Clerici, M. (2009). Genetic correlates of protection against HIV infection: The ally within. *Journal of Internal Medicine*, *265*(1), 110–124.

Pillai, S. K., Pond, S. L. K., Liu, Y., Good, B. M., Strain, M. C., Ellis, R. J., et al. (2006). Genetic attributes of cerebrospinal fluid-derived HIV-1 env. *Brain*, *129*(7), 1872–1883.

Piller, S. C., Ewart, G. D., Jans, D. A., Gage, P. W., & Cox, G. B. (1999). The amino-terminal region of vpr from human immunodeficiency virus type 1 forms ion channels and kills neurons. *J Virol*, *73*, 4230–4238.

Piller, S. C., Ewart, G. D., Premkumar, A., Cox, G. B., & Gage, P. W. (1996). Vpr protein of human immunodeficiency virus type 1 forms cation-selective channels in planar lipid bilayers. *Proc Natl Acad Sci U S A*, *93*, 111–115.

Pitas, R. E., Boyles, J. K., Lee, S. H., Hui, D., & Weisgraber, K. H. (1987). Lipoproteins and their receptors in the central nervous system. Characterization of the lipoproteins in cerebrospinal fluid and identification of apolipoprotein B,E(LDL) receptors in the brain. *Journal of Biological Chemistry*, *262*(29), 14352–14360.

Plantier, J. C., Leoz, M., Dickerson, J. E., De Oliveira, F., Cordonnier, F., Lemee, V., et al. (2009). A new human immunodeficiency virus derived from gorillas. *Nature Medicine*, *15*(8), 871–872.

Pocernich, C. B., Sultana, R., Mohmmad-Abdul, H., Nath, A., & Butterfield, D. A. (2005). HIV-dementia, tat-induced oxidative stress, and antioxidant therapeutic considerations. *Brain Research - Brain Research Reviews*, *50*(1), 14–26.

Polazzi, E., Levi, G., & Minghetti, L. (1999). Human immunodeficiency virus type 1 tat protein stimulates inducible nitric oxide synthase expression and nitric oxide production in microglial cultures. *J Neuropathol Exp Neurol*, *58*(8), 825–31.

Poli, G., Kinter, A., Justement, J. S., Kehrl, J. H., Bressler, P., Stanley, S., et al. (1990). Tumor necrosis factor alpha functions in an autocrine manner in the induction of human immunodeficiency virus expression. *Proceedings of the National Academy of Sciences of the United States of America*, *87*(2), 782–785.

Pond, S. L. K., Poon, A. F. Y., Zárate, S., Smith, D. M., Little, S. J., Pillai, S. K., et al. (2008). Estimating selection pressures on HIV-1 using phylogenetic likelihood models. *Statistics in Medicine*, *27*(23), 4779–4789.

Power, C., Gill, M., & Johnson, R. (2002). Progress in Clinical Neurosciences: The neuropathogenesis of HIV infection: Host-virus interaction and the impact of therapy. *The Canadian Journal of Neurological Sciences*, *29*(1), 19–32.

Power, C. & Johnson, R. T. (2001). Neuroimmune and neurovirological aspects of human immunodeficiency virus infection. *Advances in Virus Research*, *56*, 389–433.

Power, C., McArthur, J. C., Johnson, R. T., Griffin, D. E., Glass, J. D., Perryman, S., et al. (1994). Demented and nondemented patients with AIDS differ in brain-derived human immunodeficiency virus type 1 envelope sequences. *J Virol*, *68*(7), 4643–4649.

Power, C., McArthur, J. C., Nath, A., Wehrly, K., Mayne, M., Nishio, J., et al. (1998). Neuronal death induced by brain-derived human immunodeficiency virus type 1 envelope genes differs between demented and nondemented AIDS patients. *Journal of Virology*, *72*(11), 9045–9053.

Price, P., Keane, N. M., Stone, S. F., Cheong, K. Y., & French, M. A. (2001). MHC haplotypes affect the expression of opportunistic infections in HIV patients. *Human Immunology*, *62*(2), 157–164.

Price, P., Morahan, G., Huang, D., Stone, E., Cheong, K. Y., Castley, A., et al. (2002). Polymorphisms in cytokine genes define subpopulations of HIV-1 patients who experienced immune restoration diseases. *AIDS*, *16*(15), 2043–2047.

Quasney, M. W., Zhang, Q., Sargent, S., Mynatt, M., Glass, J., & McArthur, J. (2001). Increased frequency of the tumor necrosis factor-alpha-308 A allele in adults with human immunodeficiency virus dementia. *Annals of Neurology*, *50*(2), 157–162.

Rabkin, J. G., Ferrando, S. J., van Gorp, W., Rieppi, R., McElhiney, M., & Sewell, M. (2000). Relationships among apathy, depression, and cognitive impairment in HIV/AIDS. *Journal of Neuropsychiatry & Clinical Neurosciences*, *12*(4), 451–457.

Rao, V. R., Sas, A. R., Eugenin, E. A., Siddappa, N. B., Bimonte-Nelson, H., Berman, J. W., et al. (2008). HIV-1 clade-specific differences in the induction of neuropathogenesis. *Journal of Neuroscience*, *28*(40), 10010–10016.

Rappaport, J., Joseph, J., Croul, S., Alexander, G., Del Valle, L., Amini, S., et al. (1999). Molecular pathway involved in HIV-1-induced CNS pathology: Role of viral regulatory protein, tat. *J Leukocyte Biol*, *65*(458–465).

Reddy, R. T., Achim, C. L., Sirko, D. A., Tehranchi, S., Kraus, F. G., Wong-Staal, F., et al. (1996). Sequence analysis of the V3 loop in brain and spleen of patients with HIV encephalitis. *AIDS Research & Human Retroviruses*, *12*(6), 477–482.

Reyes, M. G., Faraldi, F., Senseng, C. S., Flowers, C., & Fariello, R. (1991). Nigral degeneration in acquired immune deficiency syndrome (AIDS). *Acta Neuropathologica*, *82*(1), 39–44.

Robertson, K. R., Kapoor, C., Robertson, W. T., Fiscus, S., Ford, S., & Hall, C. D. (2004). No gender differences in the progression of nervous system disease in HIV infection. *Journal of Acquired Immune Deficiency Syndromes: JAIDS*, *36*(3), 817–822.

Rom, I., Deshmane, S. L., Mukerjee, R., Khalili, K., Amini, S., & Sawaya, B. E. (2009). HIV-1 vpr deregulates calcium secretion in neural cells. *Brain Research*, *1275*, 81–86.

Ross, H. L., Gartner, S., McArthur, J. C., Corboy, J. R., McAllister, J. J., Millhouse, S., et al. (2001). HIV-1 LTR C/EBP binding site sequence configurations preferentially encountered in brain lead to enhanced C/EBP factor binding and increased LTR-specific activity. *Journal of Neurovirology*, *7*(3), 235–249.

Rumbaugh, J. A. & Nath, A. (2006). Developments in HIV neuropathogenesis. *Current Pharmaceutical Design*, *12*(9), 1023–1044.

Russo, R., Siviglia, E., Gliozzi, M., Amantea, D., Paoletti, A., Berliocchi, L., et al. (2007). Evidence implicating matrix metalloproteinases in the mechanism underlying accumulation of IL-1β and neuronal apoptosis in the neocortex of HIV/gp120-exposed rats. *International Review of Neurobiology* (pp. 407–421). Academic Press.

Sacktor, N., Nakasujja, N., Skolasky, R. L., Rezapour, M., Robertson, K., Musisi, S., et al. (2009). HIV subtype D is associated with dementia,

compared with subtype A, in immunosuppressed individuals at risk of cognitive impairment in Kampala, Uganda. *Clinical Infectious Diseases*, *49*(5), 780–786.

Saha, R. N. & Pahan, K. (2007). Differential regulation of mn-superoxide dismutase in neurons and astroglia by HIV-1 gp120: Implications for HIV-associated dementia. *Free Radical Biology and Medicine*, *42*(12), 1866–1878.

Saito, Y., Sharer, L. R., Epstein, L. G., Michaels, J., Mintz, M., Louder, M., et al. (1994). Overexpression of nef as a marker for restricted HIV-1 infection of astrocytes in postmortem pediatric central nervous tissues. *Neurology*, *44*(3 Pt 1), 474–481.

Samson, M., Libert, F., Doranz, B. J., Rucker, J., Liesnard, C., Farber, C. M., et al. (1996). Resistance to HIV-1 infection in Caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. *Nature*, *382*(6593), 722–725.

Sas, A. R., Bimonte-Nelson, H., Smothers, C. T., Woodward, J., & Tyor, W. R. (2009). Interferon-alpha causes neuronal dysfunction in encephalitis. *Journal of Neuroscience*, *29*(12), 3948–3955.

Sasseville, V. G., & Lackner, A. A. (1997). Neuropathogenesis of simian immunodeficiency virus infection in macaque monkeys. *Journal of Neurovirology*, *3*(1), 1–9.

Sawaya, B. E., Khalili, K., Gordon, J., Taube, R., & Amini, S. (2000). Cooperative interaction between HIV-1 regulatory proteins tat and vpr modulates transcription of the viral genome. *J Biol Chem*, *275*, 35209–35214.

Schwartz, S., Felber, B. K., & Pavlakis, G. N. (1991). Expression of human immunodeficiency virus type 1 vif and vpr mRNAs is rev-dependent and regulated by splicing. *Virology*, *183*(2), 677–686.

Shah, M., Smit, T. K., Morgello, S., Tourtellotte, W., Gelman, B., Brew, B. J., et al. (2006). Env gp120 sequence analysis of HIV type 1 strains from diverse areas of the brain shows preponderance of CCR5 usage. *AIDS Research and Human Retroviruses*, *22*(2), 177–181.

Shapshak, P., Segal, D. M., Crandall, K. A., Fujimura, R. K., Zhang, B. T., Xin, K. Q., et al. (1999). Independent evolution of HIV type 1 in different brain regions. *AIDS Research & Human Retroviruses*, *15*(9), 811–820.

Shimizu, N. S., Shimizu, N. G., Takeuchi, Y., & Hoshino, H. (1994). Isolation and characterization of human immunodeficiency virus type 1 variants infectious to brain-derived cells: Detection of common point mutations in the V3 region of the env gene of the variants. *Journal of Virology*, *68*(9), 6130–6135.

Si, Q., Kim, M., Zhao, M., Landau, N. R., Goldstein, H., & Lee, S. C. (2002). Vpr- and nef-dependent induction of RANTES/CCL5 in microglial cells. *Virology*, *301*(2), 342–353.

Singh, I. N., El-Hage, N., Campbell, M. E., Lutz, S. E., Knapp, P. E., Nath, A., et al. (2005). Differential involvement of p38 and JNK MAP kinases in HIV-1 tat and gp120-induced apoptosis and neurite degeneration in striatal neurons. *Neuroscience*, *135*(3), 781–790.

Singh, I. N., Goody, R. J., Dean, C., Ahmad, N. M., Lutz, S. E., Knapp, P. E., et al. (2004). Apoptotic death of striatal neurons induced by human immunodeficiency virus-1 tat and gp120: Differential involvement of caspase-3 and endonuclease G. *Journal of Neurovirology*, *10*(3), 141–151.

Singh, K. K., Barroga, C. F., Hughes, M. D., Chen, J., Raskino, C., McKinney, R. E., et al. (2003). Genetic influence of CCR5, CCR2, and SDF1 variants on human immunodeficiency virus 1 (HIV-1)-related disease progression and neurological impairment, in children with symptomatic HIV-1 infection. *Journal of Infectious Diseases*, *188*(10), 1461–1472.

Sippy, B. D., Hofman, F. M., Wallach, D., & Hinton, D. R. (1995). Increased expression of tumor necrosis factor-alpha receptors in the brains of patients with AIDS. *Journal of Acquired Immune Deficiency Syndromes & Human Retrovirology*, *10*(5), 511–521.

Smit, T. K., Wang, B., Ng, T., Osborne, R., Brew, B., & Saksena, N. K. (2001). Varied tropism of HIV-1 isolates derived from different regions of adult brain cortex discriminate between patients with and without AIDS dementia complex (ADC): Evidence for neurotropic HIV variants. *Virology*, *279*(2), 509–526.

Smith, D., Guillemin, G., Pemberton, L., Kerr, S., Nath, A., Smythe, G., et al. (2001). Quinolinic acid is produced by macrophages stimulated by platelet-activating factor, nef and tat. *Journal of Neurovirology*, *7*(1), 56–60.

Song, L., Nath, A., Geiger, J. D., Moore, A., & Hochman, S. (2003). Human immunodeficiency virus type 1 tat protein directly activates neuronal N-methyl-D-aspartate receptors at an allosteric zinc-sensitive site. *Journal of Neurovirology*, *9*(3), 399–403.

Sporer, B., Koedel, U., Paul, R., Kohleisen, B., Erfle, V., Fontana, A., et al. (2000). Human immunodeficiency virus type-1 nef protein induces blood-brain barrier disruption in the rat: Role of matrix metalloproteinase-9. *Journal of Neuroimmunology*, *102*(2), 125–130.

Stern, R. A., Silva, S. G., Chaisson, N., & Evans, D. L. (1996). Influence of cognitive reserve on neuropsychological functioning in asymptomatic human immunodeficiency virus-1 infection. *Archives of Neurology*, *53*(2), 148–153.

Stewart, G. J., Ashton, L. J., Biti, R. A., Ffrench, R. A., Bennetts, B. H., Newcombe, N. R., et al. (1997). Increased frequency of CCR-5 delta 32 heterozygotes among long-term non-progressors with HIV-1 infection. The Australian Long-term Non-progressor Study Group. *AIDS*, *11*(15), 1833–1838.

Su, B., Jin, L., Hu, F., Xiao, J., Luo, J., Lu, D., et al. (1999). Distribution of two HIV-1-resistant polymorphisms (SDF1–3'A and CCR2–64I) in East Asian and world populations and its implication in AIDS epidemiology. *American Journal of Human Genetics*, *65*(4), 1047–1053.

Su, B., Sun, G., Lu, D., Xiao, J., Hu, F., Chakraborty, R., et al. (2000). Distribution of three HIV-1 resistance-conferring polymorphisms (SDF1–3'A, CCR2–641, and CCR5-delta32) in global populations. *European Journal of Human Genetics*, *8*(12), 975–979.

Tarr, P. E., Taffe, P., Bleiber, G., Furrer, H., Rotger, M., Martinez, R., et al. (2005). Modeling the influence of APOC3, APOE, and TNF polymorphisms on the risk of antiretroviral therapy-associated lipid disorders. *Journal of Infectious Diseases*, *191*(9), 1419–1426.

Taylor, J., Pomerantz, R., Bagasra, O., & Amini, S. (1992). TAR-independent transactivation by tat in cells derived from the CNS: A novel mechanism of HIV-1 gene regulation. *EMBO J*, *11*(9), 3395–403.

Teja, V. D., Talasila, S. R., & Vemu, L. (2005). Neurologic manifestations of HIV infection: An Indian hospital-based study. *AIDS Reader*, *15*(3), 139–143.

Telenti, A. (2005). Adaptation, co-evolution, and human susceptibility to HIV-1 infection. *Infection, Genetics & Evolution*, *5*(4), 327–334.

Thomas, E. R., Dunfee, R. L., Stanton, J., Bogdan, D., Taylor, J., Kunstman, K., et al. (2007). Macrophage entry mediated by HIV envs from brain and lymphoid tissues is determined by the capacity to use low CD4 levels and overall efficiency of fusion. *Virology*, *360*(1), 105–119.

Thompson, K. A., Kent, S. J., Gahan, M. E., Purcell, D. F., McLean, C. A., Preiss, S., et al. (2003). Decreased neurotropism of nef long terminal repeat (nef/LTR)-deleted simian immunodeficiency virus. *Journal of Neurovirology*, *9*(4), 442–451; 442.

Toborek, M., Lee, Y. W., Flora, G., Pu, H., András, I. E., Wylegala, E., et al. (2005). Mechanisms of the blood-brain barrier disruption in HIV-1 infection. *Cellular & Molecular Neurobiology*, *25*(1), 181–199.

Toggas, S. M., Masliah, E., Rockenstein, E. M., Rall, G. F., Abraham, C. R., & Mucke, L. (1994). Central nervous system damage produced by expression of the HIV-1 coat protein gp120 in transgenic mice. *Nature*, *367*(6459), 188–193.

Tornatore, C., Chandra, R., Berger, J., & Major, E. (1994). HIV-1 infection of subcortical astrocytes in the pediatric central nervous system. *Neurology*, *44*, 481–87.

Tozzi, V., Balestra, P., Bellagamba, R., Corpolongo, A., Salvatori, M. F., Visco-Comandini, U., et al. (2007). Persistence of neuropsychologic deficits despite long-term highly active antiretroviral therapy in patients with HIV-related neurocognitive impairment: Prevalence and risk factors. *Journal of Acquired Immune Deficiency Syndromes: JAIDS*, *45*(2), 174–182.

Trillo-Pazos, G., McFarlane-Abdulla, E., Campbell, I. C., Pilkington, G. J., & Everall, I. P. (2000). Recombinant nef HIV-IIIB protein is toxic to human neurons in culture. *Brain Research*, *864*(2), 315–326.

Tscherning, C., Alaeus, A., Fredriksson, R., Bjorndal, A., Deng, H., Littman, D. R., et al. (1998). Differences in chemokine coreceptor usage between genetic subtypes of HIV-1. *Virology*, *241*(2), 181–188.

Tyor, W. R., Glass, J. D., Griffin, J. W., Becker, P. S., McArthur, J. C., Bezman, L., et al. (1992). Cytokine expression in the brain during the acquired immunodeficiency syndrome. *Annals of Neurology*, *31*(4), 349–360.

van Marle, G., Henry, S., Todoruk, T., Sullivan, A., Silva, C., Rourke, S. B., et al. (2004). Human immunodeficiency virus type 1 nef protein mediates neural cell death: A neurotoxic role for IP-10. *Virology*, *329*(2), 302–318.

van Marle, G., & Power, C. (2005). Human immunodeficiency virus type 1 genetic diversity in the nervous system: Evolutionary epiphenomenon or disease determinant? *Journal of Neurovirology*, *11*(2), 107–128.

van Rij, R. P., Broersen, S., Goudsmit, J., Coutinho, R. A., & Schuitemaker, H. (1998). The role of a stromal cell-derived factor-1 chemokine gene variant in the clinical course of HIV-1 infection. *AIDS*, *12*(9), F85–90.

van Rij, R. P., Portegies, P., Hallaby, T., Lange, J. M., Visser, J., de Roda Husman, A. M., et al. (1999). Reduced prevalence of the CCR5 delta32 heterozygous genotype in human immunodeficiency virus-infected individuals with AIDS dementia complex. *Journal of Infectious Diseases*, *180*(3), 854–857.

Vasan, A., Renjifo, B., Hertzmark, E., Chaplin, B., Msamanga, G., Essex, M., et al. (2006). Different rates of disease progression of HIV type 1 infection in Tanzania based on infecting subtype. *Clinical Infectious Diseases*, *42*(6), 843–852.

Venturi, G., Catucci, M., Romano, L., Corsi, P., Leoncini, F., Valensin, P., et al. (2000). Antiretroviral resistance mutations in human immunodeficiency virus type 1 reverse transcriptase and protease from paired cerebrospinal fluid and plasma samples. *The Journal of Infectious Diseases*, *181*(2), 740–745.

Vergote, D., Butler, G. S., Ooms, M., Cox, J. H., Silva, C., Hollenberg, M. D., et al. (2006). Proteolytic processing of SDF-1alpha reveals a change in receptor specificity mediating HIV-associated neurodegeneration. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(50), 19182–19187.

Vilades, C., Broch, M., Plana, M., Domingo, P., Alonso-Villaverde, C., Pedrol, E., et al. (2007). Effect of genetic variants of CCR2 and CCL2 on the natural history of HIV-1 infection: CCL2-2518GG is overrepresented in a cohort of spanish HIV-1-infected subjects. *Journal of Acquired Immune Deficiency Syndromes: JAIDS*, *44*(2), 132–138.

Voevodin, A., Samilchuk, E., & Dashti, S. (1998). A survey for 32 nucleotide deletion in the CCR-5 chemokine receptor gene (deltaccr-5) conferring resistance to human immunodeficiency virus type 1 in different ethnic groups and in chimpanzees. *Journal of Medical Virology*, *55*(2), 147–151.

Wadia, R. S., Pujari, S. N., Kothari, S., Udhar, M., Kulkarni, S., Bhagat, S., et al. (2001). Neurological manifestations of HIV disease. *Journal of the Association of Physicians of India*, *49*, 343–348.

Wallace, V. C. J., Blackbeard, J., Pheby, T., Segerdahl, A. R., Davies, M., Hasnie, F., et al. (2007). Pharmacological, behavioural and mechanistic analysis of HIV-1 gp120 induced painful neuropathy. *Pain*, *133*(1–3), 47–63.

Wang, Y. S. & White, T. D. (2000). The HIV glycoproteins gp41 and gp120 cause rapid excitation in rat cortical slices. *Neuroscience Letters*, *291*(1), 13–16.

Wang, Z., Pekarskaya, O., Bencheikh, M., Chao, W., Gelbard, H. A., Ghorpade, A., et al. (2003). Reduced expression of glutamate transporter EAAT2 and impaired glutamate transport in human primary astrocytes exposed to HIV-1 or gp120. *Virology*, *312*(1), 60–73.

Weiss, J. M., Nath, A., Major, E. O., & Berman, J. W. (1999). HIV-1 tat induces monocyte chemoattractant protein-1-mediated monocyte transmigration across a model of the human blood-brain barrier and up-regulates CCR5 expression on human monocytes. *Journal of Immunology*, *163*(5), 2953–2959.

Werner, T., Ferroni, S., Saermark, T., Brack-Werner, R., Banati, R. B., Mager, R., et al. (1991). HIV-1 nef protein exhibits structural and functional similarity to scorpion peptides interacting with K+ channels. *AIDS*, *5*(11), 1301–1308; 1301.

Wesselingh, S. L., Power, C., Glass, J. D., Tyor, W. R., McArthur, J. C., Farber, J. M., et al. (1993). Intracerebral cytokine messenger RNA expression in acquired immunodeficiency syndrome dementia. *Annals of Neurology*, *33*(6), 576–582.

Wiley, C. A., Masliah, E., Morey, M., Lemere, C., DeTeresa, R., Grafe, M., et al. (1991). Neocortical damage during HIV infection. *Annals of Neurology*, *29*(6), 651–657.

Williams, K. C., & Hickey, W. F. (2002). Central nervous system damage, monocytes and macrophages, and neurological disorders in AIDS. *Annual Review of Neuroscience*, *25*, 537–562.

Winkler, C., Modi, W., Smith, M. W., Nelson, G. W., Wu, X., Carrington, M., et al. (1998). Genetic restriction of AIDS pathogenesis by an SDF-1 chemokine gene variant. ALIVE study, Hemophilia Growth and Development study (HGDS), Multicenter AIDS Cohort study (MACS), Multicenter Hemophilia Cohort study (MHCS), San Francisco city cohort (SFCC). *Science*, *279*(5349), 389–393.

Wu, L., Gerard, N. P., Wyatt, R., Choe, H., Parolin, C., Ruffing, N., et al. (1996). CD4-induced interaction of primary HIV-1 gp120 glycoproteins with the chemokine receptor CCR-5. *Nature*, *384*, 179.

Wyatt, R. & Sodroski, J. (1998). The HIV-1 envelope glycoproteins: Fusogens, antigens, and immunogens. *Science*, *280*(5371), 1884–1888.

Yeung, M. C., Pulliam, L., & Lau, A. S. (1995). The HIV envelope protein gp120 is toxic to human brain-cell cultures through the induction of interleukin-6 and tumor necrosis factor-alpha. *AIDS*, *9*(2), 137–143.

Yeung, M. L., Bennasser, Y., Le, S. Y., & Jeang, K. T. (2005). siRNA, miRNA and HIV: Promises and challenges. *Cell Res*, *15*(11–12), 935–946.

Yu, W., Wang, Y., Shaw, C. A., Qin, X. F., & Rice, A. P. (2006). Induction of the HIV-1 tat co-factor cyclin T1 during monocyte differentiation is required for the regulated expression of a large portion of cellular mRNAs. *Retrovirology*, *3*, 32.

Zauli, G., Secchiero, P., Rodella, L., Gibellini, D., Mirandola, P., Mazzoni, M., et al. (2000). HIV-1 tat-mediated inhibition of the tyrosine hydroxylase gene expression in dopaminergic neuronal cells. *Journal of Biological Chemistry*, *275*(6), 4159–4165.

Zhang, K., Hawken, M., Rana, F., Welte, F. J., Gartner, S., Goldsmith, M. A., et al. (2001). Human immunodeficiency virus type 1 clade A and D neurotropism: Molecular evolution, recombination, and coreceptor use. *Virology*, *283*(1), 19–30.

Zhang, K., McQuibban, G. A., Silva, C., Butler, G. S., Johnston, J. B., Holden, J., et al. (2003). HIV-induced metalloproteinase processing of the chemokine stromal cell derived factor-1 causes neurodegeneration. *Nature Neuroscience*, *6*(10), 1064–1071.

Zhang, L., Huang, Y., He, T., Cao, Y., & Ho, D. D. (1996). HIV-1 subtype and second-receptor use. *Nature*, *383*(6603), 768.

Zhao, M. L., Kim, M. O., Morgello, S., & Lee, S. C. (2001). Expression of inducible nitric oxide synthase, interleukin-1 and caspase-1 in HIV-1 encephalitis. *Journal of Neuroimmunology*, *115*(1–2), 182–191.

Zhong, P., Peeters, M., Janssens, W., Fransen, K., Heyndrickx, L., Vanham, G., et al. (1995). Correlation between genetic and biological properties of biologically cloned HIV type 1 viruses representing subtypes A, B, and D. *AIDS Research & Human Retroviruses*, *11*(2), 239–248.

Zhu, J., Mactutus, C. F., Wallace, D. R., & Booze, R. M. (2009). HIV-1 tat protein-induced rapid and reversible decrease in [3H]dopamine uptake: Dissociation of [3H]dopamine uptake and [3H]2Î²-carbomethoxy-3-Î²-(4-fluorophenyl)tropane (WIN 35,428) binding in rat striatal synaptosomes. *Journal of Pharmacology and Experimental Therapeutics*, *329*(3), 1071–1083.

Zhu, Y., Antony, J. M., Martinez, J. A., Glerum, D. M., Brussee, V., Hoke, A., et al. (2007). Didanosine causes sensory neuropathy in an HIV/AIDS animal model: Impaired mitochondrial and neurotrophic factor gene expression. *Brain*, *130*(Pt 8), 2011–2023.

Zhu, Y., Jones, G., Tsutsui, S., Opii, W., Liu, S., Silva, C., et al. (2005). Lentivirus infection causes neuroinflammation and neuronal injury in dorsal root ganglia: Pathogenic effects of STAT-1 and inducible nitric oxide synthase. *Journal of Immunology*, *175*(2), 1118–1126.

1.5

GENETIC SUSCEPTIBILITIES FOR NEUROAIDS

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Among HIV-1 infected patients, clinical involvement of the central and peripheral nervous systems is widespread but far from universal, even among untreated patients. The precise basis for differences among individuals in the natural history of HIV-1 disease is largely unknown. Conceptually, these differences may be attributed to environmental, viral, host, and other factors. This chapter focuses specifically on host genetic polymorphisms that influence susceptibility to the spectrum of HIV-1 associated neurological disorders, peripheral neuropathies, and central nervous system (CNS) opportunistic infections. Particular attention is paid to polymorphisms for cytokines, chemokines and their receptors, and a number of other cellular products (apolipoprotein E, mannose-binding lectin). Also discussed are polymorphic determinants of drug-related CNS effects (efavirenz) and of progressive multifocal leukoencephalopathy and nondetermined leukoencephalopathy.

INTRODUCTION

One of the hallmarks of HIV infection is the involvement of the central and peripheral nervous systems. Approximately one-third of patients with advanced, untreated HIV disease develop neurological disorders, including central nervous system (CNS) opportunistic infections (OIs); HIV-1-associated neurocognitive disorders (HAND), including the most severe form, HIV-associated dementia (HAD); and HIV-related distal sensory peripheral neuropathy (SN).

The advent of highly active antiretroviral therapy (HAART) in 1996 afforded a highly salutary increase in patient survival and decrease in the incidence of CNS disorders including CNS OIs and HAD (Bhaskaran et al., 2008; Dore et al., 1999). However, the availability of HAART has attenuated but not extinguished disorders of the CNS: In HIV-infected patients receiving long-term HAART, milder HIV-associated neurocognitive disorders remain present in up to 40% of patients (Cysique & Brew, 2009; Sacktor et al., 2002); SN remains prevalent in patients in both resource-poor (Wright, 2009) and -rich countries (Ellis et al., 2010) as a consequence of HAART toxicity. Additionally, CNS immune restoration disease (IRD) occurs in up to 20% of patients initiating HAART in the setting of CNS opportunistic infections (Muller et al., 2010). Hence, neurological disorders remain an ongoing challenge for HIV-infected patients and their treating clinicians.

The precise basis for the inter-subject differences in the prevalence of CNS disorders during HIV infection is unknown. Conceptually, these differences may be due to inter-subject differences in environmental (e.g., co-infections), viral (e.g., neurotropism of infecting strain), and host (e.g., genetic polymorphisms) determinants as well as other comorbid conditions such as Alzheimer's Disease (AD) or Parkinson's Disease (PD). There is increasing evidence that genetic variations in human genes may predispose individuals to neurological disorders such as Alzheimer's disease (Bras & Singleton, 2009; Brouwers, Sleegers, & Van Broeckhoven, 2008; Cacabelos, 2009; Pardo & van Duijn, 2005; Pinholt, Frederiksen, & Christiansen, 2006; Rademakers, Cruts, & Van Broeckhoven, 2003; Rademakers & Rovelet-Lecrux, 2009; Tsuang & Bird, 2002) neurodevelopmental and peripheral nerve disorders (Baloh, 2008; Stankiewicz & Lupski, 2010; Verhoeven et al., 2006; Verpoorten, De Jonghe, & Timmerman, 2006), and epilepsy (de Kovel et al., 2010). As a corollary, there has been increasing emphasis on evaluating the role of host genetics in the pathogenesis of viral infections of the nervous system and studies have focused mainly on infections with HSV-1 (Hill, Bhattacharjee, & Neumann, 2007), West Nile virus (Ahuja & He, 2010; Glass et al., 2006; Lim et al., 2009; Lim et al., 2008; Lim et al., 2010), tick-borne encephalitis (Kindberg et al., 2008), and HIV-1 (reviewed in Hill, 2006). In this chapter, we have reviewed the host genetic polymorphisms that influence pathogenesis of HAND, including HIV-1-associated dementia, peripheral neuropathies, CNS opportunistic infections, and drug metabolism relevant to CNS disorders.

HIV-1-ASSOCIATED NEUROCOGNITIVE DISORDERS

The term HIV-associated neurocognitive disorders is an umbrella term used to describe asymptomatic neurocognitive impairment, mild neurocognitive disorder, and HAD (Antinori et al., 2007). Asymptomatic neurocognitive impairment occurs in at least 15% of patients (Antinori et al., 2007; Wojna et al., 2006) and is characterized by abnormal performance on neuropsychological testing. However, this abnormal performance is not reflected in the patients' day-to-day lives, whereby patients are asymptomatic and fully functional. Mild neurocognitive disorders occur in up to 40% of patients (Robertson et al., 2007) and are characterized by abnormal

neurocognitive performance and mild interference in performance of activities of work and daily living.

HIV-1-ASSOCIATED DEMENTIA

HIV-1-associated dementia (HAD) is one of the leading causes of dementia in young adults worldwide (Sacktor et al., 2001). Before the introduction of HAART, 15–20% of patients with advanced untreated HIV disease developed HAD. In the pre- and post-HAART eras the estimated incidence of HAD is 6.49 and 0.66 per 1000 person years, respectively (Bhaskaran et al., 2008). HAD is a subcortical dementia characterized by the triumvirate of severe cognitive impairment, psychomotor slowing, and behavioral disturbance that significantly interfere with a patient's activities of daily living and the capacity to work. HAD lies at the severe end of the spectrum of HAND. Approximately half of HIV-infected individuals with HAD had neuropathological changes at postmortem, discovered during autopsy, and a quarter had a triad of clinical cognitive, behavioral, and motor abnormalities ranging from mild motor/cognitive deficits to overt dementia (Gendelman et al., 1997; McArthur et al., 1993). The introduction of HAART has considerably reduced the incidence of HAD (Bhaskaran et al., 2008; Dore et al., 1999).

However, due to increased longevity associated with HAART there has been a significant increase in other clinical manifestations of HAND. For example, Sacktor et al. compared HIV-associated cognitive impairment before and after the advent of HAART, and found that though HAART had reduced the incidence of HIV dementia, HIV-associated cognitive impairment continued to be prevalent in patients receiving HAART (Sacktor et al., 2001). Similar findings were noted in an Australian study by Cysique et al. (2004). Recently, the prevalence rate for HAND was found to be approximately 50% in HIV-1-infected individuals from the United States (McArthur & Brew, 2010). Furthermore, additional factors such as age (Cherner et al., 2004), and comorbid conditions including co-infection with hepatitis C virus (Cherner et al., 2005), vascular disease (Becker et al., 2009; Wright et al., 2010), and substance use (Rippeth et al., 2004) are thought to contribute to the development of cognitive disorders in HIV-infected patients. Furthermore, there is evidence to suggest that neurodegenerative diseases, notably AD and PD are occurring with increased frequency in HIV-infected patients receiving antiretroviral therapy (Brew et al., 2009; Tisch & Brew, 2009). Brew et al. have hypothesized that there are pathways common to aging, HIV infection, and neurodegenerative diseases that may afford this accelerated neurodegeneration and that host factors and antiretroviral toxicity may contribute to this process (Brew et al., 2009). To support this, recent data show an overlap between protein expression from the frontal lobes of patients with AD and patients with HAD (Zhou et al., 2010).

The greater life expectancy of HIV-1-infected individuals in the HAART era coupled with the increasing age of HIV-positive populations (Murray, McDonald, & Law, 2009) suggests that the current prevalence of HAND, albeit compounded or modified by the above-mentioned factors, may persist or rise over the next decade. A proportional increase in HAD compared with other AIDS-defining illnesses and a marked increase in the median CD4 cell count at HAD diagnosis have occurred since the introduction of HAART (Dore et al., 1999). Furthermore, several recent studies suggest that HANDs are prevalent in persistently treated aviremic patients (recently reviewed by McArthur et al., 2010). It should also be noted that in resource-poor countries, the prevalence of HAD is high (Riedel et al., 2006) and hence likely has a negative impact upon the workforce and hence the social and economic framework of these countries.

Surprisingly, there is discordance between the manifested neurocognitive disorders and characteristics related to HIV, including plasma viral load, host immune status, and disease progression rates. HIV-induced neuropathology has been often linked to infiltration of mononuclear phagocytes (MP) from the periphery and inflammatory mediators produced during HIV encephalitis. Clinical disease is often, but not always, correlated with neuropathologic features of HIV-induced encephalitis (HIVE). HAD is a subcortical dementia that traditionally affects the basal ganglia and deep white matter. It is characterized by productive infection of brain macrophages and microglia, giant cell formation, macrophage infiltration into the brain, and neocortical atrophy (neuronal loss, dendritic arbor damage, and spatial neuron alterations) (Asare et al., 1996; Everall et al., 1999; Masliah et al., 1997). Glass et al. demonstrated that the best histopathologic correlate of HAD is the number of inflammatory MPs in the CNS (Glass et al., 1993). Interestingly, most patients with HIVE have HAD, but not all HAD patients have HIVE. Even minimal increases in the numbers and, perhaps even more importantly, the state of MP immune activation could be sufficient to cause neurological dysfunction. Activated MPs produce a variety of neurotoxins including arachidonic acid and its metabolites, platelet-activating factor, pro-inflammatory cytokines, quinolinic acid, neurotoxic amines and nitric oxide (Achim, Heyes, & Wiley, 1993; Adamson et al., 1996; Bukrinsky et al., 1995; Garden et al., 2002; Gelbard et al., 1994; Genis et al., 1992; Giulian et al., 1996; Grimaldi et al., 1991; Heyes et al., 2001; Nottet et al., 1995). Viral proteins such as gp120, gp41, and Tat or virions binding to chemokine receptors expressed on neurons can also affect neuronal viability and/or function. Indeed, there is widespread microglial activation and accompanying reactive astrogliosis in the areas with pronounced dendritic damage (Adle-Biassette et al., 1999; Giometto et al., 1997). Thus, the initial events that lead to entry of the virus into the brain culminate in neurodegenerative changes that manifest as neurocognitive disorders and MPs likely play an important role in both of these processes.

On the basis of the aforementioned, it can be envisaged that polymorphisms that impact on the expression of genes encoding inflammatory mediators can lead to inter-individual differences in MP recruitment and activation, and in turn influence susceptibility to developing the full spectrum of HAND. However, the precise repertoire of host genetic factors/networks that influence HAD/HAND pathogenesis remains largely unknown. Polymorphisms in genes other than

those classically involved in inflammatory response have also been implicated in HAND susceptibility (e.g., APOE alleles). Furthermore, host genetic polymorphisms have been found to be involved in CNS side effects resulting from antiretroviral therapies and have provided impetus to develop personalized therapeutic regimens as discussed in the later sections of this chapter. For example, a powerful and an exciting example of use of personalized genetics in clinical therapy is screening for patients for HLA subtype B*5701 allele, as this was found to be associated with hypersensitivity to abacavir (Mallal et al., 2008).

HOST GENETIC VARIATIONS RELATED TO HAND

Overview

Before discussing individual genes that have been implicated in the genetic susceptibility to HAND, we will provide a brief overview on the role of various cytokines and chemokines in HIV-1-induced neuroinflammation. HIV is thought to enter the CNS during very early stages of infection. It has been proposed that there is continuous recruitment of

Table 1.5.1 POLYMORPHISMS ASSOCIATED WITH HIV-ASSOCIATED NEUROCOGNITIVE DISORDERS AND NEUROPATHIES.[a]

NAME	GENETIC VARIATION	MECHANISMS	EFFECT ON HAND AND/OR PERIPHERAL NEUROPATHIES	REFERENCES
		Chemokine Receptors		
CCR5	*CCR5* Δ*32* (rs333) *CCR5 −2135*[#] T > C (rs1799988)	Truncated CCR5 protein Increased CCR5 expression	Δ32/+ genotype associated with significantly delayed disease progression, including less neurocognitive impairment and low risk of onset of AIDS dementia complex. *59353* C/C genotype associated with CNS abnormalities	Singh, 2003 Boven, 1999 van Rij, 1999 Singh, 2003
CCR2	CCR2–p.Val64Ile [†] (rs1799864)	Linkage disequilibrium with *CCR5* variants; heterologous receptor desensitization of CCR5 and CXCR4	The CCR2 64I bearing allele was associated with faster rate of progression to neuropsychological impairment in HIV-1-infected adults	Singh, 2004
CX3CR1	CX3CR1-p.Val249Ile (rs3732379)*;* CX3CR1p. Thr280Met(rs3732378)	Presence of 280Met reduces receptor expression and binding of the cognate ligand CX3CL1(fractalkine) while 249Val-280Thr does the opposite	Children with 249Ile in homozygous state experienced accelerated disease progression and a trend toward greater CNS impairment; Children with 249Val-280Thr bearing haplotype experienced significantly less disease progression and CNS impairment	Singh, 2005
DARC	*DARC −46T>C* (rs2814778)	Regulate circulating chemokine levels; influences binding of HIV-1 and transinfection of HIV target cells; in linkage disequilibrium with p.Asp42Gly which associates with serum levels of CCL2, CCL5 and CXCL8.	−46C/C protected against the rate of progression of neurocognitive deficits to HAD	He, 2008
		Chemokines		
CCL3L1	*CCL3L1* low copies plus detrimental *CCR5* genotypes	Modulation of CCR5 expression levels; HIV-1 suppression	Accelerated rate of disease progression to HAD	Gonzalez, 2005
CCL3 (MIP-1α)	rs1130371	Possible linkage disequilibrium with variation in CCL3, CCL4 or CCL18	TT genotype for the *CCL3* rs1130371 was associated with a twofold increase in risk for HAD	Levine, 2009
CCL5 (RANTES)	*CCL5−403G>A* (rs2107538)	−403A associated with increased CCL5 expression	*CCL5* −403 G/A polymorphism was significantly associated with PML-like leukoencephalopathy, known as nondetermined leukoencephalopathy (NDLE)	Guerini, 2008
CXCL12 (SDF-1)	c.*519G>A (rs1801157) Other Names: *(SDF1−3′-A)*	Increased levels of CXCL12 mRNA and enhanced mRNA stability	Children with the *SDF1−3′A/A* genotype had a faster decline in neurocognitive faculties	Singh, 2003

(*Continues*)

Table 1.5.1 (CONTINUED)

NAME	GENETIC VARIATION	MECHANISMS	EFFECT ON HAND AND/OR PERIPHERAL NEUROPATHIES	REFERENCES
CCL2 (MCP-1)	*CCL2–2578 A>G* (rs1024611) Other Names: *CCL2 –2518A>G*	*CCL2 –2578G* allele associated with increased transcription, protein production and monocyte recruitment. It is also associated with elevated CCL2 levels in CSF.	*CCL2–2578G* homozygosity was associated with a 50% reduction in the risk of acquiring HIV-1 but accelerated disease progression and a 4.5-fold increased risk of HAD *CCL2–2518G* allele was marginally associated with central nervous system (CNS) impairment in HIV-infected children	Gonzalez, 2002 Singh, 2006
		Cytokines		
TNF-α	*TNF–A –308G>A* (rs1800629) Other Names: *TNFA2* *TNFA –1031C>T* (rs1799964) Other Names: -1031*2	*TNFA* –308A is associated with increased production of TNF-α. Possible LD with SNPs in other genes involved in inflammation NA	*TNFA* –308A was more common in AIDS dementia complex (HAD) compared to HIV-negative controls. High risk of HAD in African American individuals with –308A. Carriage of *TNFA* –1031*2 is associated with highest risk for nucleoside analog-associated sensory neuropathy (NRTI-SN).	Pemberton, 2008 Quasney, 2001 Peterson, 2004
IL-12 (p40)	*IL12B* (c.*159A>C) (rs3212227) Other Names: 3'UTR (+1188 A>C) IL12B (3' UTR)*2	Increased expression? Conflicting reports.	Carriage of *IL12B* (3' UTR)*2 is protective for nucleoside analog-associated sensory neuropathy (NRTI-SN).	Cherry, 2008
IL-1β	*IL1B 3954C>T* (rs1143634) Other Names: 3953C>T	*IL1B 3954T* allele is associated with increased IL-1β expression	*IL1B* 3954C>T polymorphism was less frequent in patients with lipodystrophic syndrome compared with those without; absence of the 3954T allele was significantly associated with lipodystrophic syndrome.	Asensi, 2008
		Others		
MBL2	*MBL-2 O/O* genotype (rs5030737; rs1800450; rs1800451)	Low plasma concentration and structural damage of MBL	*MBL-2* O/O genotypes associate with more rapid HIV-1-related disease progression and CNS impairment, predominantly in children younger than 2 years	Catano, 2008 Spector, 2010
APOE	*APOE* ε2, *APOE* ε3, *APOE* ε4 alleles (rs429358; rs7412)	apoE4 enhances HIV cell entry in vitro .	*APOE* ε4 allele is associated with HAND but not in all cohorts. May be age dependent.	Burt, 2008 Corder, 1993 Spector, 2010
CYP2B6	*CYP2B6 516G>T* (rs3745274)	Efavirenz metabolism; higher plasma levels of efavirenz lead to CNS side effects	The CYP2B6 G/T and T/T genotypes were associated with greater severity of CNS symptoms	Haas, 2005 Rotger, 2005
Mitochondrial DNA	*m.4917A>G* Haplogroup T	Asparagine to aspartic acid change in the ND2 subunit of Complex I. Oxidative phosphorylation?	The mitochondrial 4917G polymorphism may increase susceptibility to antiretroviral therapy-associated peripheral neuropathy This is a common European mitochondrial haplogroup that may predict NRTI-associated peripheral neuropathy	Canter, 2008 Hulgan, 2005

NOTES: [a] Studies that showed positive associations with HAND or other neuropathies are shown. Please refer to the text for additional references.
[#]CCR5 polymorphism nomenclature is according to Mummidi et al. (2000).
[†]Abbreviations used: p, protein; c, coding DNA; m, mitochondrial DNA; LD, linkage disequilibrium.

monocyte-macrophage lineage cells into the brain. The predominant cell type that is recruited is the CD16+ activated macrophage. The CD16+ monocytes are expanded following HIV-1 infection and are elevated in patients with dementia when compared to patients without dementia (Pulliam et al., 1997). The recruitment of CD16+ cells is probably mediated through the soluble form of CX3CL1 (fractalkine) that is physiologically expressed by brain tissue (Ancuta et al., 2003). Of note, the CD16+ fraction of the monocytes express high levels of the fractalkine receptor, CX3CR1 (Ancuta et al., 2003). HIV-1 preferentially infects CD16+ cells and probably gains entry to the CNS through this physiological recruitment (the "Trojan horse" hypothesis). In addition, CD16+ monocytes may serve as latent reservoirs of the virus in vivo (Ellery

et al., 2007). The initial recruitment of HIV-1 infected CD16+ monocyte/macrophages into the brain is likely to trigger the inflammatory response leading to the expression of cytokines and chemokines. Two key pro-inflammatory cytokines, TNF-α and IL-1β are thought to be involved in initiating the inflammatory pathways leading to neuronal injury. In addition to having direct effects on blood-brain barrier permeability as well as the viability of the neuronal cells, TNF-α and IL-1β upregulate expression of several neurotoxic as well as neuroprotective molecules. One of the predominant chemokines that is released by the infected macrophages and the activated astrocytes is CCL2 (MCP-1) which leads to recruitment of CD16- and CC chemokine receptor 2 (CCR2)+ monocytes to the brain. An excellent review of the mechanisms involved in neuroinvasion by HIV-1 has been recently published (Gras & Kaul, 2010). Below, we review the genotype-HAND associations described for polymorphisms in genes that encode proteins that have been implicated in HAND pathogenesis (summarized in Table 1.5.1). The major categories are: I. Cytokines; II. Chemokines and their receptors; III. Other genes.

CYTOKINE GENES

Tumor Necrosis Factor –α

As discussed above, TNF-α that is released by infected microglia may play a key role in neuronal injury following HIV-1 infection (reviewed in (Brabers & Nottet, 2006; Saha & Pahan, 2003)). However, its exact role in the pathogenesis is controversial as it has been implicated in both neurodegeneration as well as neuroprotection. Activated macrophages in HAD show increased expression of TNF-α (Wesselingh et al., 1997) and TNF receptors. The temporal expression of TNF-α was found to correlate with dementia progression and severity (Wesselingh et al., 1993). While the key role of TNF-α in the pathogenesis of HAD and other cognitive disorders is undisputed, its role may be dependent on several factors such as TNF-receptor usage, its known role in induction of beta chemokines, effects on blood-brain barrier, potentiation of toxic effects of other neuroinflammatory molecules, and monocyte trafficking (reviewed in Saha & Pahan, 2003).

The *TNFA* gene is located in the major histocompatability complex (MHC) and polymorphic variation in its regulatory regions has been implicated in a variety of diseases. Of note, a G to A transition in the promoter region at position -308 (TNF-308A, rs1800629 G>A) is associated with increased TNF-α expression and increased susceptibility to several infectious diseases (Elahi et al., 2009). However, *TNFA* gene is proximal to several inflammatory genes such as lymphotoxin, and polymorphisms in this region exhibit strong linkage disequilibrium (linked polymorphisms). Thus, disease associations may not be ascribed a single marker or gene in this locus. Several studies have examined the association between the -308 polymorphism and HAD/HIVE. Two studies have found no association between HIV-1 encephalitis and/or dementia (Diaz-Arrastia et al., 2004; Sato-Matsumura et al., 1998); however, this may be ascribed to low patient numbers in these studies. By contrast, Quasney et al. found that *TNFA2* allele (i.e., those bearing -308A) was found to be overrepresented in adults with HAD when compared to adults without dementia (Quasney et al., 2001). Similarly, Pemberton et al. also found that *TNFA2* allele was present at increased frequency in patients with HAD when compared to both HIV-1-infected patients who did not develop symptoms of dementia as well as HIV-1-negative controls (Pemberton et al., 2008). The latter study is a meta-analysis and included data from studies conducted by Quasney et al. (2001) and Diaz-Arrastia et al. (2004).

Interleukin-1 (IL-1)

Similar to TNF-α, IL-1 is a pluripotent cytokine and has powerful inflammatory and immunomodulatory effects. Comparative studies on human brain samples from HIV-1 patients with and without HAD initially showed that there was no increase in IL-1 expression during HAD (Wesselingh et al., 1993). However in a subsequent study, Zhao et al, showed that IL-1β expression was significantly higher in brain tissues from patients with HAD when compared to HIV-1-positive patients without dementia and high expression was detected in macrophages, microglia, and multinucleated giant cells (Zhao et al., 2001). IL-1β has pleiotropic effects and modulates expression of several genes that have been implicated in neuronal injury as well as neuroprotection (reviewed in Brabers & Nottet, 2006). Both *IL1A* and *IL1B* genes are polymorphic and may be associated with altered gene expression (Pociot et al., 1992). Pemberton et al. evaluated the role of *IL1A* −889 (C>T; rs1800587) and *IL1B* 3953 (C>T; rs11143634) polymorphisms in HIV-1-infected patients and found no difference between patients with HAD and HIV-positive and HIV-negative controls (Pemberton et al., 2008).

CHEMOKINES AND THEIR RECEPTORS

CC Chemokine Receptor 5 (CCR5)

CCR5 serves as the major co-receptor for the entry of macrophage-tropic HIV-1 (R5) strains into cells of monocyte macrophage lineage as well as memory T cells (Alkhatib et al., 1996; Berger, Murphy, & Farber, 1999; Deng et al., 1996; Samson et al., 1996). A 32-bp deletion mutation (Δ32; rs333) in the *CCR5* open reading frame leads to formation of truncated protein which fails to get expressed on cell surface. Homozygosity for this mutation confers almost an absolute protection against HIV-1 infection (Berger, Murphy, & Farber, 1999; Liu et al., 1996). It also has a protective phenotype in heterozygous state and is associated with decreased surface expression and delayed disease progression (de Roda Husman et al., 1997; Gonzalez et al., 1999; Huang et al., 1996; Ioannidis et al., 2001; Mummidi et al., 1998; Zimmerman et al., 1997), although the level of protection may be determined by the partner CCR5 allele (Gonzalez et al., 1999; Hladik et al., 2005; Mangano et al., 2001; Salkowitz et al., 2003) and was not consistent in all cohorts examined (Eskild et al., 1998; Wilkinson et al., 1998).