HANDBOOK OF

Pediatric HIV Care

Edited by
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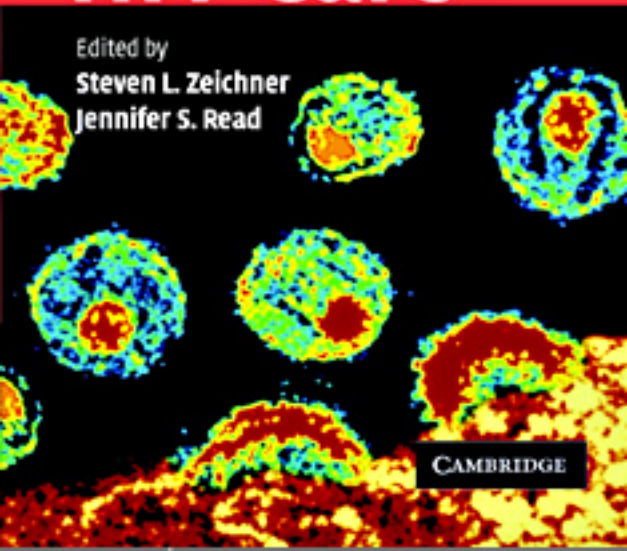

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Handbook of

Pediatric HIV Care

This portable and practical handbook provides a concise guide to the essentials of pediatric HIV care. During the past few years, many agents for the treatment and prophylaxis of HIV infection and the opportunistic infections that accompany HIV infection have been developed, and many new ways of monitoring HIV infection in children have been produced. These new therapies and approaches to management are complicated, but the long-term health of HIV-infected children depends on their correct application. This handbook presents the core information and guidelines necessary for effective management of infected children.

Dr. Stephen L. Zeichner received his undergraduate and graduate degrees at the University of Chicago. He trained in pediatrics and infectious diseases at the Children's Hospital of Philadelphia. An investigator in the HIV and AIDS Malignancy Branch, National Cancer Institute, NIH, and an adjunct family member of the George Washington University School of Medicine. Children's National Medical Center, Washington, DC, and the Uniformed Services University of the Health Sciences, he studies the basic biology of HIV and Kaposi's sarcoma-associated herpesvirus, and directs clinical trials of new therapies for HIV-infected children.

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approved drugs and recently developed drugs that may be close to approval, both for HIV and for HIV-related opportunistic infections, new information concerning the management of children infected with HIV, and information concerning the social welfare of children infected with HIV. In some fields, so much new information has become available that we included entirely new chapters in the book. There are new chapters about the evolutionary biology of antiretroviral drug resistance and the assessment and management of antiretroviral drug resistance, the interruption of mother-to-infant HIV transmission, metabolic complications of HIV infection and antiretroviral therapy, therapeutic drug monitoring for HIV infection, and the gynecology of the HIV-infected adolescent. Neither book has chapters discussing, in detail, HIV vaccines because both prophylactic and therapeutic vaccines are only in the earliest stages of clinical development, but the basic science chapters about virology, immunology, pathogenesis, and natural history describe some of the fundamental information that vaccine developers are using in their efforts. We hope that we will be able to include in a future edition chapters that outline the use of prophylactic and therapeutic vaccines for HIV infection.

The book does not include a specific chapter on the management of pediatric HIV disease in resource-poor countries. We initially contemplated including such a chapter in the book, but soon came to realize that the spectrum of resources available in 'resource-poor' countries varied tremendously from one country to another. For example, in some countries there are government-mandated commitments to essentially universal access to antiretrovirals, while in others only a tiny fraction of the population has access to the drugs, and these circumstances are changing month by month. We look forward to the day when everyone will receive the best care possible, but until then we thought it wisest to describe state-of-the-art care as practiced in the world's richer countries, and acknowledge that providers elsewhere will know best how to adapt these principles to their own local circumstances.

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Second Edition

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For Rachel, Sarah, and Elizabeth

For Alex, Samantha, and Geoffrey

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Abbreviations

AAP	American Academy of Pediatrics
ABC	abacavir
ABCD	amphotericin B colloidal dispersion
ABLC	amphotericin B lipid complex
ACCAP	AIDS Community Care Alternatives Program
ACEI	angiotensin enzyme inhibitors
ACIP	Advisory Committee on Immunization Practices
ACOG	American College of Obstetricians and Gynecologists
ACTG	AIDS Clinical Trals Group
ACTH	adrenocorticotropin hormone
ACTIS	AIDS Clinical Trials Information Service
ADCC	antibody-dependent cell-mediated cytotoxicity
ADDP	AIDS Drug Distribution Program
ADEC	Association for Death Education and Counseling
ADHD	attention deficit/hyperactivity disorder
AEGIS	AIDS Education Global Information System
AFB	acid-fast bacilli
AFXB	Association François-Xavier Bagnoud
AGCUS	atypical glandular cells of undetermined significance
AIDS	acquired immune deficiency syndrome
ALRI	acute lower respiratory tract infection
AmFAR	American Foundation for AIDS Research
AMP	amprenavir
ANC	absolute neutrophil count
ANRS	Agence Nationale de Recherches sur le SIDA
AOM	acute otitis media
ACP	antigen-presenting cell
APV	amprenavir
ARB	angiotensin blockers
ARDS	acute respiratory distress syndrome

ARF	acute renal failure
ARL	AIDS-related lymphoma
ARN	acute retinal necrosis
ART	antiretroviral therapy
ASCUS	atypical squamous cells of undetermined significance
AST	asparate aminotransferase
ATN	adolescent medicine trials network
ATP	adenosine triphosphate
ATZ	atazanavir
AUC	area under the curve
AZT	zidovudine (also known as ZDV)
BAL	broncheoalveolar lavage
BBB	blood-brain barrier
BCG	Bacille Calmette–Guerin
βHCG	serum beta human chorionic gonadotropin
BIA	bioelectrical impedance analysis
BMC	bone mineral content
BMD	bone mineral density
BMI	body mass index
BUN	blood urea nitrogen
BV	bacterial vaginosis
CARE	Ryan White (Comprehensive AIDS Resources Emergency) Act
CAT	computerized axial tomography
CBC	complete blood count
CD	cluster of differentiation
CDC	Centers for Disease Control and Prevention
CDC-GAP	Centers for Disease Control and Prevention Global AIDS Program
CHF	congestive heart failure
Cho	choline
CHOP	cyclophosphamide, doxorubicin, vincristine and prednisone
CIN	cervical intraepithelial neoplasia
CIPRA	Comprehensive International Program of Research on AIDS
C_{max}	maximum concentration/peak blood concentration
CMT	cervical motion tenderness
CMV	cytomegalovirus
CNS	central nervous system
CPAP	continuous positive airway pressure
CRF	case report form
CRH	corticotropin-releasing hormone
CRP	C-reactive protein

CSF	cerebrospinal fluid
CSOM	chronic suppurative otitis media
CT	computed tomography
CTL	cytotoxic *T*-lymphocytes **also** cytotoxic memory T-cells
CVC	central venous catheter
CXR	chest X-ray
d4T	stavudine
DC	dendritic cells
DC-SIGN	dendritic cell-specific intercellular adhesion molecule-grabbing non-integrin
ddC	zalcitabine
ddI	didanosine
DEXA	dual energy X-ray absorptiometry
DFA	direct fluorescent antibodies also direct immunofluorescence assay
DHEAS	dihydroepiandrosterone sulfate
DHFR	dihydrofolate reductase
DHPS	dihydropteroate synthase
DHSS	Department of Health and Human Services
DIC	disseminated intravascular coagulation
DLBCL	diffuse large B-cell lymphoma
DL_{co}	diffusing capacity
DLV	delavirdine
DMAC	disseminated *Mycobacterium avium* complex
DMPA	depot medroxyprogesterone acetate
DNA	deoxyribonucleic acid
dNTPs	triphosphorylated nucleosides
DOT	directly observed therapy
DOTS	directly observed therapy (short course)
DSMB	Data Safety Monitoring Board
DTH	delayed type hypersensitivity
DTP	diphtheria–tetanus–pertussis
DTaP	diphtheria–tetanus–acellular pertussis
DUB	dysfunctional uterine bleeding
EBCT	electron beam computed tomography
EBV	Epstein–Barr virus
EC	emergency contraception **also** enteric coated
ECG	electrocardiogram
ECHO	echocardiography
ED	Emergency Department **also** end diastolic

EEG	electroencephalogram
EFV	efavirenz
EGPAF	Elisabeth Glaser Pediatric AIDS Foundation
EGW	external genital warts
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assays
EMEA	European Agency for the Evaluation of Medicinal Products
ENF	enfuvirtide
Env	viral envelope
EP	extrapulmonary pneumocytosis
ERCP	endoscopic retrograde cholangiopancreatography
ES	end systolic
ESR	erythrocyte sedimentation rate
ESRD	end-stage renal disease
5-FU	5-fluorouracil
FACS	fluorescent antibody cell sorting
FAMA	fluorescent antibody membrane antigen
FDA	Food and Drug Administration
FEV_1	forced expiratory volume in 1 second
FFA	free-fatty acids
FFM	fat free mass
FRS	fat redistribution syndrome
FSGS	focal segmental glomerulosclerosis
FSH	follicle stimulating hormone
FTC	emtricitabine
FTT	failure to thrive
FVT	forced vital capacity
G-6-PD	glucose-6-phosphate dehydrogenase
GCP	good clinical practices
g-CSF	filgrastim
G-CSF	granulocyte-colony stimulating factor
GER	gastroesophageal reflux
GH	growth hormone
GI	gastrointestinal
GM-CSF	granulocyte-macrophage colony-stimulating factor
GnRH	gonatropin releasing hormone
HAART	highly active antiretroviral therapy
HAIRAN	hyperandrogenic-insulin resistant acanthosis nigricans
HAMB	HIV and AIDS Malignancy Branch

HAM/TSP	HLTV-1-associated myelopathy/tropical spastic paraparesis
HAV	hepatitis A virus
HAZ	height-for-age Z-scores
hbhA	heparin-binding hemagglutinin adhesin
HBIG	hepatitis B immunoglobulin
HBV	hepatitis virus B
HCP	healthcare personnel
HDL	high-density lipoprotein
HDL-C	high-density lipoprotein cholesterol
HHV-6	human herpesvirus-6
HHV-8	human herpesvirus-8
HIB	*Hemophbilus influenzae* type B
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HMOs	health maintenance organizations
^{1}HMRS	proton magnetic resonance spectroscopy
HPA	hypothalamic-pituitary-adrenal
HPTN	HIV Prevention Trials Network
HPV	human papillomavirus
HRCT	high-resolution computerized tomography
HRIG	human rabies immunoglobulin
HSI	HIV/AIDS and sexually transmitted infections
HSV	herpes simplex virus
HTLV-1	human T-cell leukemia virus 1
HUS	hemolytic-uremic syndrome
ICASO	International Council of AIDS Services Organizations
ICD	immune complex dissociated
ICMA	immunochemiluminescent assay
IDU	injection drug use
IDV	indinavir
IFA	immunofluorescence assay
IFN	interferon
Ig	immunoglobulin
IGFPB-3	insulin-like growth factor binding protein-3
IgFBPs	IGF binding proteins
IgF-1	insulin-like growth factor 1
IL	interleukin
ILD	interstitial lung disease
IMCI	integrated management of childhood illness
INH	isoniazid

INR	international normalized ratio
In V	intravaginal
IP	interferon inducible protein
IPAA	International Partnership Against AIDS
IPI	invasive pneumococcal infections
IPV	inactivated polio vaccine
IQ	inhibitory quotient
IRB	Institutional Review Board
IRU	immune recovery uveitis
ISA	induced sputum analysis
ITP	immune thrombocytopenia purpura
IUDs	intrauterine devices
IUS	intrauterine system
IVIG	intravenous immunoglobulin
KOH	potassium hydroxide
KS	Kaposi's sarcoma
KSHV	Kaposi's sarcoma-associated herpesvirus
LBM	lean body mass
LDH	lactate dehydrogenase
LDL	low-density lipoproteins
LDL-C	low-density lipoprotein cholesterol
LFT	liver function test
LGE	linear gingival erythema
LH	iuteinizing hormone
LIFE	leadership and investment in fighting an epidemic
LIP	lymphoid interstitial pneumonitis
LIPA	line probe assays
LP	lumbar puncture
LPN	licensed practical nurse
LPV	lopinavir
LPV/r	lopinavir plus ritonavir
LTNP	long-term non-progression
LTR	long terminal repeat (HIV promotor)
LV	left ventricular
MAC	*Mycobacterium avium* complex **also** mid-arm circumference
MACS	Multicenter AIDS Cohort Study
MALT	mucosa-associated lymphoid tissue
MAMC	mid-arm muscle circumference

MCP	monocyte chemoattractant protein
MDI	Mental Developmental Index **also** metered dose inhaler
MDR	multi-drug resistance
MEMS	medication event monitoring system
MESA	myoepithelial sialadenitis
MHC	major histocompatibility complex
MI	myo-inositol
MIG	monokine induced by interferon gamma
MIP	macrophage inflammatory protein
MIRIAD	mother–infant rapid intervention at delivery
Mo	month
MMR	measles, mumps and rubella
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
MRSA	methicillin-resistant *Staphylococcus aureus*
MSM	men who have sex with men
MTCs	multilocular thymic cysts
MTD	*Mycobacterium tuberculosis* direct test
MTCT	mother-to-child transmission
NAA	*N*-acetyl asparate
NAHC	National Association for Home Care
NAMs	nucleoside associated mutations
NAAT	nucleic acid amplification tests
NASBA®	nucleic acid sequence-based amplification
NCHS	National Center for Health Statistics
NCI	National Cancer Institute
NF-kappa B	nuclear factor kappa-B
NFV	nelfinavir
NHL	non-Hodgkin's lymphoma
NIAID	National Institute of Allergy and Infectious Diseases
NICHD	National Institute of Child Health and Human Development
NIH	National Institutes of Health
NK	natural killer
NMDA	*N*-methyl-D-asparate
NNRTIs	non-nucleoside reverse transcriptase inhibitors
NPA	nasopharyngeal aspirate
nPEP	non-occupational postexposure prophylaxis
NPO	nothing by mouth
NRTIs	nucleoside reverse transcriptase inhibitors
NSAIDs	non-steroidal anti-inflammatory drugs
NSS	normal saline solution

NUG	necrotizing ulcerative gingivitis
NUP	necrotizing ulcerative periodontitis
N/V	nausea/vomiting
17-OHP	17-hydroxyprogesterone
OCs	oral contraceptives
OD	optical density
OGTT	oral glucose tolerance test
OHL	oral hairy leukoplakia
OHRP	Office of Human Research Protections
OIs	opportunistic infections
OLA	oligonucleotide ligation assays
oPEP	occupational postexposure prophylaxis
OPV	oral polio vaccine
OSHA	Occupational Safety and Health Administration
PACTG	Pediatric AIDS Clinical Trials Group
PACTS	Perinatal AIDS Collaborative Transmission Study
PAHO	Pan American Health Organization
PAP	Papanicolaou (Smear)
PBLD	polymorphic B-cell lymphoproliferative disorder
PBMC	peripheral blood mononuclear cells
PCM	protein–calorie malnutrition
PCNS	primary central nervous system
PCOS	polycystic ovary syndrome
PCP	*Pneumocystitis jiroveci* pneumonia **also** primary healthcare provider
P Cr	plasma creatinine
PCR	polymerase chain reaction
PCV	pneumococcal conjugate vaccine
PCV7	heptavalent pneumococcal conjugate vaccine
PEL	primary effusion lymphoma
PENTA	The Pediatric European Network for the Treatment of AIDS
PEP	postexposure prophylaxis
PFC	persistent fetal circulation
PFTs	pulmonary function tests
PGE_2	prostaglandin E_2
PGP	p-glycoprotein
PHA	phytohemaglutinin
PHC	preventive health care
PHS	public health service
PI	pentamidine isothionate
PIs	protease inhibitors

PIC	pre-integration complex
PID	pelvic inflammatory disease
PIT	pills identification test
PJ	*P. jiroveci*
PLH	pulmonary lymphoid hyperplasia
PMDD	premenstrual dysphoric disorder
PML	progressive multifocal leukoencephalopathy
PMPA	9-[2-(R)-(phosphonylmethoxy)propyl] adenine
PMS	premenstrual syndrome
PMTCT	prevention of mother-to-child transmission
P Na	plasma sodium
PNS	peripheral nervous system
PORN	progressive outer retinal necrosis
POS	point of service
PPD	purified protein derivative
PPOs	preferred providers organizations
PPV	pneumococcal polysaccharide vaccine
PMN	polymorphonuclear leukocyte
PRA	peripheral renin activity
PRAMS	pregnancy risk assessment monitoring system
PSD	Pediatric Spectrum of Disease
PT	prothrombin time
PTH	parathyroid hormone
PTT	partial thromboplasin time
PTX	spontaneous pneumothorax
PWAs	persons with AIDS
PZA	pyrazinamide

RAD	reactive airway disease
RBC	red blood cells
RDA	recommended dietary allowance
REACH	reaching for excellence in adolescent care and health
RER	rough endoplasmic reticulum
RN	registered nurse
RNA	ribonucleic acid
ROspA	recombinant outer surface protein
RPE	retinal pigment epithelium
RR	relative risk
RRE	rev responsive element
RSV	respiratory syncytial virus
RTI	reverse transcriptase inhibitor

RT-PCR	reverse transcription-polymerase chain reaction
RTV	ritonavir
SBIs	serious bacterial infections
Sc	subcutaneous
SDF	stromal-cell derived factor
SHBG	sex hormone-binding globulin
SIADH	syndrome of inappropriate secretion of antidiuretic hormone
SILs	squamous intraepithelial lesions
siRNA	small interfering ribonucleic acids
SIV	simian immunodeficiency virus
SMM	Sooty Mangabey monkey
SOIs	sharp object injuries
SPECT	single photon emission computed tomography
SPNS	special projects of national significance
SQV	saquinavir
SSDI	Social Security Disability Income
SSI	Supplemental Security income
SSRIs	selective serotonin reuptake inhibitors
STIs	sexually transmitted infections
SUDS	single use diagnostic system
3TC	lamivudine
T4	free levothyroxine
TAMS	thymidine analogue mutations
TANF	temporary assistance for needy families
TAR	transactivation responsive
TB	tuberculosis
TCA	trichloroacetic acid
TCR	t-cell receptors
Td	tetanus and diphtheria toxoids
TDF	tenofovir disoproxil fumarate
TDM	therapeutic drug monitoring
Th	T-helper
TIG	tetanus immunoglobulin
TMP/SMX	trimethoprim-sulfamethoxazole
TNF	tumor necrosis factor
TOA	tubo-ovarian abscess
TPN	total parenteral nutrition
TREAT	treatment regimens enhancing adherence in teens
TRH	thyrotropin-releasing hormone

TSF	triceps skinfold thickness
TSH	thyroid stimulating hormone
TST	tuberculin skin test
TTP	thrombotic thrombocytopenia purpura
U Cr	urine creatinine
UDPGT	uridine diphosphoglycronyltransferase
U Na	urine sodium
URIs	upper respiratory infections
USAID	United States Agency for International Development
USPHS	United States Public Health Service
UTI	urinary tract infection
VCAM-1	vascular cell adhesion molecule-1
VGC	valganciclovir
VLA-4	very late activation antigen-4
Vif	virion infectivity factor
VLDL	very low density lipoprotein
VZIG	varicella-zoster immunoglobulin
VZV	varicella-zoster virus
WAZ	weight-for-age Z-scores
WBCs	white blood cells
VVC	vulvovaginal candidiasis
WHO	World Health Organization
WITS	Women and Infants Transmission Study
XR	extended release
ZDV	zidovudine (also known as AZT)

Foreword

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More than two decades have passed since this devastating infection was first identified. We have come from a time when no diagnosis could be made and there was no treatment, to an era when the development of multiple therapeutic agents and advances in the prevention of HIV infection is commonplace in the developed world. Foremost amongst these accomplishments is our ability to prevent mother-to-child transmission of HIV infection. Seldom is it possible to chronicle such advances in knowledge, which materially affect the lives of thousands of people on a daily basis. All of this speaks to the commitment of scientists and care providers and the rapid evolution of information and technology. There is, however, a pervasive recurrent theme of needing to advocate for the health of children infected and affected by HIV infection.

This handbook provides accessible information at a time when the developed world has succeeded in dramatically decreasing the number of children who acquire infection from their mothers. The need for this information is greater now than ever before. First, because the evolution of information continues at a rapid rate. Second, because the complexity of treatment requires expertise and access to the most current information. Third, because the numbers of HIV-infected children have decreased in the USA and the probability that a physician will have cumulative experience with substantive numbers of these children has diminished. It is important that pediatricians continue to be sensitive to the possibility that a child is HIV-infected and be attuned the specific medical needs and support systems required.

There is an index to Web sources of information, convenient summary tables, and eloquent discussions of antiretroviral drugs conveniently separated from therapeutic decision making. The material is readable, concise, and thorough.

I would wish that this information was accessible, in demand, and essential in the parts of the world where there is so much HIV infection of adults and children. One must reflect on the fact that as many infants are born with HIV infection in sub-Saharan Africa every day as were born in the USA in an entire year prior to the availability of interventions to prevent mother-to-child transmission. Progress is being made to bring

these effective interventions to the developing world. We would hope we can entice a new generation of pediatricians, public health authorities, and other providers to devote their lives to addressing the problem as effectively in the developing world as has been done in the developed world. This handbook contributes to the knowledge, and hopefully will provide additional incentive to take these advances to the entire world of children.

Preface

When Cambridge University Press decided to undertake the publication of the second edition of the *Handbook of Pediatric Care*, they told us that they were very enthusiastic about the book, but that they thought that, while the handbook was too large to be a true "handbook," they still valued and appreciated the more comprehensive content of the book. The Press therefore asked us, for the second edition, to both shorten the material to a more manageable size to make a new handbook and to augment the material in the handbook to make an even more comprehensive *Textbook of Pediatric HIV Care*. We hope that we have achieved these goals in these two books, a second edition of the *Handbook of Pediatric HIV Care* and the first edition of the *Textbook of Pediatric HIV Care*.

Our goals for both books are to provide the clinician with the information needed to provide excellent care to children infected with HIV. Neither book is meant to be an exhaustive treatise on the subject of pediatric HIV disease, covering all the many societal and policy issues that are involved necessarily in a complete discussion of HIV and children. Rather, we aim to provide helpful management information for the frontline clinician. While we have focused on the management of pediatric HIV disease, we believe that effective management requires a solid understanding of the basic and applied virology, immunology, and pathophysiology of the disease, so that the practitioner can thoughtfully and rationally apply the management information supplied in the other chapters. Our authors have included more detailed discussions in their *Textbook* chapters, and have tried to condense their presentations in their *Handbook* chapters to include the most clinically pertinent details. Some of the information presented in more than one *Textbook* chapters has been condensed into a single chapter for the handbook, but we hope that we have been able to include the information in the handbook that will enable clinicians to provide optimum care for their HIV-infected patients.

The HIV epidemic changes quickly. The authors of the individual chapters have attempted to include a significant amount of new information, including new basic science findings, new information concerning the pathogenesis of the disease and the opportunistic infections that affect children with HIV, descriptions of recently

Part I

Scientific basis of pediatric HIV care

1 The scientific basis of pediatric HIV care

Combined from the following chapters in the *Textbook of Pediatric HIV Care: Normal development and physiology of the immune system* Sherilyn Smith and Ann Melvin. *HIV basic virology for clinicians* Steven L. Zeichner. *The immunology of pediatric HIV disease* Elizabeth McFarland. *The clinical virology of pediatric HIV disease* Paul Palumbo. *The natural history of pediatric HIV disease* Grace Aldrovandi

Normal development and physiology of the immune system

Components and function of the immune system

The immune system can be divided into two components. The "innate arm" of the immune system provides a rapid, non-specific pathogen response. It acts as a surveillance system and initiates the antigen-specific phase of the immune system. The major components of innate immunity include physical barriers, complement and other opsonins, the spleen, phagocytes and NK (natural killer) cells. Many responses are triggered by the Toll-like receptor (TLR) with the molecules they bind.

The antigen-specific phase of immunity is directed at specific pathogen antigens. The inducible portions of the immune system include cellular and humoral immune responses. These components control infection and form long-term immunity. Table 1.1 summarizes the major functions of the immune system and the infections that can result from its dysfunction.

Innate immune system

Barriers

The initial defense against microbes is an intact physical mucosal and epithelial barrier. Specialized cells (including ciliated respiratory epithelia), and localized chemical barriers (stomach acid, mucus layers in respiratory and gastrointestinal tracts, and skin

Handbook of Pediatric HIV Care, ed. Steven L. Zeichner and Jennifer S. Read.
Published by Cambridge University Press.

Table 1.1. The immune system: functions, developmental aspects and infections associated with dysfunction

Immune system component	Function	Developmental differences	Infections associated with dysfunction
Innate			
Epithelial barriers/ mucosal defense	• Impede entrance of microorganisms • Present antigen • Sample environment	• Epithelial barriers decreased in premature infants • Decreased IgA–adult levels by 6–8 years	• Low virulence organisms: coagulase negative staphylococcus opportunistic gram negative bacteria fungi
Complement/opsonins	• Amplify the immune response • Facilitate phagocytosis • Chemoattractants	• Terminal complement levels decreased in neonates	• Encapsulated organisms • Recurrent infections with *Neisseria* species • Recurrent/recalcitrant skin infections
Phagocytes	• Engulf and kill microorganisms • Present antigens to T-cells (macrophages) • Elaborate immune active substances including cytokines and chemotactic factors	• Monocytes: decreased chemotaxis, decreased cytokine production–adult function by 6 years • Neutrophils: decreased bone marrow pool in neonates, decreased chemotaxis–adult levels by 1 year	• *S. aureus* • Low virulence organisms: other staphylococci gram negative opportunistic bacteria fungi
Spleen	• Filters intravascular organisms • Aids with opsonization • Antibody formation		• Encapsulated organisms (*S. pneumoniae*, Salmonella, *H. influenzae*) • Develop severe or recurrent infections

Natural killer (NK) cells	• Lyse cells presenting "non-self" antigens (e.g., tumor or viral proteins)	• Decreased ADCC, decreased cytolytic activity	• Recurrent/severe viral infections with members of the Herpesvirus family
Dendritic cells			
	• Capture and present antigens to lymphocytes	• Decreased ability to present antigen	?
Antigen specific			
T-cells	• Cell-mediated immunity • Elaboration of cytokines • Regulation of the immune response • Cytolysis • Increases the efficiency of B-cell function by providing "help"	• Increased absolute numbers – decline to adult levels by late childhood • Naive phenotype in neonate (90%) decreased cytokine production, costimulatory molecule expression, and ability to provide "help" to B-cells – normalizes throughout infancy with antigenic exposure	• Infections with "unusual" organisms: intracellular bacteria (listeria, mycobacteria) • Fungi (aspergillus, candida) • Viruses (esp HSV, VZV, CMV, HHV-8) • Protozoa (giardia, *Pneumocystis carinii*)
B-cells	• Humoral immunity (formation of antibody to specific antigens)	• Unable to respond to polysaccharide antigens until ~2 years of age	• Encapsulated organisms • Enteroviral infections • Recurrent GI or sinopulmonary infections • Inability to respond to vaccines

fatty acids and cerumen) impede pathogen entry. Breaches in these barriers may result in infection by low virulence organisms.

Mucosal immunity

Mucosal-associated lymphoid tissues are located at sites close to the environment. These lymphoid aggregates (e.g., Peyer's patches) sample the environment, allowing for early initiation of antigen-specific responses [1, 2]. Secretory IgA, synthesized in these tissues, adds to the local mucosal defense.

Opsonins

Opsonins are proteins that bind to pathogen surfaces, facilitating phagocytosis. They include acute phase reactants (C-reactive protein, fibronectin), complement and antibody. Complement is a protein that is sequentially activated by proteases. Complement plays an important role in the killing of invasive bacteria. Two pathways activate complement: the classical pathway (antibody binds bacterial antigen which then is complexed with C1, a complement component), which begins a series of proteolytic reactions activating additional complement components, and the alternate pathway (bacterial antigen directly binds the C3b complement component). Both pathways produce a complex that lyses bacteria [3].

Spleen

The spleen efficiently filters opsonized bacteria and is an antibody production site. Absence or dysfunction of the spleen predisposes to overwhelming infection with encapsulated organisms.

Macrophages

Macrophages and monocytes clear invading microbes. Macrophages migrate to sites of infection and phagocytose foreign substances. Macrophages elaborate many cytokines and growth factors that modify evolving immune responses [4].

Neutrophils

Neutrophils are blood phagocytes that migrate to sites of infection and phagocytose pathogens, notably immunoglobulin- or complement-coated microbes, including bacteria and fungi. Neutrophils kill phagocytosed pathogens via the respiratory burst (reactive oxygen metabolites generation) or by degranulation with release of substances that directly kill pathogens.

Natural killer (NK) cells

NK cells are specialized lymphocytes that recognize non-self proteins, important in early responses to viral infections. Viruses often down-regulate host major histocompatibility complex molecules (see below) on infected cells surfaces, which causes NK cells to recognize them as foreign, making them targets for lysis.

Dendritic cells (DC)

DC capture antigen and present it to lymphocytes. There are three major DC populations: (a) Langerhans cells (interdigitating cells) reside in tissues and migrate to T-cell areas of lymphoid organs after antigen uptake; (b) myeloid DCs (interstitial or dermal DCs), which become germinal center DCs in lymphoid follicles; and (c) plasmacytoid DCs, which reside in T-cell lymphoid tissues areas [5]. Immature DCs take up and process antigen. Migrating to lymphoid tissues, they mature to become antigen-presenting cells [5]. Different populations of DCs have different functions. Langerhans cells activate CD8+ cytotoxic T-cells [6] and promote T-helper type 1 (Th1) responses in CD4+ T-cells. Myeloid DCs (known as DC1 cells) also promote Th1 responses; plasmacytoid DCs (DC2 cells) induce Th2 responses [7].

Toll-like receptors

Toll-like receptors are a family of transmembrane proteins that help initiate the innate immune response. Ten Toll-like receptors (TLR1–10) have been cloned. They serve as an "early warning system" for recognition of microbial antigens and molecules produced by microbes, such as lipopoly saccharides, CpG DNA, and double-stranded RNA. Activation releases chemokines and other inflammatory mediators from dendritic cells and macrophages and modulates expression of chemokine receptors on dendritic cells. Several toll-like receptors can probably act in concert [8].

Antigen-specific immunity

Cell-mediated immunity

T-cells

T-lymphocytes (thymus-dependent lymphocytes) mediate delayed-type hypersensitivity reactions, regulate the development of antigen-specific antibody responses, and provide specific defense against many organisms. Distinct T-lymphocyte subpopulations express different cell surface proteins (see Table 1.2).

T-cell receptor complex

T-cells bear antigen-specific T-cell receptors (TCR), required for foreign antigen recognition and binding. TCRs have either α- and β-chains or γ- and δ-chains. Each chain has a variable amino-terminal involved in antigen recognition and constant carboxy-terminal regions. As T-lymphocytes mature, the TCR genes rearrange [9], creating unique TCRs within T-cells with specific antigen recognition capacity, generating TCR diversity to recognize many antigens. Lymphocytes with α/β-chain TCRs (α/β-cells) locate in lymphoid organs and peripheral circulation, those with γ/δ TCR chains (γ/δ T-cells) locate in mucosa.

MHC molecules

Antigen-presenting cells present antigen to T-cells as short peptides complexed with major histocompatibility complex (MHC) molecules. These cell surface molecules were

Table 1.2. Lymphocyte function and phenotype

Lymphocyte type	Function	Type of antigen receptor	Common cell surface markers
T-lymphocytes	• Regulation of the immune response	αβ T-cell receptor	CD3+,CD4+, CD8−
Helper	• Development of "memory" response to antigens		
Th1	• Cell mediated immunity – control of intracellular pathogens, DTH response • Activates macrophages via cytokine elaboration (IFN-γ and IL-2)	αβ T-cell receptor	CD3+,CD4+, CD8−
Th2	• Stimulates B-lymphocyte differentiation and proliferation (humoral immunity) • Elaborates cytokines involved primarily in the allergic response (IL-4, IL-5, IL-10)	αβ T-cell receptor	CD3+,CD4+, CD8−
Cytotoxic	• Lysis of tumor cells, virus-infected cells • Stimulates cell-mediated immunity via cytokine production	αβ T-cell receptor	CD3+, CD4–, CD8+
B-lymphocytes	• Production of antigen-specific immunoglobulins (humoral immune response)	Immunoglobulin molecules (IgG, IgM, IgE, IgA)	Fc receptors, MHC II molecules CD20, CD19
Natural killer (NK) lymphocytes	• Lysis of virus infected cells and tumor cells lacking MHC class I; antibody-dependent cellular cytotoxicity		CD16, CD56

initially identified as major antigens involved in transplant rejection. Cells expressing different MHC molecules are recognized as "non-self" and rejected. When foreign antigens are complexed with MHC molecules, the complex is recognized as non-self, initiating an immune response [10]. Class I MHC molecules are expressed on the surface of most cells and present intracellular antigens (e.g., antigens derived from infecting viruses). Class II MHC molecules exist primarily on "professional" antigen presenting cell (monocyte, macrophage, dendritic, and B-cell) surfaces and present proteins originating outside or within the cell (e.g., phagocytosed bacterial protein). CD4+ (helper/inducer) T-cells recognize exogenous antigen bound to class II molecules, and CD8+ (cytotoxic) T-cells recognize endogenous antigen bound to class I molecules [11].

Antigen presentation

The initiation of specific immune responses begins when the T-cell TCR recognizes short peptides processed and bound to an antigen-presenting cell (APC) MHC molecule. The TCR-associated CD3 molecule transduces a signal into the cell. Proper antigen recognition requires the TCR/CD3 complex. Other T-cell accessory molecules (CD4 and CD8) must also interact with the APC [12]. They bind the invariant regions of class I or class II MCH molecules. Other molecules, including CD28 and integrins, act as costimulatory signals to induce certain immune responses. TCR–MHC–antigen interaction produces T-cell activation and differentiation, initiating the response. T-cell surface markers change once the T-cell TCR encounters specific antigen. A subset of peripheral CD4+ T-cells (CD45RA+ $CD29^{low}$) includes naïve cells that have not encountered specific antigen, forming the pool of cells responding to novel antigens. After an initial antigen encounter they develop into memory T-cells (CD45RO+$CD29^{hi}$) [13]. These memory T-cells rapidly proliferate and produce cytokines when rechallenged with previously encountered antigens, yielding rapid, expanded secondary responses.

CD4+ T-cells

Most peripheral α/β T-cells express CD4 or CD8 antigens. CD4+ T-cells (helper/inducer cells) help regulate the immune response. CD4+ T-cells help B-cells to produce antigen-specific antibody. B-cells process antigen and present self-MHC-bound antigen fragments, activating CD4+ T-cells. During interactions between B- and CD4+ T-cells, membrane molecules that increase the efficiency of the interaction are upregulated [14]. CD40 ligand appears on the activated T-cell surface, which acts on B-cells, promoting humoral immune response [15]. CD4+ cells activate B-cells into antibody-secreting cells and help generate CD8+ T-cell cytotoxic and suppressor functions (see below). Memory T-cells (see above) are CD4+ T-cells.

T_H1 vs. T_H2 T-cells

Two functionally distinct CD4 cell subsets are distinguished by their cytokine expression [16]. T_H1 cells produce interferon-γ and IL-2, and enhance cellular immunity

Table 1.3. Selected cytokines, cell source and principal effects

Cytokine	Cell source	Target cell/principal effects
IL-2	T-cells	T-cells: proliferation and differentiation; activation of CTL and macrophages
IL-3	T-cells, stem cells	Cell colony stimulating factor
IL-4	T-cells	T/B-cells: B-cell growth factor, isotype selection
IL-6	T/B-cells	B-cells/hepatocytes: B-cell differentiation, acute phase reactant production
IL-8	Monocytes	Granulocytes, basophils, T-cells: chemotaxis, superoxide release, granule release
IL-12	Monocytes	T-cells: induction of TH1 cells
IFN-γ	T-cells, NK cells	Leukocytes, macrophages: MHC induction, macrophage activation and cytokine synthesis
TNF-α	Macrophages, mast cells, lymphocytes	Macrophages, granulocytes: activation of monocytes, granulocytes, increase adhesion molecules, pyrexia, cachexia, acute phase reactant production

IL – interleukin; IFN – interferon; TNF – tumor necrosis factor.

and macrophage activity. T_H1 cells regulate delayed-type hypersensitivity, granuloma formation, and intracellular pathogen killing. T_H2 cells produce IL-4, IL-5, and IL-10, and regulate humoral immunity, which mediates the development of allergic diseases: IL-4 promotes IgE production; IL-5 induces eosinophil proliferation and differentiation [12, 17]. Differentiation of naïve CD4+ T-cells into T_H1 or T_H2 cells depends on the cytokine milieu, antigen dose, and the specific antigen.

CD8+ T-cells

CD8+ (cytotoxic/suppressor) T-cells act as cytotoxic T-cells and can suppress immune responses [11]. Class I MHC-bound antigen activates CD8+ T-cells to generate antigen-specific cytolytic activity. Cytolytic T-lymphocytes (CTL) respond to viral infection of most host cells [12]. CD4+ cells help CD8+ T-cells to develop a CTL response by producing several cytokines, particularly IL-2 [18].

Cytokines

Cytokines are soluble proteins that modulate immune responses. They interact with specific membrane receptors. Different cytokines may perform similar functions and affect multiple cell types. Cytokine functions include (a) regulating lymphocyte growth and differentiation, (b) mediating inflammation, and (c) regulating hematopoesis. Cytokines affecting T-cells include the interleukins (IL), interferons, growth factors, and tumor necrosis factor (TNF) [12] (Table 1.3).

Table 1.4. Selected chemokines, their receptors and target cells

Chemokine	Receptors	Target cell
MIP-1α	CCR1 – 7	Eosinophils, monocytes, activated T-cells, dendritic cells, NK cells
MIP-1β	CCR1 – 7	Monocytes, activated T-cells, dendritic cells, NK cells
RANTES	CCR1 – 7	Eosinophils, basophils, monocytes, activated T-cells, dendritic cells, NK cells
Fractalkine	CX_3CR1	Monocytes, activated T-cells, NK cells
SDF-1	CXCR4	Monocytes, resting T-cells, dendritic cells
MIG	CXCR3	Activated T-cells, NK cells
IL-8	CXCR1 and 2	Neutrophils
IP-10	CXCR3	Activated T-cells
MCP-1	CCR2 and 5	Monocytes, activated T-cells, dendritic cells, NK cells
Eotaxin-1	CCR1-3	Eosinophils, basophils

MIP – macrophage inflammatory protein; SDF – stromal-cell derived factor; MIG – monokine induced by interferon gamma; IL – interleukin; IP – interferon inducible protein; MCP – monocyte chemoattractant protein
Adapted from [19].

Chemokines

Chemokines are a family of cytokines that regulate chemotaxis [19]. Over 40 chemokines are grouped into four families, including the α- and β-chemokines. β-chemokines have two adjacent cysteine residues (CC); α-chemokines have one amino acid separating the first two cysteine residues (CXC). Almost all cell types produce chemokines, particularly in response to inflammation. Proinflammatory cytokines (IL-1 and TNF-α), lymphokines (INF-γ and IL-4), and bacterial LPS and viral infection stimulate chemokine production. Chemokines bind specific target cell receptors. Most chemokine receptors bind more than one chemokine; however, CC chemokine receptors bind only CC chemokines and CXC receptors bind only CXC chemokines. Different leukocyte types express different chemokine receptors. Some receptors are restricted to specific cell types; others are expressed widely (Table 1.4).

Infiltrating inflammatory cells are determined partly by chemokines in affected tissue. Chemokines link innate and adaptive immune systems. Dendritic cells internalize antigens in tissues to carry them to lymph nodes, where naïve B- and T-cells are activated. Activated cells traffic back to inflammation sites. Chemokines regulate DC and lymphocyte trafficking [19].

Humoral immunity

Immunoglobulins

Immunoglobulins (antibodies) are proteins that bind antigen with high affinity and specificity. An immunoglobulin molecule is made up of two heavy and two light chains,

aligned in parallel, and covalently linked by disulfide bonds. (IgM is a pentamer of the basic immunoglobulin). The heavy and light chains have variable (V) and constant (c) regions. The variable regions of the chains (V_H and V_L) form the antigen-binding region. During B-cell development, rearrangement of the genes within individual B-cells yields unique, specific antibodies recognizing particular antigens [20]. Heavy chain constant regions determine antibody isotype: IgG, IgM, IgA, IgE, IgD. Immunoglobulin isotype functions include: (a) opsonization or binding to a microbe or particle to facilitate phagocytosis or killing (IgM, IgG, IgA, IgE); (b) complement fixation (activated via the classical pathway) (IgM, IgG); (c) direct inactivation of toxins or viruses (IgG, IgM, IgA); (d) antigen clearance via the reticuloendothelial system (IgG, IgM); and (e) release of chemical mediators following antibody receptor binding (IgG, IgE).

B-cells

B-cells bind antigen via cell surface immunoglobulin variable regions. Naïve B-cells express both cell surface IgD and IgM before encountering cognate antigen (antigen recognized by the immunoglobulin receptor). Surface immunoglobulin is associated with two other proteins, Ig-α and Ig-β; this complex forms the signaling pathway [21]. Most B-cells require T-cell help (via cytokines, contact of B- and T-cells through co-stimulatory molecules like CD40) for activation. B-cells may differentiate terminally into plasma cells, which can produce large amounts of specific immunoglobulin [20].

The primary immune response

When the immune system first encounters an antigen, few cells specifically recognize the antigen. The primary immune response is slow and produces low affinity antibodies [20]. Antigen is endocytosed and processed by an APC (monocyte, macrophage or DC, usually not B-cells), and is then presented to an antigen-specific T-cell. The T-cell must then contact and activate B-cells specific for the antigen/TCR complex, which proliferate or differentiate into plasma cells. Low affinity, mainly IgM is produced during this immune response phase. B-cells expressing higher affinity antibody are selected for activation and differentiation. Most B-lymphocytes differentiate into plasma cells; the remainder revert to memory B-cells.

The secondary immune response

When the B-cell re-encounters its cognate antigen, the antigen is endocytosed, loaded onto MHC class II molecules, presented on the surface of the B-cell to CD4 cells, which activate the B-cell. The B-cells proliferate and differentiate further, including class switching (DNA rearrangement that results in different heavy chain isotypes linked to variable regions). These B-cells can produce different types of high affinity antigen-specific immunoglobulins – IgG, IgE or IgA. Clonal proliferation and plasma cell differentiation occur in an accelerated manner, resulting

in rapid production of large amounts of high affinity antibodies when antigen is encountered [14].

Immune system development

Both innate and adaptive immune systems are less efficient in infants than in adults. Mucosal barriers are less effective, particularly in premature infants. Immunoglobulin levels are lower, and the specific immune response is decreased. Adult level immune responses are achieved within the first few years of life. Table 1.1 summarizes the major immune system developmental differences.

Innate immunity

Epithelial barriers/mucosal defenses

The epidermis increases in cell layers and thickness during gestation; infant skin is thinner than adult. Secretory IgA is undetectable at birth, but occurs in secretions by 2 weeks, reaching adult levels by 6–8 years [22]. Decreased secretory IgA permits greater adherence of pathogens to mucosa.

Complement

Complement synthesis begins early in gestation (6–14 weeks); by birth, levels and biologic activity of some complement cascade components equal adult levels. Some elements of the alternative pathway (C8, C9) are <20% adult levels, perhaps contributing to the susceptibility of infants to infection with organisms like *N. meningitides* [22]. Infants have decreased levels of C3b, contributing to increased encapsulated organism susceptibility.

Phagocytes

Macrophages and monocytes are derived from common stem cells. From bone marrow, mature monocytes migrate to peripheral blood. Monocytes circulate for 1–4 days, then migrate into tissue to differentiate into tissue macrophages. The monocytes circulating in blood vary, reflecting egress into tissues, margination along endothelial surfaces, and new cell migration from bone marrow. Tissue macrophages have long half-lifes (60 days to years); they are differentiated terminally.

The number of monocytes is higher in neonates than in adults. The number decreases from the neonatal period, reaching adult levels by early childhood. Tissue macrophages from infants may not kill pathogens as successfully as adult macrophages. There are modest differences in the generation of reactive oxygen intermediates [23].

Lymphokines from activated T-cells prime monocyte function. Neonatal monocytes respond less to IFN-γ production by NK and T-lymphocytes than do adult monocytes, decreasing lymphokine production by the T-cells [23]. Neonatal monocyte/macrophages produce less of some cytokines and growth factors (TNF-α,IL-8, IL-6 and G-CSF) [22, 23]. Immature macrophages have decreased chemotaxis,which persists until approximately 6–10 years of age.

Neutrophils

Neutrophils arise from bone marrow stem cells and differentiate into granulocytes. Their development depends upon cytokines and growth factors (granulocyte-colony stimulating factor (G-CSF)). Mature neutrophils are detected by 14–16 weeks' gestation. The blood neutrophil pool has equal circulatory and marginated components. In adults, neutrophil maturation takes approximately 9–11 days, accelerated by stress or infection. The number of neutrophils in the peripheral circulation rises after birth, but the ability to expand the neutrophil pool is limited in neonates, which may contribute to the inability of infants to increase circulating neutrophils with infection. Chemotaxis of neonatal neutrophils is decreased compared with adults. This deficit is multifactorial, reflecting decreased ability to adhere to vascular endothelium, decreased monocyte cytokine production, and additional chemotactic deficiencies; chemotaxis reaches adult competence by 2 years [24].

Natural killer cells

Neonatal NK cells have reduced cytolytic activity (~50% of adults), not reaching adult levels until 9–12 months of age, and decreased antibody-dependent cell-mediated cytotoxicity (ADCC). The decreased NK cell activities may increase susceptibility to infection by herpes simplex and by cytomegalovirus [22].

Dendritic cells

The reduced ability of neonatal T- and B-cells to respond to antigens (discussed below) may partly be due to reduced ability to present antigen. Cord blood dendritic cells express fewer MHC and ICAM-1 molecules and are less effective than adult cells at supporting antigen-stimulated T-cell proliferation [25].

Cell-mediated immune response

Thymic development

The thymus descends to its position in the anterior mediastinum between 7 and 10 weeks' gestation. By 10–14 weeks' gestation, it is highly organized, and emigration of mature T-cells has been established. The thymus has a cortex, containing immature T-cells, and a medulla where mature T-cells migrate. Thymic stromal cells play a role in the differentiation, development, and selection of T-cells [26]. Proper development of T-cells bearing the $\alpha\beta$ TCR depends on an intact thymus; some T-cells bearing $\gamma\delta$ TCRs undergo thymus-independent development [22].

T-cell phenotype

Neonatal T-cells have a naive (CD45RA+ $CD29^{low}$) phenotype. Ninety percent of neonatal T-cells have this phenotype, compared to 60% of adult T-cells [13]. Memory T-cells (CD45RO+, $CD29^{hi}$) migrate to sites of inflammation, depend less on costimulatory molecules for activation, proliferate more rapidly, and produce cytokines more

efficiently [27]. These attributes permit rapidly expanded T-cell responses with antigenic rechallenge. Neonates experience delayed T-cell-dependent responses.

T-cell numbers

T-cell numbers increase from mid-gestation until 6 months of age (median CD4 cell count at 6 months is ~3000 cells/mm^3). Counts decline throughout childhood until adult levels (~1000 cells/mm^3)are reached by late childhood. Changes in CD4 percentage are less dramatic, declining from ~50% to ~40% between infancy and adulthood. The CD4+ to CD8+ ratio changes throughout childhood, achieving the adult ratio of 2:1 at ~4 years (Fig. 1.1) [28, 29].

Cytokine production

TNF-α and GM-CSF are modestly reduced in neonates, while others, critical for a rapid integrated immune response (IFN-γ, IL-3, IL-4, IL-5 and IL-12) are markedly decreased in neonates [30]. IL-2 and TNF-β are at near adult levels [31]. Neonatal CD4+ T-cells preferentially develop a Th2 phenotype [32], but increased CD28 costimulation in high Th1 cytokine levels, suggesting that the neonatal T-cell defect is not intrinsic, but is related to activation conditions. Administration of IL-12 can induce an adult-type Th1 response in neonates. Cytokine synthetic ability increases with age. TNF-α production normalizes within the first few months of life; IFN-γ and IL-12 production normalizes by 1 year [22, 30].

T-cell help for antibody production

Infant T-cells provide less help to B-cells. This reflects reduced cytokine production and reduced expression of costimulatory molecules (CD40 ligand) [33]. Diminished T-cell help produces delayed infection and immunization.

DTH

Delayed type hypersensitivity requires the integration of T-lymphocytes with APCs. At birth, there is no detectable DTH, due to absent memory T-cells, defective monocyte chemotaxis, and/or decreased numbers of efficient APCs. A reliable DTH response exists after 1–2 years [34].

Cytotoxic T-lymphocytes (CTL)

CTL development and the magnitude of CTL activity are decreased during most natural infections in infants. CTL activity matures within the first year of life [30].

Humoral immune response

Development

The fetus can mount humoral immune responses by 6–7 months' gestation; humoral immune function reaches adult competence after 2 years of age. B-cell maturation continues in bone marrow throughout life, although only a small fraction of B-lymphocytes

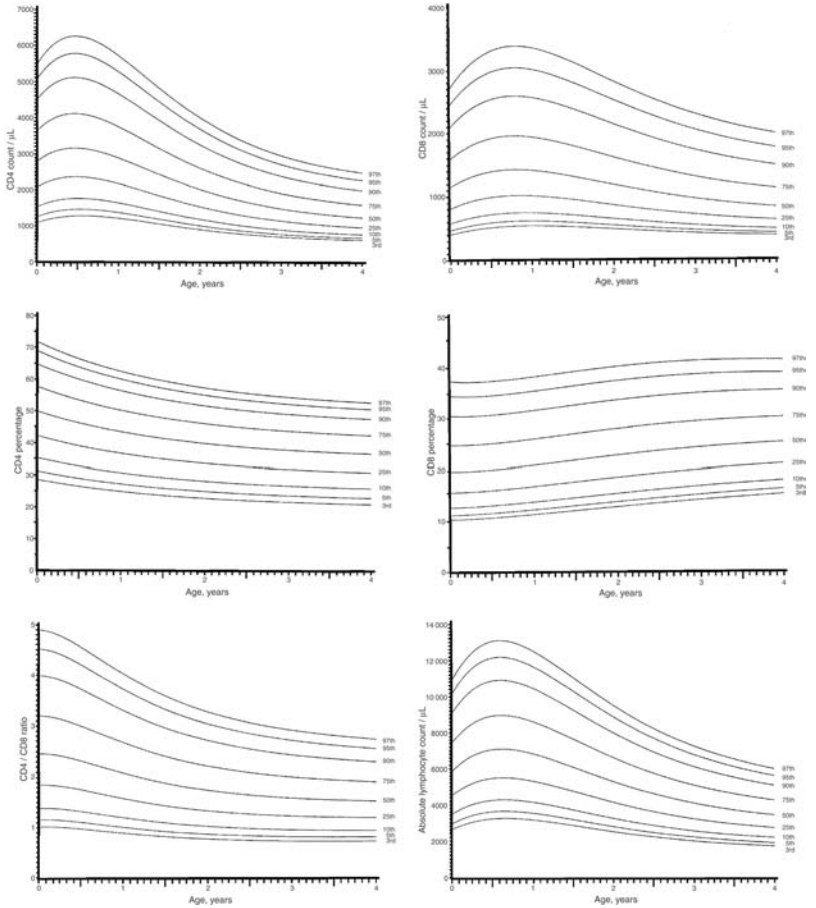

Fig. 1.1. Lymphocyte parameters as a function of age. The panels show values for the CD4+ and CD8+ lymphocyte counts and percentages, the CD4+/CD8+ lymphocyte ratio, and the absolute lymphocyte count during the first 4 years of life. Figure modified from reference [29].

circulates. An ongoing process eliminates B-cells with non-functional or self-reactive immunoglobulins [35].

Immunoglobulins

Neonatal B-cells show Ig diversity similar to adult cells. Neonatal B-cells undergo somatic mutation at the same rate as adult cells [36]. Immunoglobulin concentrations

gradually rise with age (Table 1.5) although the repertoire of IgG, especially IgG2a, achieves adult phenotypes after 6–12 months [30].

B-cells

In vitro, neonatal B-cells can differentiate into plasma cells secreting IgM, but not those secreting IgA or IgG. This limitation probably reflects the lack of appropriate T-cell help (discussed above), rather than an inability of B-cells to class switch [37].

B-cell response to specific antigens

Responses to antigens that need T-cell help generally mature faster than those occurring independently of T-cells. Infants generally mount protective immune responses against protein antigens (T-cell-dependent responses, e.g., tetanus, diphtheria, polio). Thymus-dependent immune responses mature rapidly, in many cases by 2 months. IgG antibody responses to protein antigens approach adult levels after 1 year [30]. However, the response to polysaccharide antigens (e.g., *H. influenzae* or *S. pneumoniae*) (T-cell independent antigens) only matures at 2–3 years. Reduced levels of complement receptors on infant B-cells, low complement activity (C3d), reduced IgG2a production, and immaturity of the splenic marginal zone may cause these delays [30]. These differences help explain the age-specific risk for development of invasive bacterial disease.

HIV basic virology

Classification and origin of HIV

HIV-1 belongs to the *Lentivirus* genus of retroviruses (for review see [38]). The virus entered the human population in Africa about 70 years ago [39], probably as humans hunted and butchered chimpanzees. HIV-2, a less pathogenic relative of HIV-1, infects some human populations mostly in western Africa. The material in this book refers to HIV-1 (usually just "HIV") unless otherwise noted.

HIV-1 is grouped into several clades or subtypes (A, B, C, D, E, F, G, H, J, and K) [38], and three groups (M (main) group, O (outlier); N (non-M, non-O)). Certain clades predominate in certain areas. Clade B predominates in North America, and represents the major subtype in Europe and Australia. Clade A predominates in West Africa, clade D in Central Africa, and clade C in Southern and Eastern Africa and the Indian Subcontinent. Clade E is a major subtype in Thailand. Some assays optimized for one clade (clade B) may not detect other clades. Immunologic responses aimed at one clade may not affect other clades, complicating vaccine development.

HIV virion structure

The virion's capsid is composed of viral capsid (CA or p24) protein, enclosing two copies of the RNA genome, and two copies of reverse transcriptase (RT or p66/p51) (Fig. 1.2). Within the capsid, viral nucleocapsid (NC or p9) proteins are complexed with the viral

Table 1.5. Levels of immunoglobulins in normal subjects by age

	Total immunoglobulins		IgG		IgM		IgA	
Age	mg/dl	% of adult level	mg/dl	% of adult level	mg/dl	% of adult level	mg/dl	% of adult level
Newborn	1044±201	67±13	1031±200	89±17	11±5	11±5	2±3	1±2
1–3mo	481±127	31±9	430±119	37±10	30±11	30±11	21±13	11±7
4–6mo	498±204	32±13	427±186	37±16	43±17	43±17	28±18	14±9
7–12mo	752±242	48±15	661±219	58±19	54±23	55±23	37±18	19±9
13–24mo	870±258	56±16	762±209	66±18	58±23	59±23	50±24	25±12
25–36mo	1024±205	65±14	892±183	77±16	61±19	62±19	71±37	36±19
3–5 yr	1078±245	69±17	929±228	80±20	56±18	57±18	93±27	47±14
6–8 yr	1112±293	71±20	923±256	80±22	65±25	66±25	124±45	62±23
9–11 yr	1334±254	85±17	1124±235	97±20	79±33	80±33	131±60	66±30
12–16 yr	1153±169	74±12	946±124	82±11	59±20	60±20	148±63	74±32
Adult	1457±353	100±24	1158±100	100±26	99±27	100±27	200±61	100±31

Mean values ± one standard deviation – normal levels may vary at different reference laboratories.
Modified with permission from [299].

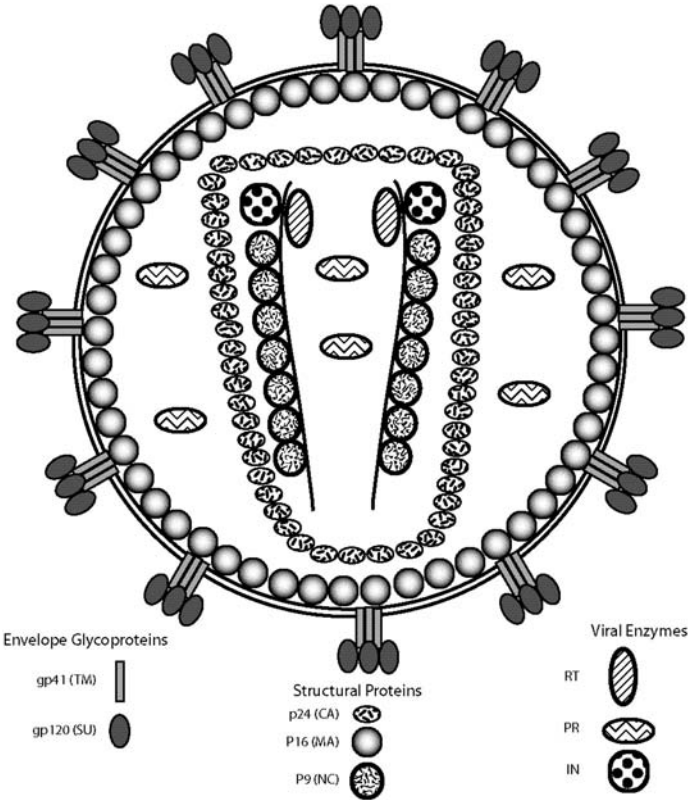

Fig. 1.2. A schematic diagram of the HIV virion. Individual viral proteins and their functions are described in detail in the text.

genomic RNA, via zinc finger domains in NC and a specific viral RNA "packaging signal" (the "Ψ-site") (see below). Also within the capsid lie viral integrase (IN or p31) and viral protease (PR or p11). RT, PR, and IN derive from gag–pol preprotein precursor (Pr160), cleaved into its subunits by PR (see below). Other core proteins derive from gag preprotein (Pr55).

The core outer region contains matrix (MA or p16) protein. MA lies inside the envelope, tethered to the interior side of the envelope via myristic acid. The envelope is a lipid bilayer, derived from host cell plasma membrane. Anchored into the lipid bilayer and extending out into extracellular space is the gp41 transmembrane portion of the viral envelope (or Env) glycoprotein (TM or gp41). TM associates non-covalently with the gp120 envelope (Env) glycoprotein (SU or gp120); gp120 and gp41, processed from

a precursor, gp160, are highly glycosylated. gp120+gp41 exist as trimers on the virion surface, and mediate viral entry and syncytium formation. gp120 has constant (C) regions, with amino acid sequences remaining relatively constant among viruses, and variable (V) regions.

The virion also contains host cell macromolecules derived, and incorporated, during virion assembly [40]. Some of these (cellular lysine transfer RNA, the protein cyclophillin A) play crucial roles in viral replication. Figure 1.2 outlines virion structure. Table 1.6 lists the viral proteins.

The HIV life cycle

Viral entry into the host cell

Figure 1.3 depicts the life cycle. Infection begins when gp120 binds CD4 on the future host cell (for review see [41]). Binding triggers a change in gp120, facilitating interaction with a viral coreceptor, CXCR4 or CCR5 (which physiologically serve as chemokine receptors, see above and Table 1.4). Coreceptor binding triggers changes in gp41, inserting part of gp41, the "fusion peptide," into the host cell membrane, and the formation of a specialized structure in gp41, bringing the membranes into apposition and engendering envelope-membrane fusion [42], releasing capsid into the cytoplasm. The newly developed antiretroviral agent enfuvirtide (T-20, Fuzeon), a 36-amino acid peptide, is homologous to gp41 [43]. T-20 binds to a gp41 instead of to a native gp41 helix, preventing gp41 from forming structures producing fusion. Some small molecule inhibitors are also under development. Decreasing expression of cell surface CD4 can also block *in vitro* infection [44], but clinical utility lies far in the future.

HIV has tropisms for different host cell types: macrophage-tropic (M-tropic) and T-cell-tropic (T-tropic). Coreceptor usage determines tropism. M-tropic strains infect macrophages, monocytes, and primary T-cells, but not CD4+ T-cell lines (for review see [45]). M-tropic (or R5) viruses use CCR5 as the coreceptor. T-tropic (or X4) viruses infect CD4+ T-cells, but not macrophages and monocytes, using CXCR4 as the coreceptor. Some dual tropic (R5X4) viruses exist. Certain Env V-region envelope sequences are associated with particular tropisms. Most patients are first infected with R5 viruses. Later, the predominant virus may shift to X4, a shift associated with clinical deterioration.

The α-chemokines (or C–C chemokines, for their juxtaposed cysteines) (RANTES, MIP1-α, and MIP1-β) bind CCR5 (Table 1.4), or A β-chemokine (C–X–C chemokine, for adjacent cystein-X-cysteine residues), stromal derived factor 1 (SDF-1), or binds CXCR4. Other chemokine coreceptors may mediate HIV entry into certain cells, perhaps including the central nervous system (CNS) [46].

The coreceptors are drug development targets. Some inhibitors block viral replication *in vitro* and are currently in early stage human trials, including AMD-3100, which targets CXCR4, and Schering-C and -D, targeting CCR5 [47].

Individuals with mutant coreceptors are less likely to become HIV-infected and HIV-infected patients bearing coreceptor mutations have slower disease progression

Table 1.6. HIV viral genes and gene products: existing and potential targets for antiretroviral agents

HIV genes and gene products as targets for antiretroviral drug development. The table lists the viral genes, the proteins the viral genes encode, and the function of the proteins. The table also notes whether the proteins have been used as targets for the development of antiretroviral drugs. The proteins that are targeted by approved drugs or drugs in advanced stages of clinical development are listed in bold.

Viral protein	Gene	Function	Inhibitors
p16 (MA)	*gag*	Matrix protein; lies beneath envelope; targeted to membrane via myristoylation; recruits envelope into virion; aids in PI localization to nucleus	Nuclear localization site inhibitors; myristoylation inhibitors; transdominant negative gag mutants
p24 (CA)	*gag*	Capsid protein; viral core	
p9 (NC)	*gag*	Nucleocapsid protein; interacts with viral RNA via zinc fingers	Zinc chelators
p6 (NC)	*gag*		
Protease (PR)	*pol*	Cleaves gag (Pr55) and gag–pol (Pr160) precursor proteins during virion maturation	Protease inhibitors
Reverse transcriptase (RT)	*pol*	Catalyzes synthesis of viral cDNA from viral genomic RNA	Nucleoside analogue reverse transcriptase inhibitors (NRTIs); nucleotide analoge reverse transcriptase inhibitors (tenofovir); non-nucleoside analogue reverse transcriptase inhibitors (NNRTIs)
Integrase (IN)	*pol*	Catalyzes integration of viral cDNA into host cell genomic DNA to create provirus	Integrase inhibitors
gp120	*env*	Mediates interaction of virus with CD4 and chemokine co-receptors. Initial steps of viral binding and entry.	Chemokine co-receptor inhibitors (e.g., Schering C and D); binding inhibitors (soluble CD4)
gp41	*env*	Integral membrane envelope glycoprotein; contains fusion domain mediating virion envelope-host cell plasma membrane fusion	Fusion inhibitors (e.g., enfuvirtide (T-20)

(*cont.*)

Table 1.6. (*cont.*)

Viral protein	Gene	Function	Inhibitors
Tat	*tat*	Transactivates viral gene expression; binds to TAR structure in nascent viral RNA and cellular kinase leading phosphorylation of celluar RNA polymerase II, increasing processivity	Kinase inhibitors (cellular enzyme); Small molecule inhibitors; Tat-TAR interaction blockers; TAR decoys; antisense oligo-nucleotides; ribozymes, small interfering RNAs
Rev	*rev*	Mediates nuclear export of singly spliced and unspliced viral RNAs	Small molecule inhibitors, inhibitors of Rev-RRE binding (aminoglycosides); RRE decoys; transdominant Rev; antisense oligonucleotides; ribozymes; inhibitors of nuclear export, small interfering RNAs
Vif	*vif*	Viral infectivity factor	
Vpu	*vpu*	gp160/CD4 complex degradation; CD4 downregulation; virus release	
Vpr	*vpr*	Cell cycle arrest; transactivation; PIC entry into nucleus	
Nef	*nef*	CD4 downregulation; stimulates cellular signal transduction pathways	

Targets of drugs with current clinical utility (licensed drugs and drugs in advanced clinical development) are shown in **bold**.

(including some "long-term non-progressors" (LTNP)). Important mutations include a 32- base pair CCR5 gene deletion (Δ32CCR5) [48]. Δ32CCR5 does not localize to the cell membrane. Δ32CCR5 cells do not resist infection by T-tropic viruses, since they use CXCR4. Δ32CCR5 homozygotes are highly resistant to initial infection, because M-tropic viruses may be primary mediators of transmission. Δ32CCR5- individuals exhibit no discernable deleterious phenotype. Other mutations that decrease CCR5 are also seen in LTNP.

Reverse transcription

After entry, viral capsid releases the RNA genome, with proteins and the tRNA to prime reverse transcription, into the cytoplasm. RT catalyzes reverse transcription, through which it produces a cDNA of the viral genome. RT consists of a dimer, p51 and p66 (the

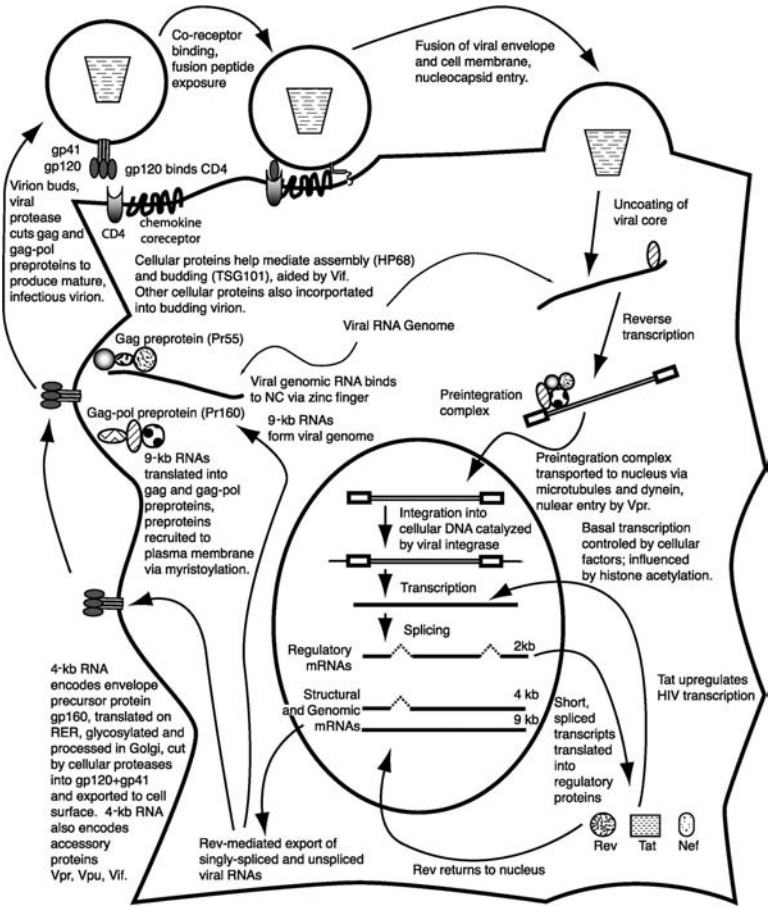

Fig. 1.3. A schematic diagram of the HIV replication cycle. The features of the viral life cycle are described in detail in the text.

catalytic subunit). Many drugs target RT, including nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) (see Chapter 14).

The HIV RNA genome begins 5′ with the "R" (repeat) region, followed by the U5 (5′ unique) region, then sequences encoding viral proteins and, at the 3′ end, U3 (3′ unique) and another R (Fig. 1.4) [49]. RT synthesizes a double-stranded cDNA version of the genome, with two long terminal repeats (LTRs) at either end. Each LTR consists of the repeated versions of U3, R, and U5.

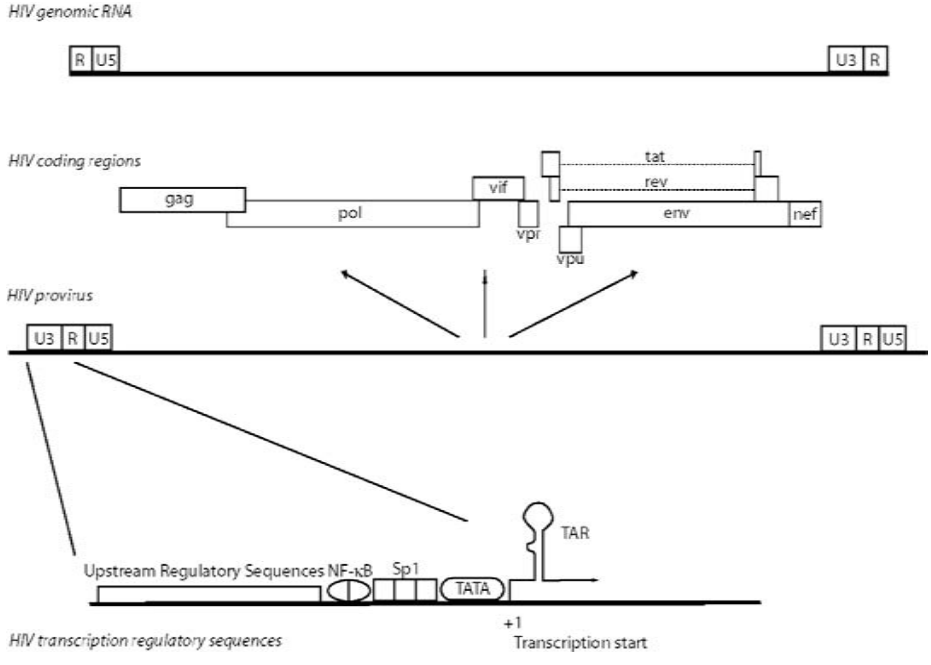

Fig. 1.4. The genomic organization of HIV. The top of the figure shows the organization of the viral genomic RNA. The middle of the figure shows the HIV provirus, with the reading frames of the different HIV genes identified. The lower portion of the figure shows the location of selected HIV transcription regulatory sites, including both selected sites active in the HIV LTR (NF-kB, Sp1, and TATA), and the TAR site as it exists in the newly transcribe viral RNA.

Reverse transcription is an essential step in replication. NRTIs were the first antiretrovirals. NRTIs are converted to the active triphosphate form by cellular kinases. The triphosphate-NRTIs compete with native nucleotide triphosphates. When RT incorporates the NRTIs into cDNA, no further nucleotides can be added, truncating the cDNA because the ribose 3′-OH is replaced by another group incapable of forming a covalent bond with the next nucleotide. For example, the 3′-OH is replaced by an azido group in zidovudine (ZDV or AZT). These drugs are termed "chain terminators."

The non-nucleoside RT inhibitors (NNRTIs) represent another class of RT inhibitors, with three licensed drugs, efavirenz, nevirapine, and delavirdine. NNRTIs act differently, binding a hydrophobic pocket near the enzyme active site (see Chapter 14).

RT is a "low fidelity" enzyme, inserting the wrong base (termed "misincorporation") in the growing cDNA chain every 1 per 1700 to 1 per 4000 bases, producing mutant virus (see Chapter 14).

Nuclear localization and entry

After reverse transcription, cDNA associates with viral proteins (IN, RT, MA, and NC, the viral accessory protein Vpr, and host cell proteins Ku, INI 1 and HMGa1 (or HMG I(Y)), to form the pre-integration complex (PIC) [50]. Some PIC components (IN) are essential for later replication cycle steps. Others (the cellular proteins) may not be essential. Following reverse transcription, cellular machinery transports PICs to the nucleus, with help from Vpr [51]. The PIC interacts with the nuclear membrane, via Vpr docking with host cell nucleoporin protein, hCG1 [52], producing nuclear entry.

Integration

After nuclear entry, HIV cDNA integrates into the host cell genomic DNA. A cDNA preintegration form with a recessed 3′ end interacts with 5′ overhanging ends in cellular DNA, generated by IN, which then joins the cDNA and the cellular DNA, in a process called strand transfer [53].

Since provirus formation is an essential life cycle feature and since IN is an essential enzyme in HIV replication [54], IN is a drug development target [55].

Control of viral gene expression

After integration, the provirus can either remain quiescent, or it can begin replication. *In vitro*, treatment of latently infected cells with host cell signal transduction activating or histone acetylation altering agents (phorbol esters, butyrate) [56], or certain cytokines (IL-2) can induce the infection cycle completion [57].

During lytic infection cycle progression HIV regulates its gene expression in a tightly controlled pattern of three phases (Fig. 1.5). In the initial phase, low levels of full-length transcripts are produced. Some transcripts are retained in the nucleus, spliced, and exported to the cytoplasm. These short transcripts encode viral regulatory proteins, notably Tat. Next, Tat, with cellular factors, transactivates viral gene transcription, dramatically increasing expression. Finally, Rev mediates nuclear export of unspliced

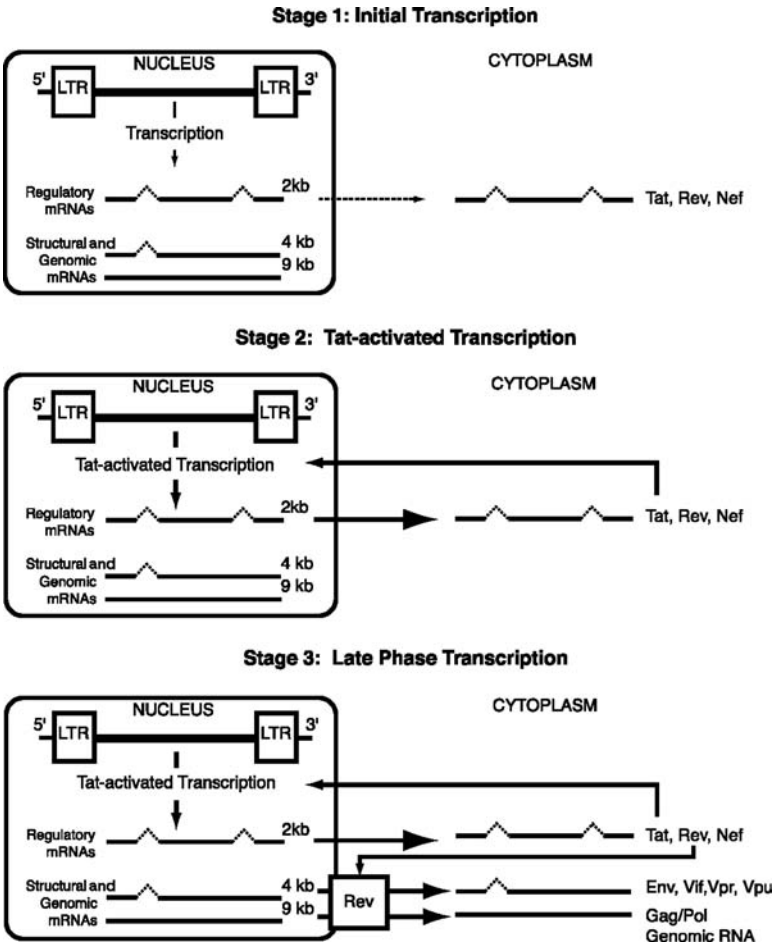

Fig. 1.5. Phases of HIV gene expression. Initially, only small amounts of transcription occur and only small quantities of short, multiply spliced viral messages encoding the viral regulatory genes Tat and Rev and the accessory gene Nef are exported to the cytoplasm. When the HIV promoter is activated, more messages are produced and sufficient Tat protein returns to the nucleus to produce a large increase in viral gene expression. Later, when sufficient quantities of Rev are present in the nucleus, the longer singly spliced and unspliced messages encoding the viral structural proteins and comprising the viral genomic RNA are exported to the cytoplasm.

and singly spliced RNAs encoding viral structural proteins, including future viral genomes.

Regulation of transcription from the HIV LTR by cellular factors

The provirus 5′ HIV LTR is the viral promoter. It contains regulatory sites homologous to those in cellular promoters (Fig. 1.4) [58, 59]. Some sites regulate basal HIV expression in lymphocytic cells [60]; others may modulate expression in different cell types [61]. The critical regulatory sequences are TATA and Sp1 [62]. NF-B sequences contribute to basal expression and mediate viral promoter responses to stimulatory signals. TATA serves as the transcription machinery assembly site, including transcription factors and RNA polymerase II. Targeting certain HIV RNAs can inhibit HIV replication [44], but clinical applicability is probably distant.

Sequences 5′ to the TATA, Sp1, and NF-B sites can affect HIV expression, but their function is less clear. Some 5′ sequences are critically important for maximal expression in certain non-lymphocytic cell types [63].

Chromatin structure is another factor influencing HIV gene expression. The location and acetylation state of histones help control expression from the integrated provirus [64]. Altering histone acetylation can greatly increase expression [65].

Regulation of transcription by Tat

The short (2 kb) viral transcripts first produced by HIV encode three viral gene products, Tat, Rev, and Nef. Tat produces a dramatic increase in expression. Rev regulates HIV gene expression post-transcriptionally, controlling the export of HIV RNA from the nucleus. Nef has several effects on the virus and host cell.

Tat binds a distinctive stem–loop–bulge RNA secondary structure, the trans-activation responsive (TAR) region, in HIV RNAs (Figs. 1.4 and 1.6). Without Tat, RNA polymerase II stalls, producing mostly very short transcripts. If transcription is activated, so that Tat and the TAR-containing transcript are present simultaneously, Tat binds to the bulge in TAR [66], recruiting additional cellular factors, P-TEFb (or TAK, Tat-associated kinase), including the cellular protein kinase CDK9 (or PITALRE) and cyclin T [67]. CDK9 phosphorylates the RNA polymerase II C-terminal domain, making the enzyme more processive [68].

Tat can be secreted from infected cells and taken up into cells from the extracellular environment, affecting cells [69] in ways that may contribute to pathogenesis.

Inhibition of Tat activity is another potential target for drug development. Small molecule inhibitors could block Tat–TAR interaction [70]. cdk9 inhibitors can block Tat activity, inhibiting replication [71].

Post-transcriptional regulation of gene expression by Rev

Rev controls the switch from the early pattern of viral gene expression where multiply spliced 2-kb messages are expressed, to the late pattern of viral gene expression where longer 4-kb and 9-kb messages are expressed (for review see [72]) (Figs. 1.4 and 1.7).

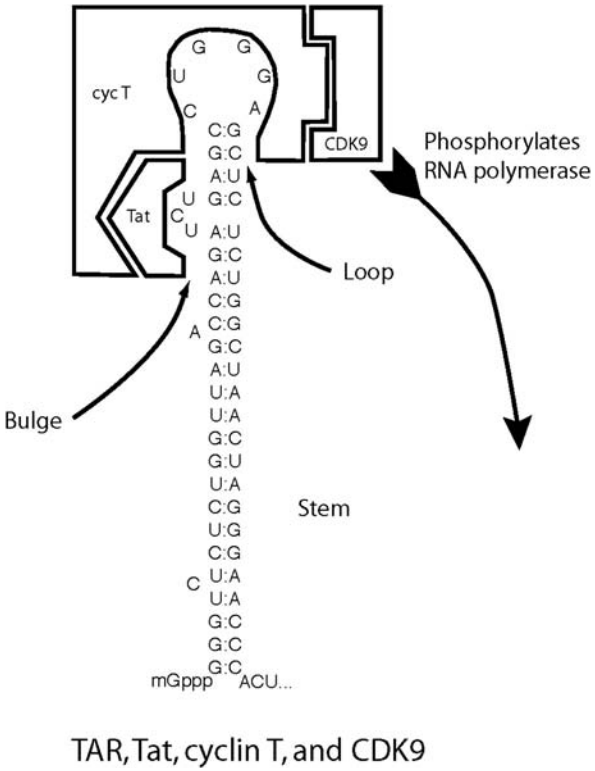

Fig. 1.6. TAR, Tat, and associated cellular factors. Tat binds to the "bulge" region of the stem–loop–bulge structure in the TAR RNA. The cellular protein cyclin T interacts with Tat and with the TAR loop region. The cellular kinase cdk9 interacts with cyclin T and then goes on to phosphorylate the C-terminal domain of RNA polymerase II, greatly increasing the processivity of the polymerase, causing a large increase in the expression of the viral RNAs.

HIV RNA is initially produced as a full-length transcript, but if introns are not spliced out of the RNA, without Rev, the 4-kb and 9-kb singly spliced and unspliced messages are not exported from the nucleus. Rev enables the nuclear export of these messages. The HIV RNA contains a region within env that forms a complicated stem–loop secondary structure, the Rev-responsive element (RRE). Revs cooperatively bind RRE RNA. With enough Rev bound, the longer unspliced messages exit the nucleus.

Rev has two regions, nuclear localization /RNA binding region, and another mediating nuclear export (Fig. 1.7). Rev, with bound HIV RNA, binds a cellular protein, CRM1. CRM1 in turn binds another cellular protein, Ran, a small GTPase, but only when Ran

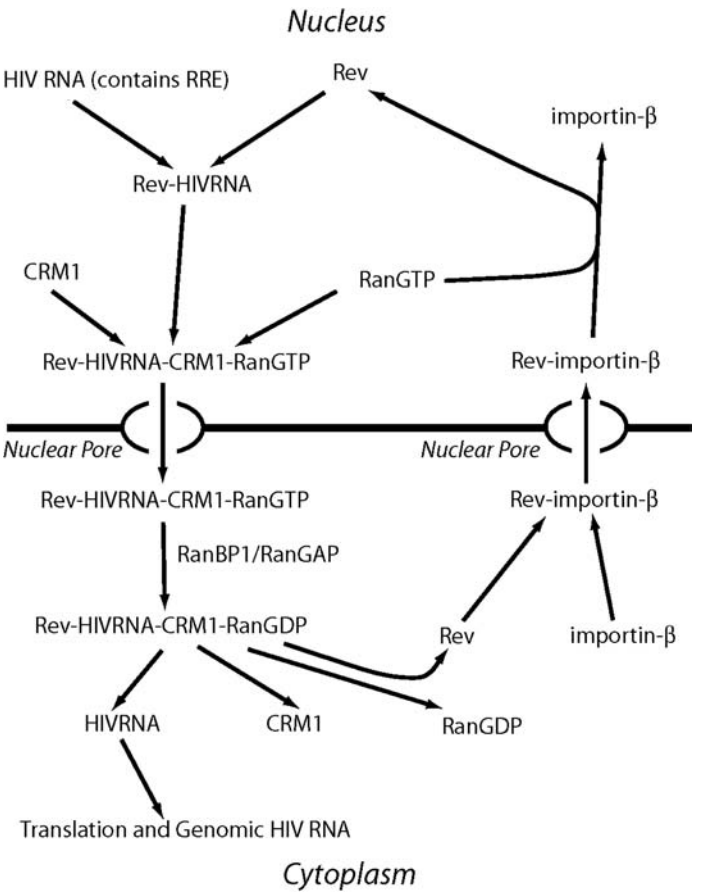

Fig. 1.7. Rev and the export of HIV RNA from the host cell nucleus. Singly spliced and unspliced HIV RNAs are exported from the host cell nucleus via a specialized host cell nuclear pore apparatus, after Rev binds to the RRE sequence in the HIV RNA. See text for details.

has bound GTP (Ran-GTP) [73]. This complex is translocated into the cytoplasm where two proteins, Ran GTPase activating protein 1 (Ran GAP1) and Ran binding protein 1 (Ran BP1) hydrolyze GTP bound to Ran, dissociating the complex bound to HIV RNA, freeing RNA [74]. Rev re-enters the nucleus by binding a cellular protein called importin-β. In the nucleus, RanGTP interacts with importin-β freeing Rev to start the cycle again.

Rev is a potential drug development target. Some small molecules inhibit Rev activity *in vitro*, but none have entered clinical development. Some Rev mutants can inhibit Rev function [75].

Translation of structural (late) viral messages and post-translational modification of the late viral proteins

The long singly spliced and unspliced RNAs encode viral structural and enzymatic proteins. A Gag preprotein, Pr55, and a Gag–Pol fusion preprotein, Pr160, are translated from full-length RNA. The capsid structural components, p16 (MA), p24 (CA), p9 (NC), and p6 (NC), are first translated in the form of Pr55 preprotein, cleaved by the viral protease during virion maturation. The Gag–Pol fusion protein is also cleaved during maturation, forming the *pol* products: RT, IN, and PR (see Chapter 14).

HIV uses a translational mechanism to regulate the relative amounts of *gag*- and *pol*-derived protein production. In the HIV virion, there are more *gag*-derived structural proteins than there are pol-derived enzymatic proteins.

The 4-kb RNA encodes the envelope preprotein gp160 and the HIV accessory proteins Vpr, Vpu, and Vif. gp160 is translated in the rough endoplasmic reticulum (RER), glycosylated, transits through the Golgi complex, and is cleaved by furin family cellular proteases, forming gp41, which remains membrane anchored, and gp120, which remains non-covalently gp41-associated [76]. The glycosylation pattern helps influence viral coreceptor usage [77]. From the Golgi, gp120/gp41 moves to the external cell surface. Blocking gp160 proteolytic processing inhibits infectious virus production.

Viral envelope glycoprotein glycosylation is essential for viral pathogenicity. Glycosylation appears to mask critical sites from host immune responses [76]. Unglycosylated glycoproteins become much more immunogenic. The resulting virus is less pathogenic, probably because the sugar residues guarding critical conserved immunogenic sites are gone [78].

gp160 and CD4 can become non-covalently associated within the RER during translation and processing, preventing the appearance of gp120 and gp41 at the surface. The viral accessory protein Vpu interacts with CD4 in the gp160/CD4 complex, inducing proteolysis [79], freeing gp 160 from the complex and for processing into envelope glycoproteins. This also causes cell surface CD4 downregulation, which may prevent host cell superinfection [80].

Virion assembly, budding, and maturation

Virion assembly occurs at the interior face of the plasma membrane, where Gag and Gag–Pol preproteins assemble beneath the envelope glycoproteins [81]. Following translation, Gag preproteins are myristoylated: a fatty acid, myristic acid targets the preprotein to the plasma membrane. The preprotein preferentially inserts into cholesterol-rich microdomains or "rafts" in the plasma membrane, where new virions bud [82]. Agents that deplete cholesterol from the plasma membrane, including approved agents

like simvastatin, decrease virion production [82], but cholesterol is an obligatory component of many membranes and serves other important functions.

About 1500 Gag molecules assemble together to form a functional viral core. A cellular protein (HP68) helps assemble the Gag molecules and promotes viral core formation [83].

Other aspects of virion assembly are mediated by CA, which contains a domain enabling Gag to multimerize [84], another functioning viral core condensation, and another binding the host protein cyclophilin A [85]. Mutations in the multimerization domain or the domain involved in core condensation produce defective virions. The cyclophilin A interaction appears essential for infectious virus formation [86].

The viral RNA genome is packaged into virions by interactions with the Pr55 Gag preprotein [87]. The p9 (NC) region contains two zinc-finger amino acid motifs (cys–X_2–cys–X_4–his–X_4–cys, where X is any amino acid), distinct from cellular zinc finger domains. The full-length genomic RNA contains the RNA packaging signal sequence (Ψ site) at the 5′ end. The sequence is spliced out of the short and intermediate length viral RNAs, preventing packaging into virions. Viral RNA packaging constitutes another drug development target. Zinc chelators block zinc from binding the NC zinc finger, making it in turn unable to bind HIV RNA, blocking infectious virion formation [88].

HIV proteins are essential for budding, but the virus also uses a cellular pathway, used to form the multivesicular body (MVB), to mediate virion budding [89, 90]. Certain *env* mutations prevent Env from associating with assembling viral proteins and being incorporated into virions, implying that gp41 and MA interact to promote Env incorporation [91].

After budding, the newly formed virion undergoes maturation, required for viral infectivity, involving viral protease-catalyzed proteolytic processing. PR cleaves Pr55 (Gag) and Pr160 (Gag–Pol) proteins to produce mature virion proteins (see Fig. 1.3, and Chapter 14). PR is essential for infectious virion production and has become a favored drug development target. HIV protease inhibitors (PIs) are a remarkably effective class of antiretrovirals. PI resistance is described in more detail in Chapter 14.

HIV accessory proteins

The HIV accessory proteins, Nef, Vpu, Vif, and Vpr, have important functions in the viral life cycle. *In vitro*, mutations in the accessory genes do not abolish viral replication, but are critical *in vivo*.

Vpu enhances virus production by downregulating CD4 post-translationally by binding the cytoplasmic tail of CD4 while CD4 is in the endoplasmic reticulum (ER) [92, 93].

Vif (virion infectivity factor) is required in certain cell types during the late stages of infection for infectious virus production [94]. Some lymphocytes have innate antiretroviral activity, yielding non-infectious virus. Vif suppresses this innate antiretroviral activity. This antiretroviral activity results from a cellular gene, CEM15 (Apobec-3G), which inhibits the production of infectious virus lacking Vif [95]. APOBEC-3G

(CEM 15) deaminates cytidine to produce uracil, an activity that can cause mutations that decrease viral infectivity.

Vpr has several functions, in addition to PIC nuclear localization and import, which contribute to infection of non-dividing cells [96]. Vpr is incorporated in large amounts into virions. Vpr can modestly increase HIV gene expression and alters the expression of some cellular genes [97]. Vpr causes cell cycle arrest at G_2 [98], which may increase expression and make more precursors available for virion production.

Nef has several activities during replication and is incorporated into virions. Nef is required for the virus to be fully pathogenic. Nef augments the infectivity of HIV virions, induces downregulation of cell surface CD4 molecules [99] by targeting CD4 for incorporation into endosomes through interactions with a cellular protein, β-COP [100], and interacts with cellular signal transduction pathways [101]. Nef also decreases the surface expression of cellular MHC Class 1 [102].

Immunology of pediatric HIV disease

HIV-1 damages the immune system by harming the infected host cells, harming non-infected cells via the effects of virions and parts of virions, and by chronic cell activation. This can lead to dysfunction of other cell types, since the immune system is highly interconnected. The main target cells of HIV-1 include cells that are critical in the immune control of the virus.

Immunopathogenesis

Primary infection

Most HIV-1 infections result from exposure of HIV-1 to mucosal surfaces [103]. Mucosal dendritic cells transport HIV-1 to regional lymph nodes within 48 hours of exposure, where CD4+ T-cells become infected. Infected T-cells and virus can be found throughout the body 4–11 days after infection.

HIV in blood increases rapidly over the first weeks after infection in adults, then declines dramatically, reaching a stable set point after approximately 6 months. HIV-1-specific cytotoxic T-lymphocytes (CTL) in the peripheral blood correlate with the decline in HIV-1 (for review see [104]). Cytotoxic T-lymphocyte responses appear before antibody responses, suggesting that cell-mediated responses are the key immune activities leading to suppression of initial viremia [105].

Innate immune responses also control plasma HIV-1 levels. CD8+ T-cells produce soluble factors that suppress HIV-1 replication, including β-chemokines (RANTES, macrophage inflammatory protein-1) that compete with HIV-1 for binding to coreceptors, blocking entry [106], and the α-defensins 1, 2, and 3 [107].

The immunology of perinatal primary infection is less well understood. Most infants reach peak viremia at 1–2 months of life but, unlike adults, have only minimal declines in plasma virus over the next several months [108]. Some children with rapid disease

Table 1.7. Mechanisms used by HIV-1 to evade immune responses

Mutations no longer recognized by cytotoxic T-lymphocytes (CTL escape mutations)
Mutations no longer recognized by neutralizing antibodies (neutralizing antibody escape mutations)
Inherent resistance to neutralization
Downregulation of MCH class I expression (mediated by viral gene products, e.g., Nef, acting within the infected cell)
Preferential infection and destruction of HIV-1-specific CD4+ T-lymphocytes
Dysregulation of cytokine production (IL-2, IFN-γ, IL-12, IL-10)

progression have no decrease in viral load over the first year of life. Children with slow progression show declines in viral RNA, but usually not more than 0.5–1 $\log_{10}$.

Among proposed explanations for the absence of a significant decline in viral load in some vertically infected infants is that the infant fails to mount an effective response. HIV-1-specific CTL may be delayed in perinatally infected infants [109]. However, children who have survived to age 2 years have HIV-1-specific CTL frequencies comparable to adults [110]. The ADCC responses in infants are also less vigorous [111, 112].

Another possible explanation for the relatively high viral loads following vertical infection is transmission of virus that has mutated to escape the maternal immune response. Transmission of this type of escape mutant has been observed [113]. The observation that virus from infants with rapid progression develops fewer new mutations over time suggests that rapid progressors' virus experiences less immune pressure [114].

Chronic/progressive infection/non-progressive infection

HIV-1 replication damages the immune system. Lymph nodes harbor actively replicating HIV-1 and large quantities of antibody–virus complexes bound to follicular dendritic cells (FDC) in the germinal centers [115], which is highly infectious. In advanced HIV-1 infection, lymph node architecture becomes grossly abnormal, with complete loss of germinal center organization. Ongoing viral replication results in generalized immune activation, with higher levels of programmed cell death and T-cell turnover and, perhaps, impaired ability of the thymus to generate new T-cells. Eventually, the immune system becomes unable to respond to infectious pathogens.

HIV-1 infected adults and children can maintain detectable cytotoxic T-cell-mediated immune responses and HIV-1-specific antibody into advanced disease. However, HIV-1-specific lymphoproliferative responses are notably low or absent. This may be an indication of relative deficiency of CD4+ helper T-cell responses (for review see [104]). CD4+ T-cells that are HIV-1-specific are infected preferentially by HIV-1 [116]. Thus, HIV-1 may directly delete some of the T-cells required for generating an immune response against it.

HIV-1 uses other mechanisms to evade the immune response (Table 1.7). High mutation rates allow for outgrowth of virus with variant epitopes that escape recognition by CTL (for review see [104]). HIV-1-specific CTL have phenotypes differing from phenotypes responding to other chronic viral infections. HIV-1-specific CTL may be not fully functional effectors [117–119]. The viral accessory protein Nef downregulates expression of MHC class I on infected cells [120]. MHC class I expression is critical for CTL recognition and infected cell killing. HIV-1 is resistant to antibody-mediated neutralization due to characteristics of the HIV-1 envelope protein (for review see [121]).

A small number of HIV-1 infected adults and children have no evidence of disease progression for 10 or more years, with low levels of plasma HIV-1. This has been termed long-term non-progression (LTNP). Genetic and viral factors have been associated with LTNP. Some patients have had mutated viral co-receptors (see above); a few were infected with Nef-deleted virus (for review see [122]). Some HLA alleles are associated with slower progression. LTNP patients are more likely to have HIV-1-specific lymphoproliferative responses and many HIV-1-specific CTLs [104].

Effects of antiretroviral therapy

Antiretroviral treatment during acute infection in adults can improve the anti-HIV immune response, producing higher HIV-1-specific lymphocyte proliferative responses [123], with improvement in viral control at least temporarily. It is unclear whether this applies to infants. Infants with good viral suppression before 3 months of life do not maintain detectable HIV-1-specific immune responses [124]. Neither HIV-1-specific cell-mediated nor HIV-1 antibody responses are detected when tested at age 12–15 months. Normal responses to other antigens are found. The infants can generate HIV-1-specific responses, as interruptions of treatment result in rapid appearance of HIV-1-specific antibodies [125]. Infants beginning antiretroviral therapy after 3–6 months have higher levels of HIV-1-specific CD8+ T-cell responses and maintain HIV-1 antibodies.

Immune abnormalities associated with HIV-1 infection

Both cell-mediated and humoral immune functions are affected during HIV-1 infection (Table 1.8).

Cell-mediated immunity

Cell-mediated immunity primarily defends against intracellular pathogens, notably viral infections, and malignancies. Abnormal cell-mediated immunity in HIV-1-infected children leads to more severe or recurrent disease from pathogens such as varicella zoster virus, herpes simplex virus, cytomegalovirus (CMV), *Mycobacterium* species, and *Salmonella* species. Lymphomas and certain soft tissue malignancies are also more common. Abnormal cellular immune function contributes to abnormal humoral immunity.

Table 1.8. Immunologic abnormalities associated with HIV-1 infection

Cellular
- Decreased delayed-type hypersensitivity skin reaction
- T-lymphocytes
  - Decreased absolute numbers of CD4 positive (helper) T-lymphocytes
  - Increased relative numbers of CD8 positive (killer/suppressor) T-lymphocytes
  - Decreased CD4/CD8 ratio
  - Decreased numbers of cells with naïve phenotype (CD45RA+/CD62L+)
  - Increased % cells with memory phenotype (CD45RO+)
  - Increased % CD8+ cells with diminished proliferative capacity ($CD28^-$; CD95+)
  - Increased CD8+ T-cells with activated phenotype (CD38+/HLA-DR+)
  - Decreased proliferative responses to antigen and mitogens
  - Altered cytokine production (see below)
- Natural Killer (NK) cells
  - Decreased number of NK cells (CD16+/CD56+)
  - Decreased cytotoxic activity
- Antigen Presenting Cells (monocytes and dendritic cells)
  - Decreased stimulation of T-cell proliferative response to antigen
  - Decreased HLA-DR expression
  - Altered cytokine production
- Phagocytes
  - Monocytes
    - Decreased clearance of RBC
    - Decreased Fc receptor expression
    - Decreased chemotaxis
    - Decreased intracellular killing
    - Decreased superanion production
  - Polymorphonuclear cells
    - Neutropenia
    - Increased or decreased chemotaxis
    - Decreased staphylococcus killing
    - Increased or decreased phagocytosis
    - Altered surface adhesion proteins and receptors

Humoral
- B-lymphocytes
  - Decreased number of antigen-responsive B-cells (CD23+/CD62L+; $CD21^{hi}$)
  - Polyclonal activation of B-cells
  - Increased spontaneous immunoglobulin secretion from B-cells
  - Decreased immunoglobulin secretion after stimulation of B-cells
  - Increased IgG, IgA, IgM
- Specific antibody responses
  - Decreased antibody response to immunization: hepatitis B, HIB conjugate, measles, influenza
  - Declining antibody titers after immunization: diphtheria, tetanus, *Candida*, measles

Cytokines
- Decreased production of IL-2, IFN-γ
- Decreased production of IL-12
- Decreased IFN-α
- Increased production of IL-1β, IL-6, and TNF-α
- Increased production of IL-10, transforming growth factor-β

Defects in helper T-lymphocyte cell function

Decline in the absolute number and percentage of helper T-lymphocytes (CD4+ T-lymphocytes) is the hallmark of HIV-1 disease. Since CD4 is used by HIV-1 as the receptor, helper T-lymphocytes are a main target of HIV-1 infection. Direct cytopathic and indirect effects of HIV-1 probably both contribute to the abnormalities in CD4+ T lymphocyte function and number. As CD4+ T-lymphocyte numbers decline, the risks of opportunistic infections increases.

Preceding the CD4+ T-lymphocyte decline, alterations in helper T-lymphocyte function are observed. Lymphocytes from asymptomatic, HIV-1-infected children have reduced proliferation to common antigenic stimulants [126]. As the disease progresses, reduced proliferative responses to specific antigens are followed by decreased responses to allo-antigens and, then eventually diminished responses to mitogens [127].

In HIV-1 disease, helper T-lymphocytes have an abnormal pattern of cytokine secretion (for review see [128]) and decreased post-stimulation production of IL-2 and IFN-γ, which contribute to cell-mediated function defects. Patients with advanced disease have poor DTH responses to memory antigens (e.g., tetanus, *Candida*, mumps)[129]. *Mycobacterium tuberculosis* skin testing may be unreliable.

Changes in CD4+ T-lymphocyte phenotypes also occur with HIV-1 infection. The most notable phenotypic change is an increase in proportion of memory CD4+ T-lymphocytes (CD45RO+) relative to naïve CD4+ T-lymphocytes (CD45RA+CD62L+) [130]. However, due to the overall decline in CD4+ T-lymphocyte numbers, the absolute number of both naive and memory CD4+ T-lymphocytes decreases. Loss of naïve cells may compromise the ability of the immune system to handle new pathogens.

Defects in cytotoxic/suppressor T-lymphocyte cell function

Cytotoxic/suppressor T-lymphocytes mediate direct cytotoxic activity against pathogen-infected and malignant cells, and release soluble factors that inhibit pathogens. CD8+ T-lymphocytes may play a role in downregulating the immune response after an infection has been controlled.

During acute infection, a large increase in CD8+ T-lymphocytes occurs, probably due to vigorous CD8-mediated primary immune response. Large expansions of HIV-1-specific CD8+ T-cell clones – are identified in acute and chronic infection [131, 132]. The number and percentage of CD8+ T-lymphocytes may remain high, particularly in symptomatic patients. The increased number of CD8+ T-lymphocytes can result in decreased CD4/CD8 ratios (normally > 1), even before significant declines in CD4+ T-cell number occur. In advanced disease, the absolute number of CD8+ T-lymphocytes may decline due to lymphopenia. The majority of cells accounting for the CD8+ cell increase are activated (CD38+, HLA-DR+) memory cells (CD45RO+) [133]. A higher proportion of CD8+ T-cells in HIV-1 infected people have phenotypic markers of decreased proliferative potential and increased programmed cell death [134, 135].

Defects in natural killer lymphocyte cell function

The number of NK cells is lower during HIV-1 infection, declining with disease progression [136, 137]. Early in HIV-1 infection, patients have decreased NK lytic activity and decreased production of IFN-γ [137]. Exogenous cytokines (IL-2, IL-12, IL-15) can restore these NK cell functions in vitro, suggesting that an altered cytokine milieu may account for abnormal function [138].

Defects in antigen-presenting cell function

Antigen-presenting cells (APC), including monocytes, macrophages, and dendritic cells, present antigen in the context of either MHC class I or class II antigens to lymphocytes. The presentation and the type of cytokines produced by the APC at the time of interaction with lymphocytes may determine the type of immune response. Monocytes can be directly infected with HIV-1, resulting in abnormal function and dissemination of the infection. The association of HIV-1 with dendritic cells facilitates infection of CD4+ T-cells [139]. The number of dendritic cells in peripheral blood is decreased in acute and chronic HIV-1 infection in adults [140]. Dendritic cells and monocytes from HIV-1-infected patients have a decreased T-lymphocyte proliferation capacity [141].

HIV-1 infection results in abnormal monocyte and dendritic cell cytokine secretion. Interleukin-12 is produced by antigen-presenting cells that promote cellular immune responses (for review see [142]). HIV-1 infection results in decreased IL-12 production [143]. Since IL-12 promote cellular immunity, decreased production may contribute to defective cell-mediated immunity [144]. HIV -1-infected adults have decreased dendritic cell production of interferon-α (IFN-α) [145]. Interferon-α is an important component of the innate pathogen immune response. Increased tumor necrosis factor (TNF)-α serum levels and TNF-α production have been observed, PBMC, brain, and monocytes infected *in vitro* [146]. Increased TNF-α and -β may contribute to wasting disease and encephalopathy. HIV-1 patients also have increased plasma levels of pro-inflammatory cytokines (IL-1β, IL-6) and anti-inflammatory cytokines (IL-10, transforming growth factor-β) (for review see [146]). Some cytokines enhance HIV-1 replication. Cytokine dysregulation also likely impairs normal immune development.

Defects in phagocyte cell function

Phagocytes engulf and kill extracellular pathogens, generate granulomas, and localize infection. Mononuclear phagocyte and polymorphonuclear leukocyte (PMN) defects observed during HIV-1 infection include decreased chemotaxis, diminished intracellular killing, and decreased superanion radical production; these defects may contribute to poor granuloma formation observed during HIV-1-infection [147].

PMNs defend against bacterial and fungal pathogens. Patients with advanced disease often have neutropenia resulting from drug toxicity and HIV-1 disease. HIV-1 infected patients may also have defects in PMN function (decreased phagocytosis, decreased bactericidal activity, altered superoxide production, altered chemotaxis, and altered surface adhesion molecules and activation receptors) [148].

Table 1.9. Serum immunoglobulin levels (median and upper 95% confidence limit) in uninfected, asymptomatic and symptomatic HIV-1-infected children

		Children		
	Age (mo)	Uninfected	Asymptomatic	Symptomatic
IgG (mg/dl)	0–1	554 + 757	836 + 1091	952 + 1122
	1–6	437 + 630	551 + 657	1360 + 1514
	7–12	565 + 711	615 + 918	1893 + 2422
	13–24	725 + 998	774 + 1120	2125 + 2855
IgA (mg/dl)	0–1	16 + 30	16 + 27	54 + 69
	1–6	24 + 32	27 + 39	74 + 98
	7–12	27 + 68	26 + 42	141 + 191
	13–24	42 + 69	45 + 89	149 + 188
IgM (mg/dl)	0–1	47 + 78	47 + 77	103 + 152
	1–6	59 + 89	85 + 109	134 + 159
	7–12	79 + 104	108 + 134	167 + 183
	13–24	105 + 130	120 + 177	149 + 191

Modified from [151].

Humoral immunity

Abnormal humoral immunity occurs in HIV-1-infected adults and children, but is more significant in children. Children have increased rates of minor and invasive bacterial infections. HIV-1 probably destroys the ability to produce antibodies against new antigens before the child is exposed to pathogens. An HIV-1-infected adult may have generated memory B-cells before HIV-1 infection. These adult memory cells can produce protective antibody upon repeat exposure. Hyperglobulinemia is a notable feature of the disease, particularly in many pediatric HIV-1 patients. Hypoglobulinemia can be seen in some pediatric and adult HIV-1 patients with advanced disease.

Defects in B-lymphocyte function

B-lymphocytes are not infected by HIV-1, but have abnormal function, probably because of direct effects of HIV-1 gp120, altered cytokine levels, and impaired CD4+ T-cell-mediated help. A minority of patients will have hypogammaglobulinemia, particularly with advanced disease [149]. The more common abnormality is a relatively non-specific polyclonal B-cell activation, resulting in hypergammaglobulinemia [150]. Elevated IgG, particularly IgG1 and IgG2, can be observed by age 6 months (Table 1.9). Elevated IgA and IgM are also observed, particularly in rapidly progressive disease [151]. The B-lymphocytes in vitro have increased spontaneous immunoglobulin production and cell proliferation, but decreased specific immunoglobulin production and cell proliferation in response to recall antigens or B-lymphocyte-specific mitogens [150]. Most cells produce polyclonal, low affinity antibody not directed against

a discernable pathogen, although 20–40% make HIV-1-specific antibody. This HIV-1-specific antibody often does not neutralize HIV-1 found in the plasma contemporaneously, although it may neutralize HIV-1 isolated from earlier in the infection. gp120 may act as a superantigen for B-lymphocytes that bear a particular variable heavy chain, overstimulating these cells [152].

Defects in specific antibody production

Despite hypergammaglobulinemia, HIV-1-infected children have functional hypogammaglobulinemia because of diminished ability to produce specific antibody. Both T-independent and T-dependent antigens antibody responses are decreased. Asymptomatic children < 2 years have antibody responses similar to uninfected children [126]. After 2 years, untreated HIV-1-infected children have decreased antibody responses even with normal CD4+ T-lymphocyte numbers. Responses to hepatitis B, measles, influenza, and *Haemophilus influenzae* type B vaccines are decreased in untreated HIV-1-infected infants [153–156]. Disease progression is associated with decline in B-lymphocyte numbers, with preferential loss of B-lymphocytes that respond to antigen (CD23 +/CD62L+; $CD21^{low}$), leading to decreased antigen-specific antibody production [157, 158]. Soluble gp120 and HIV infection of CD4+ T-cells impairs T-cell help for specific B-cell responses [159].

Technique for CD4+ T-lymphocyte number and percentage determinations

CD4+ T-lymphocyte number is determined by cell surface marker (i.e., surface protein) analysis via flow cytometry, with a concurrent complete blood count (CBC) [160]. Peripheral blood cells are incubated with antibodies specific for cell surface proteins that identify the cells of interest. The monoclonal antibodies are conjugated to fluorescent molecules that fluoresce upon excitation by the flow cytometer light source. For CD4+ T-cell determinations, the fraction of total lymphocytes that express both cell surface markers CD3 (a marker shared by all T-cells) and CD4 (marker of helper T-cells) is determined. This value is reported as the CD4+ percentage (percent CD4+ T-cells). Absolute CD4+ T-lymphocyte counts are determined by multiplying the percentage of CD3+/CD4+ lymphocytes by the absolute lymphocyte count from concurrent CBC results:

Absolute CD4+ T-lymphocyte count = absolute lymphocyte count x %CD3+/CD4 + lymphocytes = WBC x % lymphocytes x %CD3+/CD4 + lymphocytes.

To standardize CD4+ T-lymphocyte determinations, the US Public Health Service has published detailed guidelines for laboratories performing the test [160, 161].

Many laboratories determine CD4+ T-lymphocyte numbers together with a larger surface marker panel, including killer/suppressor T-lymphocytes (C3+/CD8+ lymphocytes) and B-lymphocytes (CD19+ lymphocytes). This permits calculation of the CD4/CD8 ratio, which has been used as an early marker of immunologic abnormality and was once a key indicator used to diagnose AIDS.

Several biologic and analytic factors can introduce variability into CD4+ T-lymphocyte results. The absolute count depends on three separate measurements, each of which can introduce error. Biologic sources of variability include diurnal variation, acute illness, immunizations, and drug therapy, particularly corticosteroids [162]. The CD4+ T-lymphocyte values vary diurnally, with lowest values at noon and an evening peak. Between 8:00 am and 4:00 pm (usual clinic hours), an average 19% increase can occur. CD4+ T-lymphocyte determinations should be obtained at a consistent time of day. Acute illnesses and immunizations may increase or decrease total WBC, and cause transient changes in the % CD4+ T-lymphocytes. Corticosteroid therapy is associated with decreases in absolute CD4+ T-lymphocyte counts. Even stable patients may have a ±22% variation in absolute CD4+ T-cell counts [163]. CD4 T-cell percentage values fluctuate less than absolute counts, primarily because variations in total white cell count result in changes in absolute counts [164]. CD4+ T-lymphocyte values differing substantially from previous values should be evaluated critically, especially if obtained during acute illnesses or soon after immunizations.

Interpretation of CD4+ T-lymphocyte values

Interpretation of CD4+ T-lymphocyte numbers in children requires recognition that normal cell number declines over the first 6 years of life; values should be assessed relative to age-specific normal values (Fig. 1.1). The age-related change is marked for absolute CD4+ T-lymphocyte counts; % CD4+ T-lymphocyte values change less [29, 164, 165].

Pediatric guidelines recommend monitoring both percentage and absolute CD4+ T-lymphocyte counts and determining disease staging based on the lowest of the two values. Owing to CD4+ T-lymphocyte count variation, major therapeutic decisions should only be made after the changes have been confirmed and other sources of variability have been ruled out. A sustained 50% decrease in the absolute CD4+ T-cell count or percentage is evidence of disease progression. Smaller changes may also be significant if a trend is observed.

HIV-1 infection in children is associated with progressive decline in CD4+ T-lymphocyte values greater than the normal physiologic decline [29, 151, 165]. (Table 1.10) [151]. Symptomatic children have early declines, beginning by 2–3 months; by 13–24 months, CD4+ T-lymphocyte values are significantly lower even among asymptomatic children [165].

Immune restoration after highly active antiretroviral therapy

Successful treatment with highly active antiretroviral therapy (HAART) reverses most clinical and many immunologic signs of disease progression (for review see [166]). Effectively treated patients have lower incidence of opportunistic infections, resolution of HIV-1-related organ dysfunction (i.e., encephalopathy), and improved growth.

Clinical improvements correlate with CD4+ T-cell percentage and cell count increases. During the first 4 to 8 weeks after initiating HAART, the number of

Table 1.10. CD4+ and CD8+ T-lymphocyte numbers (mean ± standard deviation) in uninfected, asymptomatic and symptomatic HIV-1-infected children

		Children		
	Age (mo)	Uninfected	Asymptomatic	Symptomatic
CD4+ lymphocytes (cells/mm^3)	0–1	2900 ± 1541	2580 ± 1501	2317 ± 1317
	1–6	3278 ± 1401	3482 ± 1234	1706 ± 1215
	7–12	3051 ± 1285	2769 ± 1326	1951 ± 882
	13–24	2584 ± 1105	2030 ± 1481	1680 ± 1089
CD8+ lymphocytes (cells/mm^3)	0–1	1418 ± 791	1404 ± 974	1499 ± 1046
	1–6	1626 ± 985	1457 ± 709	1613 ± 958
	7–12	514 ± 300	394 ± 334	523 ± 266
	13–24	1374 ± 663	1902 ± 844	2242 ± 1290

Modified from [151].

CD4+ T-lymphocytes and B-lymphocytes increases rapidly (Fig. 1.10) [167, 168]. CD4+ T-lymphocytes continue to increase over the following 12–18 months. The rapid increase observed early following treatment suggests that the initial increases results from redistribution of cells from lymphoid tissue (for review see [169]). CD4+ T-cells with memory phenotype largely account for the increase [167]. Younger children have an early increase in naïve and memory cells, suggesting they may mobilize naïve cells from the thymus early after initiation of treatment [167, 170]. After the initial rise, continued increase in CD4+ T-cells is mostly composed of naïve cells [167, 168, 171]. These cells are probably newly derived from thymus, although some derive from proliferation of peripheral naïve cells [172, 173].

Since mostly naïve cells increase with HAART, children may be able to generate responses to new antigens, but be less able to reconstitute immune responses to recall antigens without renewed exposure [168, 170, 174, 175]. Proliferative responses to antigens not usually encountered (e.g., tetanus) are less likely to increase with HAART. Responses to uncommon antigens can be generated through reimmunization [168, 176]. Adults treated with HAART have decreases in quantitative IgG, IgM, and IgA levels, which are elevated during HIV-1 infection, indicating improvements toward more normal B-lymphocyte function [177]. However, not all patients achieve normal levels of immunoglobulins [178].

The number of CD8+ lymphocytes increases transiently in the first 8 weeks, but then returns to baseline by 12 weeks (Fig. 1.8) [167]. The percentage of CD8+ T-cells declines as the CD4+ T-cells increase and the CD4/CD8 ratio normalizes. There are significant changes in phenotype, with decreased numbers of cells with surface markers for activation and programmed cell death and increases in the number of CD8+ T-cells with naïve phenotype, and there is a decline in the frequency of HIV-1-specific

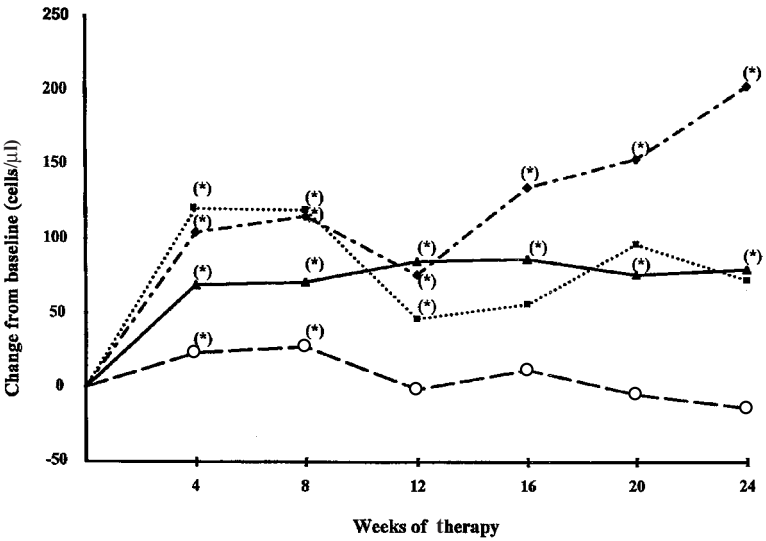

Fig. 1.8. Changes in lymphocyte populations after highly active antiretroviral therapy. Median change from entry values of CD8+ T-cell (squares), CD4+ T-cells (circles), B-cells (triangles), and NK cells (diamonds) over the course of 24 weeks. Values shown as cells per microliter. A statistically significant ($P < 0.05$) changes from baseline is identified with an asterisk. Reprinted with permission from [167].

CD8+ T-cells [179]. This probably results from decreased viral antigen levels as replication is suppressed.

In most children, HAART suppresses plasma HIV-1 RNA to low levels or below the limits of detection. However, many children will not maintain durable viral load suppression, and plasma HIV-1 RNA levels return to near pretreatment levels [168, 180, 181]. Many children will have increased CD4+ T-cell counts despite ongoing viremia. The CD4+ T-cell count increases may be as great in these virologic nonresponders/immunologic responders as in children who achieve complete viral suppression [181]. Children with ongoing viremia still derive clinical benefit from HAART if their CD4+ T-cell counts are increased [182]. The explanation for partial immune restoration with continued high level viral replication is not understood. Some evidence points to decreased viral fitness or a switch of virus strain to less pathogenic phenotypes.

Adults with prolonged low CD4+ lymphocyte counts are less likely to have immune restoration following HAART. This is less apparent for children, who exhibit increases to normal ranges, even following profoundly decreased CD4+ lymphocyte counts (Fig. 1.9) [174]. Increased thymic function in children probably contributes to the

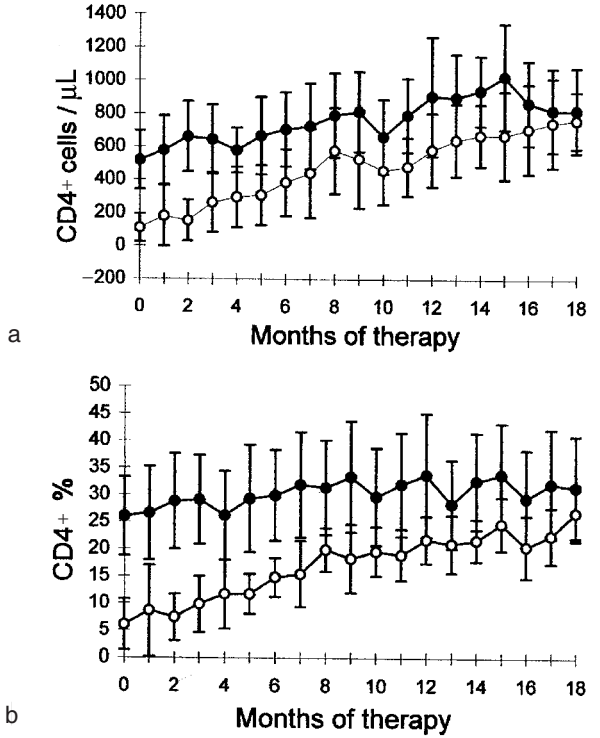

Fig. 1.9. Changes in CD4+ T-cells depend on baseline values. Changes (mean, SD) in CD4+ T-cell counts (*a*) and CD4+ T-cell percentages (*b*) in patients in CDC class 2 (filled circles) and patients in CDC class 3 (open circles) during 18 months of stavudine, lamivudine, and indinavir treatment. Reprinted with permission from [174].

greater capacity for restoration of T-lymphocytes [172, 180]. These children have a lowered incidence of opportunistic infections, indicating the recovered cells are functional.

The immune system probably cannot reconstitute completely; subtle abnormalities persist. Cell activation decreases, but remains above normal. T-cell receptors distribution may not completely normalize, a possible indication of limited T-cell diversity or persistence of expanded clones [183]. Cytokine production remains abnormal [184]. Treatment with immunologically active agents, such as IL-2, has been proposed as a possible intervention. The clinical correlates of these persistent abnormalities is unknown.

Virologic assays for diagnosis

Serology

Enzyme immunoassays (EIAs) detect antibody responses to infection [185–187]. They are used to screen blood products and diagnose HIV infection. EIAs used for diagnosis require confirmation by Western blot to verify that the EIA-detected immune response is HIV specific. Test performance characteristics (false-negative and false-positive rates, positive predictive value) depend critically on HIV infection prevalence in the population tested. Tests are adjusted to be very sensitive due to use in blood product screening, so positive predictive values of EIAS are relatively low in low-risk populations [188–190]. Rapid antibody tests [191] can be performed in 10–30 minutes, potentially providing results at the same visit. These rapid assays have similar performance characteristics to standard EIAs, with a troublesome false-positive rate varying with HIV prevalence. Overall, the false-positive rate has been estimated as 0.4% of all persons tested and as high as 18% of initially reactive results.

The United States (US) Public Health Service recommends consideration of an alternative rapid diagnostic approach, i.e., rapid screening with reporting of results during the same clinic visit, followed by confirmatory assays for reactive assays [191]. Currently, two rapid tests are licensed by the FDA (a) Murex Single Use Diagnostic System (SUDS) HIV-1 test; Abbott Laboratories, Inc., Abbott Park, Illinois; and (b) OraQuick Rapid HIV-1 Antibody Test; OraSure Technologies, Inc., Bethlehem, Pennsylvania). The former requires an onsite laboratory; the latter is a self-contained system that can be performed at the bedside or in clinic on saliva specimens. Many additional rapid tests and assay formats have not yet received US regulatory approval. A possible future approach is confirmation of rapid test results with a second rapid test [192].

The utility of serodiagnosis is compromised in young infants due to transplacental passage of HIV antibody from their mothers. Virtually all infants born to such mothers will test positive for HIV antibody, regardless of viral transmission. Relatively rare exceptions include hypo- or agammaglobulinemia and extreme prematurity. (See also Chapter 3.)

Viral detection

Viral culture

Peripheral blood mononuclear cells are cocultivated with equal numbers of uninfected peripheral blood mononuclear cells (PBMCs). Cells are first stimulated with phytohemaglutinin (PHA) and cultured in the presence of IL-2 to enhance viral replication. Supernatant is tested periodically for p24 antigen (see below); p24 detection on two sequential samplings defines a positive. Most positive specimens will be detected by 7–14 days; declaring a culture negative requires 3–4 weeks. Culture can also be performed in a quantitative format using serial dilutions.

p24 antigen detection

p24 antigen is an HIV core protein that can be detected using commercial EIAs. Antibody to p24 is affixed to a solid phase – a bead or microtiter plate, which is incubated with the patient specimen, usually plasma or serum. If p24 antigen is present, an antigen–antibody complex forms, capturing the p24 antigen on the solid phase. The antigen–antibody complex can be detected by a second, enzyme-labeled, anti-p24 antibody. This assay is straightforward and rapid, but has two shortcomings for early pediatric diagnosis: (a) false-positives can occur in the first month of life, most probably due to placental transfer of maternal p24 antigen without infection; and (b) antibody-complexed antigen is not detectable. This is especially problematic in infancy where there is much maternal antibody. This problem can be circumvented by immune complex dissociation prior to p24 antigen detection, which can be accomplished by acid treatment or boiling [193–195] and sensitivity can be enhanced by combination with signal amplification [196, 197]. Currently, there is considerable interest in p24 assays in resource-poor settings for diagnosis and disease monitoring because it costs less and is simpler to perform than other measures, but definitive studies assessing the utility of p24 assays in such settings have not been completed.

HIV DNA PCR

HIV DNA polymerase chain reaction (PCR) to detect HIV provirus was developed in the late 1980s and is used extensively for neonatal diagnosis. Diagnostic PCR assays are optimized for extreme sensitivity, and can detect 1–10 viral targets/sample. False-positives can result from specimen contamination. Laboratory procedures and quality-controlled commercial reagent kits have been developed that greatly reduce false-positive results [198].

Heel-stick blood samples on filter paper (Guthrie cards) for newborn diagnosis by DNA PCR have been validated [199, 200]. This approach has appeal for studies conducted in technically challenging environments and may be employed for routine infant screening.

Studies have shown that virus culture and DNA PCR are sensitive and specific [201–203]. Newborn diagnosis is discussed in more detail in Chapter 3. DNA PCR assays were developed initially to detect HIV strains (clade B) prevalent in the USA. Diagnostic PCR kits have been developed that can recognize target DNAs essentially from all HIV clades. This holds true for disease-monitoring HIV RNA quantitation kits (both PCR and other non-PCR-based methodologies such as branched DNA and nucleic acid sequence-based amplification assays – see below). These disease-monitoring assays are not marketed with performance characteristics optimized for diagnostic use, but have been employed for newborn HIV diagnosis in some settings due to their widespread availability.

Table 1.11. Quantitative HIV RNA assays

Assay	Version	Dynamic range	Quantitation limit
RT-PCR	Amplicor HIV-1Monitor	$10^{2.6}-10^{5.9}$	400
(Roche)	Amplicor HIV-1Monitor Ultrasensitive	$10^{1.7}-10^{5.0}$	50
NASBA	HIV-1 RNA QT	$10^{2.6}-10^{7.6}$	400
(Organon-Teknika)	NucliSens	$10^{1.9}-10^{7.6}$	80
Branched DNA	Version 1	$10^{4.0}-10^{6.2}$	10,000
(Chiron/Bayer)	Version 2	$10^{2.6}-10^{6.2}$	400
	Version 3	$10^{1.7}-??$	50

(All units are copies/ml).

Viral assays for monitoring HIV infection

HIV RNA quantitation

Reverse transcription-polymerase chain reaction (RT-PCR) is commercially available as the Roche Amplicor HIV-1 Monitor and Amplicor HIV-1 Monitor Ultrasensitive® assays. In these assays, reverse transcription of viral RNA into a DNA copy is followed by DNA amplification by PCR. An internal control is co-amplified. The regular Amplicor HIV-1 Monitor assay has been in use most extensively and has a lower limit of quantitation of 400 viral copies/ml. More recently, Roche has introduced the Ultrasensitive assay, with a lower quantitation limit of 20–100 copies/ml.

A second assay, NASBA® (nucleic acid sequence-based amplification), (Organon-Teknika) also amplifies target RNA. NASBA is an isothermal amplification reaction that employs three enzymes: reverse transcriptase, RNAse H, and T7 DNA polymerase. It also utilizes internal quantitation standards, and has a lower limit of quantitation of 1000 copies/ml. A second generation assay – NucliSens® – can quantitate as little as 80–400 copies/ml of plasma, depending on sample input.

The third commercially available, quantitative assay is the branched DNA assay (Chiron/Bayer), which uses a different technique from the previous two assays: it amplifies the signal created following capture of viral RNA by nucleic acid hybridization. It is technically the easiest assay to perform, resulting in high reproducibility. Unfortunately, the standard assay requires one ml of plasma, which makes its use impractical for pediatrics. The initial version of the assay had a quantitation limit of 10 000 copies/ml, but newer versions have a lower detection limit of 200 copies/ml.

All of the assays have been tested extensively and validated (Table 1.11), but there is some sample-to-sample variation. Samples must show at least a threefold (0.5 $\log_{10}$) difference for the difference to be considered significant. For example, two samples from a child drawn 1 month apart are reported as 40 000 and 100 000 copies/ml. These two results are not significantly different from each other since they do not differ by threefold. In addition to this performance feature, clinicians should be aware

that intercurrent infections and immunizations can activate viral replication and raise RNA levels briefly [204–206]. As with many laboratory tests, clinical decisions should prudently be based on reproducibility between sequential specimens over time and on a composite of clinical and laboratory observations. It would be clinically unwise to make a major change in management on the basis of a single viral load assay [207, 208]. Soon after the introduction of HIV protease inhibitors, some postulated that relatively short periods of antiretroviral therapy would be sufficient to eradicate infection. This enthusiasm has been tempered by the demonstration of long-lived cellular reservoirs in patients with long-term suppression of plasma virus [209–211]. Individuals in whom plasma virus is non-detectable for years remain positive for proviral DNA within cells and for culturable virus [212, 213]. These studies demonstrated that quiescent memory CD4+ T-lymphocytes are reservoirs of latent infection and their frequency is quite low (one in 1–10 million cells). While difficult to measure, their calculated decay rates are very slow, with elimination times measured in decades or lifetimes. Treatment methods for eliminating virus from these long-lived reservoirs will almost certainly be necessary if eradication of infection is to be achieved.

Additional quantitative assays

Newer assays that quantitate infected circulating cells have been developed. These are based on DNA PCR, with internal standards for accurate quantitation. These assays report "numbers of infected cells" or "copies of viral genomes/microgram of cellular DNA" and may help to monitor viral suppression and the amount of virus in long-lived cellular reservoirs [210, 213].

Qualitative plasma RNA assays are being developed to monitor low levels of viral replication once individuals attain a non-detectable state. Several investigators have reported that quantitative HIV RNA assays can sometimes permit earlier newborn diagnosis than DNA PCR [214–216].

Natural history of pediatic HIV infection

Natural history in adults

Infection of adults with HIV-1 is followed by three distinct virologic stages: (a) primary or acute infection, (b) clinical latency, and finally (c) progression to AIDS (see Fig. 1.10). The interval between primary infection and AIDS varies, with a mean of 10 to 11 years [217]. About 20% of individuals will progress in less than 5 years; a few (<5%) will remain immunologically normal for over 10 years [218]. The biological basis for this variability is unclear.

Soon after HIV-1infection, a non-specific clinical syndrome ("primary infection", "acute infection syndrome," or "acute retroviral syndrome"), including fever, pharyngitis, cervical adenopathy, fever, myalgias, rash, and lethargy, and sometimes neurologic or gastrointestinal symptoms is observed in some adults. Symptomatology may have prognostic significance [219–221]. These symptoms resolve spontaneously over days

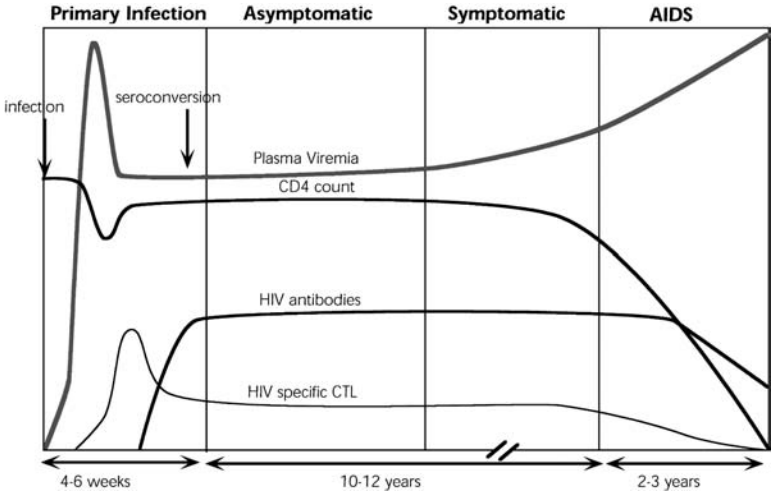

Fig. 1.10. Schematic diagram of the changes in plasma viremia, CD4+ lymphocyte count, and humoral and cellular immune response in an HIV-infected patient during the time following infection.

to weeks. These persons may be antibody negative, but have detectable plasma p24 antigen or HIV RNA. During primary infection, patients may have extremely high levels of plasma viremia (viral load) (>10^7 copies/ml), dropping to 100 000 copies/ml or less after several weeks [222, 223]. During this period of high viremia, CD4+ lymphocyte numbers decrease, occasionally to below 200 cells/μl. As viremia declines, the CD4+ lymphocyte count increases, often to normal levels. Decreasing viral loads precede development of antibody response and coincide with a measurable cytotoxic lymphocyte (CTL) response directed against HIV [224]. After some fluctuation, the viral load stabilizes around a "set point," usually between 10 000 and 100 000 copies/ml, frequently remaining fairly constant for many years [220], a period termed "clinical latency." However, much viral replication and CD4+ lymphocyte destruction occurs during clinical latency. An estimated 10 billion virions, with estimated half-lives of less than 6 hours, are produced daily [225]. Eventually, plasma viral levels increase and CD4+ lymphocytes decrease, heralding advancing immunodeficiency and AIDS [220]. The onset of the rapid fall in CD4+ cells is known as the "inflection point."

Plasma viral concentration, CD4+ cell level and disease progression in adults

Plasma viral load measurements offer imprecise assessments of viral replication, since the largest viral replication compartment, the lymphoid tissue, is not directly sampled

Table 1.12. Relationship between baseline HIV-1 RNA copy number and absolute CD4+ lymphocyte number and probability of developing AIDS

Baseline RNA (copies/ml)	Absolute CD4+ lymphocyte number (cells/μl)	% with AIDS		
		by 3 years	by 6 years	by 9 years
<500	>750	0	1.7	3.6
	<750	3.7	9.6	22.3
501–3000	ND	2	16.6	35.4
3001–10 000	>750	3.2	14.2	40.4
	<750	8.1	37.2	59.7
10 001–30 000	>750	9.5	36.7	62.4
	351–750	16.1	54.9	76.3
	<750	40.1	72.9	86.2
>30 000	>500	32.6	66.8	76.3
	351–500	47.9	77.7	94.4
	201–350	64.4	89.3	92.9
	<200	85.5	97.9	100

HIV-1 RNA was quantified using the bDNA system. Data are derived from [230], with permission.

[226]. Virus produced in lymphoid tissues is released into plasma; plasma viral load appears to sample lymphoid tissue viral replication indirectly [227]. Other organ systems where HIV replication occurs (central nervous system, genital tract, breast milk) may behave as distinct compartments. Plasma viral RNA levels may not reflect viral replication accurately in these compartments; virus present in these compartments may be genotypically distinct from virus found in plasma. These distinct viral genotypes may have differing pathogenic properties and differing sensitivities to antiretroviral agents [228, 229]. Since antiretroviral agents may not penetrate well into these compartments, they represent a potential source of resistant virus.

Absent antiretroviral therapy (ART), plasma viral RNA levels vary widely. There is an inverse, but variable, correlation between plasma viral RNA and CD4+ lymphocyte counts. Viral load and CD4+ count are independent prognostic markers. Before HAART, the steady-state level of plasma viral RNA after initial infection ("set point") was the best single predictor of progression to AIDS and death [230]. Combining plasma RNA levels and CD4+ count more accurately predicts prognosis than either alone [231] (see Table 1.12).

ART often produces decreases in plasma RNA and increases in CD4+ lymphocyte number. These changes have clinical and prognostic significance. After beginning ART, decreases in viral load or increases in CD4+ lymphocyte count predict the development of AIDS better than baseline values [232, 233]. For each tenfold decrease in plasma RNA, the risk of disease progression decreases by 56%; for every twofold increase in absolute CD4+ cell count it decreases by 67% [233].

Although ART has dramatic effects on plasma viremia, a small pool of long-lived latently infected CD4+ lymphocytes persist (for review see [234]). Replication-competent virus can be recovered from these latently infected cells. This reservoir is established early in infection and is relatively impervious to the effects of current ART. The rate of turnover of these cells is controversial, with estimates ranging from 6 months to 43 months. The persistence of this compartment represents a major obstacle to the eradication of HIV.

Factors associated with progression in adults

The determinants of the viral "set point" and "inflection point" are poorly understood, but factors may include effectiveness of the host immune response, including CD8+ CTL responses, and patient age. Certain combinations of HLA genes are associated with either relatively rapid (HLA B35) or delayed progression to AIDS (HLA B27 or −57) [235, 236], and HLA locus heterozygosity appears to be protective, perhaps because HLA heterozygotes are able to present a greater variety of antigenic peptides to cytotoxic T-lymphocytes. Inheritance of genes other than HLA have also been implicated in disease progression (for review see [237, 238]), notably including mutations in the HIV co-receptors and their promoters (see above).

Other host factors influence disease progression, including co-infection and other sources of immune activation. Factors leading to immune activation increase viral replication and hasten immune deterioration [239]. Infections and exposure to antigens have been associated with plasma viremia increases [240–242]. Some, but not all studies have shown that immunizations (influenza, tetanus toxoid) lead to transient bursts of viremia followed by return to baseline levels [204, 205, 243], but with no apparent long-term effects [244]. The benefits of vaccination preventing infection, which would undoubtedly result in much higher levels of immune activation, outweigh the unproven risks of transient increases in plasma RNA.

Viral factors have also been associated with disease progression. HIV-1 strains have been broadly classified as being M-tropic/NSI (non-syncytium-inducing) strains or T-tropic/SI (syncytium-inducing). The syncytium-inducing classification refers to production syncytia during *in vitro* growth of virus under certain conditions. M-tropic strains replicate in primary CD4+ T-cells and macrophages and use the β-chemokine receptor CCR5 (less often CCR3) as their coreceptors (for review see [245]). The T-tropic viruses can also replicate in CD4+ T-cells but can also infect established CD4+ T-cell lines *in vitro* via the α-chemokine receptor CXCR4 (fusin). Regardless of the transmission mode, over 90% of transmitted strains are M-tropic; transmission or systemic establishment of CXCR4-using (T-tropic) strains is rare. However, CXCR4-using strains are especially virulent and, once they emerge within an infected person, disease progression is accelerated [246]. At the inflection point, about 50% of adults infected with subtype B will have a switch in the phenotype of the virus from M-tropic viruses to T-tropic viruses [247]. Most of our understanding of HIV pathogenesis is based on subtype B, the predominant strain in developed countries. Whether different subtypes

differ in transmissibility, infectivity and pathogenicity is an important area of active research.

Classification of pediatric HIV-1 infection

The Center for Disease Control and Prevention (CDC) has established a classification system for HIV-infected children according to clinical and immunologic status (Tables 1.13, 1.14, 1.15).

The classification system is based on signs, symptoms, or diagnoses. Children are assigned to one of four mutually exclusive clinical categories: N, no signs or symptoms; A, mild signs or symptoms; B, moderate signs or symptoms; C, severe signs or symptoms [248]. The AIDS-defining illnesses in the 1987 CDC definition of pediatric AIDS are included in stage C, except lymphoid interstitial pneumonitis (LIP), a stage B illness. Although most children pass from N to A, B, and C in order, others can pass directly from N to B or N to C (see below).

The immunological classification uses the CD4+ lymphocyte count. Patients are assigned to mutually exclusive categories numbered 1 through 3, with lower CD4+ lymphocyte counts having higher numbers (Table 1.15). The normal CD4+ lymphocyte count changes dramatically during the first few years of life (Fig. 1.1). Assessing immune suppression is complicated because children may develop opportunistic infections at higher CD4+ cell counts than adults [249].

The current CDC immunologic classification system is based on age, on the absolute CD4+ cell count, and on the percentage of CD4+ lymphocytes [248] (see Table 1.14). If CD4+ cell count and percentage place a child in different immunologic categories, the more severe category is used. Currently, children with immune reconstitution following therapy are not reclassified to a less severe category. Values that result in a classification change should be confirmed. Since CD4+ lymphocyte values can vary with even mild illness, values are best obtained when children are clinically well.

HIV-1 natural history in children

The pace of HIV disease progression in children is accelerated compared with adults, presumably because of infection during immunologic immaturity, and/or the availability of increased numbers of target cells. Older children infected via non-vertical routes may have a course more similar to adults [250, 251].

Vertically infected children exhibit a bimodal pattern of disease progression [252–254]. About 10–25% of infected children develop profound immunosuppression, significant symptoms, and opportunistic infections within the first year; without treatment few of these children survive more than 2 years [255]. The majority of children have a slower progression to AIDS (mean time 6–9 years) [252, 254, 256, 257]. Investigators hypothesize that children with rapid progression may acquire their infection in utero and are therefore less able to mount effective immune responses [258, 259]. A working definition has been proposed wherein in utero transmission is defined as viral detection at less than 48 hours and intrapartum transmission is defined as viral detection

Table 1.13. Conditions associated with clinical categories according to the CDC 1994 Revised HIV Pediatric Classification System

Category A	Category B	Category C
Have two or more of the conditions listed below but no category B and C conditions • Lymphadenopathy (>0.5 cm at more than two sites) • Hepatomegaly • Splenomegaly • Dermatitis • Parotitis • Recurrent or persistent upper respiratory infection, sinusitis, or otitis media	Examples of conditions in clinical category B • Anemia (< 8 g/dl), neutropenia (< 1000/mm^3), or thrombocytopenia (< 1 000 000/mm^3) persisting >30 days • Bacterial meningitis, pneumonia or sepsis (single episode) • Candidiasis, oropharyngeal (thrush), persistent (>2 m) in a child >6 m • Cardiomyopathy • Cytomegalovirus infection, with onset >1 m of age • Diarrhea, recurrent or chronic • Hepatitis • HSV stomatitis, recurrent (>2 episodes within 1 yr) • HSV bronchitis, pneumonitis, or esophagitis with <1 month of age • Herpes zoster involving a > 2 distinct episodes or > 1 dermatome • Leiomyosarcoma • LIP or pulmonary lymphoid hyperplasia • Nephropathy • Nocardiosis • Persistent fever (lasting > 1 m of age) • Toxoplasmosis, onset < 1 m of age • Varicella, disseminated	• Multiple or recurrent serious bacterial infections (septicemia, pneumonia, meningitis, bone or joint infection, or abscess of an internal organ) • Candidiasis, esophageal or pulmonary • Coccidiomycosis, disseminated • Cryptococcosis, extrapulmonary • Cryptosporidiosis or Isosporiasis with diarrhea persisting for >1 month • Cytomegalovirus disease with onset of symptoms prior to 1 month of age • Cytomegalovirus retinitis (with loss of vision) • Encephalopathy in the absence of a concurrent illness other than HIV infection that could explain the findings • HSV infection causing a mucocutaneous ulcer persisting for >1 month; or as the etiology of bronchitis, pneumonitis or esophagitis • Histoplasmosis, disseminated • Kaposi's sarcoma • Primary CNS lymphoma • Lymphoma, Burkitt's, large cell, or immunoblastic • *M. tuberculosis*, disseminated or extrapulmonary • Mycobacterium infections other than tuberculosis, disseminated • *Pneumocystiis jiroveci* pneumonia • Progressive multifocal leukoencephalopathy • Toxoplasmosis of the brain with onset at >1 m of age • Wasting syndrome in the absence of a concurrent illness other than HIV infection

Adapted from [248], with permission.

Table 1.14. Immunologic categories according to the CDC 1994 Revised HIV Pediatric Classification

	No evidence of immune suppression		Evidence of moderate immune suppression		Evidence of severe immune suppression	
Age of child	CD4+ lymphocytes (cells/μl)	% CD4+ lymphocytes (cells/μl)	CD4+ lymphocytes (cells/μl)	% CD4+ lymphocytes (cells/μl)	CD4+ lymphocytes (cells/μl)	% CD4+ lymphocytes (cells/μl)
< 1 year	≥ 1,500	≥5	750–1499	15–24	<750	<15
1–5 years	≥1,000	≥25	500–999	15–24	<500	<15
6–12 yrs	≥500	≥25	200–499	15–24	<200	<15

Adapted from [248].

Table 1.15. HIV Pediatric classification incorporating both clinical and immunologic categories

	Clinical categories			
Immunologic categories	N: No signs/ symptoms	A: Mild signs/ symptoms	B: Moderate signs/ symptoms	C: Severe signs/ symptoms
1. No evidence of suppression	N1	A1	B1	C1
2. Evidence of moderate suppression	N2	A2	B2	C2
3. Severe suppression	N3	A3	B3	C3

Adapted from [248], with permission.

Table 1.16. Proportion of children progressing to Category C disease or death within a 5-year period from the beginning of each stage (before the availability of highly active antiretroviral therapy)

Stage	Percent developing category C disease within a 5-year period from the beginning of each stage	Percent mortality within 5 years from the beginning of each stage
N	50%	25%
A	58%	33%
B	60%	35%
C	-	83%

Adapted from [256], with permission.

at greater than 7 days [260]. Approximately 10–30% of children have detectable virus in the first 2 days of life and these children may have a shorter median time to onset of symptoms and death [253, 261] and higher levels of virus, but not all studies agree on this. The most striking characteristic of vertically infected infants is the extremely high levels of plasma viremia observed during the first few months of life. The viremia declines substantially in the first year of life and then more slowly over several years [108, 262, 263] (see below). At approximately 4–6 years of age, RNA levels appear to stabilize at lower levels.

The pediatric classification system (Table 1.15) has been used to describe the natural history of HIV-1 disease in the absence of ART. Most perinatally infected children remain relatively asymptomatic for the first year of life. In the second year, most progress to moderate symptoms (category B), typically remaining at this stage for over 5 years. After entering stage C, mean and median survival times are 34 months and 23 months, respectively [256] (see Table 1.16).

Natural history of HIV-1 infection in children in Africa

The vast majority of HIV -1 infected persons live in sub-Saharan Africa, where seroprevalence rates of over 25% are increasingly common. Over 80% of children infected with HIV live in this region and over 90% of new pediatric infections occur in this region. Although children account for less than 8% of those infected with HIV worldwide, 20% of deaths due to AIDS have been in children less than 15 years [264]. HIV infection has reversed the significant gains in child survival that had been achieved during the last few decades.

It has been hypothesized that HIV progression rates are more rapid in Africa than in industrialized countries. The immune activation associated with chronic infection, and the immunosuppression accompanying malnutrition have been postulated to result in higher levels of viremia and in a more rapid disease course. Differences in HIV subtype and host genetic factors have also been considered to be important variables. However, more recent data strongly argue that the natural history of HIV infection among adults in Africa is not fundamentally distinct from that of adults in resource-rich settings prior to the advent of ART [265, 266]. Africans, like members of disadvantaged groups in rich countries, tend to present for care later and have much higher levels of general morbidity. Moreover, the burden of infectious diseases such as tuberculosis is much higher. Nevertheless, the median time from seroconversion to AIDS in HIV-infected African adults is 9.4 years, and most die severely immunosuppressed, with clinical features of AIDS [267]. Pathophysiologically, HIV in Africa is not unique.

HAART and HIV natural history

HAART dramatically reduced disease progression in children and adults. When protease inhibitors were introduced, the annual mortality rate among US children enrolled in Pediatric AIDS Clinical Trials Group (PACTG) studies decreased from 5.3% to 0.7% [268]. Two ART drugs decreased the risk of death by 30%, three ART drugs resulted in a 71% decrease [269]. Progression to AIDS is also significantly reduced with HAART [270].

Unfortunately, many children who begin HAART are unable to achieve and/or sustain viral suppression, perhaps because of higher levels of viremia, sequential use of insufficiently potent ART, inability to achieve adequately high ART levels, and problems with adherence. Many of these children have viruses resistant to all available classes of antiretrovirals. The prognosis for these children will be significantly worse than for children who achieve good viral suppression unless new therapies become available. As many as 40% of children treated with HAART who do not achieve virologic suppression appear to derive some immunologic benefit from therapy, as judged by sustained CD4+ lymphocyte count increases, perhaps because resistant virus is "less fit."

Natural history of horizontally acquired HIV infection in pediatrics

Children and youth who acquire HIV infection horizontally appear to have a disease course more similar to adults than perinatally infected infants, but the pathogenesis

Table 1.17. Relationship between baseline RNA copy number and percentage CD4 and mortality in HIV-1 infected children

Baseline HIV RNA (copies/ml)	Baseline CD4+ cell percentage	Number deaths per number patients	Percent mortality
≤100 000	≥15%	15/103	15%
≤100 000	<15%	15/24	63%
>100 000	≥15%	32/89	36%
>100 000	<15%	29/36	81%

HIV RNA was quantified using the NASBA HIV-1 QT amplification system on samples obtained during the NICHD intravenous immunoglobulin clinical trial. Mean age of subjects was approximately 3 years, and mean follow-up was about 5 years. Data was taken from [274], with permission.

of other viral infections in HIV-infected adolescents appears to be distinct. Adolescent girls appear to be more susceptible to human papilloma virus (HPV) infection than older women. HPV strains persist longer in HIV-infected adolescents and the incidence of squamous intraepithelial lesions is higher (see Chapter 8). The seroprevalence of hepatitis B in HIV-infected youth in the USA is much higher than in the population at large [271]. Moreover, the serologic response to hepatitis B vaccine appears to be suboptimal in adolescents [272].

Relationship between viral load CD4+ cell level and disease progression in children

Plasma RNA concentration appears to be a critical determinant of pediatric disease progression [273]. Plasma viral RNA kinetics in the first few months of life correlates with disease course. Rapid progressors have marked increases in viral RNA, which do not decline [262]. Infants with plasma RNA levels above the median had an increased risk of disease progression and death. There was a 44% rate of progression by 24 months for children with an early peak value above 300 000 copies/ml and only a 15% rate of progression for children below this value [108]. Nevertheless, considerable overlap exists in RNA values between rapid and non-rapid progressors, and no threshold value could be identified in the first 2 years of life. However, no infant with less than 70 000 copies/ml in the first 4 months of life had rapidly progressive disease [108, 274] (see Table 1.17). The association of mortality with HIV-1 RNA levels varied with age. For children under 2 years of age, mortality is increased only when the baseline RNA is over 1 million copies/ml; for children over 2 years of age, mortality increases when plasma RNA exceeds 100 000 copies/ml [273, 274] (see Table 1.18). A linear relationship independent of age exists between plasma RNA levels and risk for disease progression. For example, an 8-month-old and an 8-year-old, both with plasma RNA values of 100 000 copies/ml, would have similar risks for future disease progression. Studies have

Table 1.18. Relationship between baseline HIV RNA copy number and risk of disease progression or death stratified by age

Baseline HIV RNA (copies/ml)	Number with disease progression or death/ number patients	Percent with disease progression or death
Age <30 months at entry		
<1000–150 000	9/79	11%
150 001–500 000	13/66	20%
500 001–1 700 000	29/76	38%
>1 700 000	42/81	52%
Age ≥30 months at entry		
<1000–15 000	0/66	0%
15 001–50 000	7/54	13%
50 001–150 000	13/80	16%
>150 000	22/64	34%

HIV RNA was quantified using NASABA HIV-1 QT amplification on samples obtained from children participating in Pediatric AIDS Clinical Trials Group protocol 152. Mean age of children in the <30 months group was 1.1 years. Mean age of children in the group >30 months was 7.3 years. All children received ART. Data taken from [273], with permission.

demonstrated that high peak levels attained during infancy and long duration of high levels predict poor outcomes [108, 275]. Although plasma RNA is a strong independent predictor of the clinical course, additional variables such as CD4+ lymphocyte count. Use of CD4+ lymphocyte count and plasma RNA together for prediction surpasses using either alone [273, 274, 276–278, 279, 280].

Factors associated with disease progression

Several clinical factors are associated with disease progression. Age at the time of infection is probably an important factor (see above) [281]. Route of infection during the perinatal period may be a significant factor. Infants infected by vertical transmission tend to progress more rapidly than those who acquire infection by blood products [250, 251, 282]. Infants born to women with advanced disease and higher viral loads also tend to be rapid progressors [283–285]. Whether this results from emergence of more virulent strains in persons with advanced disease and/or is a reflection of shared immunogenetic factors is unknown. Certain clinical manifestations of HIV-1 disease appear to be important prognostically. Children who present at a young age with PCP or encephalopathy have a worse prognosis than those presenting with lymphoid interstitial pneumonia (LIP) or bacterial infections [255, 286–288]. Infants with enlarged lymph nodes, hepato- and/or splenomegaly at birth have almost a 40% chance of developing category C disease by 1 year compared with a 15% risk without these signs [253]. Growth failure is another clinical sign of poor prognostic import [289], and has recently

been correlated with viral load [290]. Although none of these clinical findings has been found to be as predictive as the combination of plasma RNA concentration and CD4+ cell percentage, the information is easy and inexpensive to obtain [291].

Transient infection

A few pediatric centers prospectively following HIV-1 seroreverting children have described infants who had transient evidence of HIV infection, including positive DNA and RNA PCR and viral culture, in the immediate postpartum period [292–295]. Others have described the detection of cell-mediated immune responses in seroreverting infants (for review see [296]). Studies of macaques infected with Simian immunodeficiency virus (SIV) would suggest that transient infection is possible [297]. However, a detailed phylogenetic analysis of such putative human cases could not confirm transient infection [298], suggesting that, if transient HIV infection occurs, it is rare.

Conclusions

Vast amounts of information about HIV and the immune system have been accumulated since the beginning of the HIV epidemic. Advances in basic knowledge of the immune system and its development, HIV virology, and the responses of the host to HIV infection, constitute the foundation upon which the remarkable therapeutic advances of the last decade have been built. A thorough knowledge of the basic science of HIV virology and immunology will help clinicians provide the best care to their patients.

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