NEUROINFLAMMATION

New Insights into Beneficial and Detrimental Functions

Samuel David

WILEY Blackwell

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Edited by
SAMUEL DAVID, PhD

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Contents

Contributors

Stanley H. Appel
Department of Neurology
Methodist Neurological Institute
The Methodist Hospital
Weill Cornell Medical College
Houston, TX, USA

Nathalie Arbour
Department of Neurosciences
Université de Montréal
Centre de Recherche du Centre Hospitalier de l'Université de Montréal
Montreal, QC, Canada

Lukas Andereggen
Laboratories for Neuroscience Research in Neurosurgery and F.M. Kirby Neurobiology Center
Boston Children's Hospital
Boston, MA, USA

Department of Neurosurgery
Harvard Medical School
Boston, MA, USA

Ivan Ballesteros
Department of Pharmacology (Medical School)
Universidad Complutense de Madrid
Instituto de Investigación Hospital 12 de Octubre (i+12)
Madrid, Spain

David R. Beers
Department of Neurology
Methodist Neurological Institute
The Methodist Hospital
Weill Cornell Medical College
Houston, TX, USA

Larry I. Benowitz
Laboratories for Neuroscience Research in Neurosurgery and F.M. Kirby Neurobiology Center
Boston Children's Hospital
Boston, MA, USA

Departments of Neurosurgery and Ophthalmology
Program in Neuroscience
Harvard Medical School
Boston, MA, USA

Bibiana Bielekova
Neuroimmunology Branch
National Institute of Neurological Disorders and Stroke
National Institutes of Health
Bethesda, MD, USA

Nathalie Castanon
Laboratory of Nutrition and Integrative Neurobiology
INRA UMR 1286
Bordeaux, France

University of Bordeaux
Bordeaux, France

María Isabel Cuartero
Department of Pharmacology (Medical School)
Universidad Complutense de Madrid
Instituto de Investigación Hospital 12 de Octubre (i+12)
Madrid, Spain

James C. Cronk
Center for Brain Immunology and Glia and Department of Neuroscience
University of Virginia School of Medicine
Charlottesville, VA, USA

Samuel David
Department of Neurology and Neurosurgery
Faculty of Medicine
Centre for Research in Neuroscience
The Research Institute of the McGill University Health Centre
Montreal, QC, Canada

Noël C. Derecki
Center for Brain Immunology and Glia and Department of Neuroscience
University of Virginia School of Medicine
Charlottesville, VA, USA

Ashley M. Fenn
Department of Neuroscience
The Ohio State University
Columbus, OH, USA

Robin J.M. Franklin
Department of Clinical Neurosciences
Wellcome Trust-MRC Cambridge Stem Cell Institute
University of Cambridge
Cambridge, UK

Andrew D. Greenhalgh
Department of Neurology and Neurosurgery
Faculty of Medicine
Centre for Research in Neuroscience
The Research Institute of the McGill University Health Centre
Montreal, QC, Canada

Jonathan P. Godbout
Department of Neuroscience and Institute for Behavioral Medicine Research
The Ohio State University
Columbus, OH, USA

Janos Groh
Department of Neurology and Developmental Neurobiology
University of Wuerzburg
Wuerzburg, Germany

Renu Heir
Department of Neurology and Neurosurgery
Centre for Research in Neuroscience
The Research Institute of the McGill University Health Center
Montreal, QC, Canada

Kristopher G. Hooten
Department of Neurological Surgery
University of Florida
Gainesville, FL, USA

Dennis Klein
Department of Neurology and Developmental Neurobiology
University of Wuerzburg
Wuerzburg, Germany

Jonathan Kipnis
Center for Brain Immunology and Glia and Department of Neuroscience
University of Virginia School of Medicine
Charlottesville, VA, USA

Graduate Program in Neuroscience and Medical Scientist Training Program
University of Virginia School of Medicine
Charlottesville, VA, USA

Antje Kroner
Department of Neurology and Developmental Neurobiology
University of Wuerzburg
Wuerzburg, Germany

	Department of Neurology and Neurosurgery Faculty of Medicine Centre for Research in Neuroscience The Research Institute of the McGill University Health Centre Montreal, QC, Canada
Steve Lacroix	Centre de Recherche du Centre Hospitalier Universitaire (CHU) de Québec –CHUL Québec, QC, Canada
	Département de Médecine Moléculaire Faculté de médecine Université Laval Québec, QC, Canada
Hans Lassman	Division of Neuroimmunology Center for Brain Research Medical University of Vienna Vienna, Austria
Sophie Layé	Laboratory of Nutrition and Integrative Neurobiology INRA UMR 1286 Bordeaux, France
	University of Bordeaux Bordeaux, France
Ignacio Lizasoain	Department of Pharmacology (Medical School) Universidad Complutense de Madrid Instituto de Investigación Hospital 12 de Octubre (i+12) Madrid, Spain
Giamal Luheshi	Department of Psychiatry Douglas Mental Health University Institute McGill University Montreal, QC, Canada
Rudolf Martini	Department of Neurology and Developmental Neurobiology University of Wuerzburg Wuerzburg, Germany
María Ángeles Moro	Department of Pharmacology (Medical School) Universidad Complutense de Madrid Instituto de Investigación Hospital 12 de Octubre (i+12) Madrid, Spain
Muktha Natrajan	Department of Clinical Neurosciences Wellcome Trust-MRC Cambridge Stem Cell Institute University of Cambridge Cambridge, UK

Diana M. Norden
Department of Neuroscience
The Ohio State University
Columbus, OH, USA

Alexandre Paré
Centre de Recherche du Centre Hospitalier Universitaire (CHU) de Québec - CHUL
Québec, QC, Canada

Alexandre Prat
Department of Neurosciences
Université de Montréal
Centre de Recherche du Centre Hospitalier de l'Université de Montréal
Montreal, QC, Canada

Christopher Power
Department of Medicine (Neurology)
University of Alberta
Edmonton, AB, Canada

Harald Prüss
Department of Neurology and Experimental Neurology
Clinical and Experimental Spinal Cord Injury Research (Neuroparaplegiology)
Charite
Universitatsmedizin Berlin
Berlin, Germany

German Center for Neurodegenerative Diseases (DZNE)
Berlin, Germany

Catarina Raposo
Department of Neurobiology
Weizmann Institute of Science
Rehovot, Israel

Jan M. Schwab
Department of Neurology and Experimental Neurology
Clinical and Experimental Spinal Cord Injury Research (Neuroparaplegiology)
Charite - Universitatsmedizin Berlin
Berlin, Germany

Spinal Cord Injury Center
Trauma Hospital Berlin
Berlin, Germany

Department of Neurology and Neuroscience
Center for Brain and Spinal Cord Repair
The Ohio State University Medical Center
Columbus, OH, USA

Michal Schwartz
Department of Neurobiology
Weizmann Institute of Science
Rehovot, Israel

Charles N. Serhan
Department of Anesthesiology
Perioperative and Pain Medicine
Center for Experimental Therapeutics and Reperfusion Injury
Harvard Institutes of Medicine
Brigham and Women's Hospital and Harvard Medical School
Boston, MA, USA

David Stellwagen
Department of Neurology and Neurosurgery
Centre for Research in Neuroscience
The Research Institute of the McGill University Health Center
Montreal, QC, Canada

Ephraim F. Trakhtenberg
Laboratories for Neuroscience Research in Neurosurgery and
F.M. Kirby Neurobiology Center
Boston Children's Hospital
Boston, MA, USA

Department of Neurosurgery
Harvard Medical School
Boston, MA, USA

John G. Walsh
Department of Medicine (Neurology)
University of Alberta
Edmonton, AL, Canada

Yuqin Yin
Laboratories for Neuroscience Research in Neurosurgery and
F.M. Kirby Neurobiology Center
Boston Children's Hospital
Boston, MA, USA

Department of Neurosurgery
Harvard Medical School
Boston, MA, USA

Ji Zhang
The Alan Edwards Centre for Research on Pain and Department of Neurology and Neurosurgery
Faculty of Dentistry and Medicine
McGill University
Montreal, QC, Canada

Weihua Zhao
Department of Neurology
Houston Methodist Neurological Institute
Houston Methodist Hospital Research Institute
Houston Methodist Hospital
Houston, TX, USA

Preface

When I was approached by the publisher, Wiley, to edit a book on neuroinflammation I felt it was a timely project and one that would have a wide appeal. As a researcher whose work focuses on inflammation in spinal cord injury (SCI), central nervous system (CNS) autoimmune disease, peripheral nerve injury, and stroke, I have a broad perspective on the role of neuroinflammation. Moreover, as someone who has run a graduate level course on neuroinflammation for the past 10 years at McGill University, I have had a close-up view of the wide ranging impact of inflammation in neurology.

This book is divided into three sections. The first part begins with two general chapters, the first chapter provides a broad overview of neuroinflammation and immune pathology in patients with multiple sclerosis (MS) and stroke. It discusses the concept of neuroinflammation and the basic principles of immune surveillance and inflammation by adaptive immune responses. The second chapter provides an overview of *in vivo* imaging of immune and glial cell responses in animal models of CNS injury and disease. The use of intravital microscopy to study CNS inflammation is providing new insights into cell-to-cell interactions and behavior of immune and CNS cells *in situ*. The second part of the book focuses mainly on the detrimental aspects of inflammation, although discussions in many chapters also note some of the beneficial aspects of inflammation that one could modulate to improve outcomes. This section consists of eight chapters ranging from MS and experimental autoimmune encephalomyelitis, SCI, stroke, aging, obesity, neuropathic pain subsequent to peripheral nerve injury, inherited peripheral neuropathies, and CNS viral infections such as human immunodeficiency virus (HIV) and West Nile virus. The third part of the book focuses on areas in which the beneficial aspects of neuroinflammation are seen more prominently. This section consists of seven chapters ranging from CNS injury, remyelination in the CNS, Rett syndrome, amyotrophic lateral sclerosis (ALS), and the role of tumor necrosis factor (TNF) in synaptic plasticity and neuronal function. The book ends with a chapter on the mechanisms underlying resolution of inflammation in CNS. The key reasons for choosing these topics are summarized in the subsequent text and will give the reader an idea of the main objectives of this book.

It is becoming increasingly evident that inflammation plays a role in many if not most neurological disorders. Certain conditions such as MS have long been recognized as a neuroinflammatory condition involving a prominent autoimmune response to CNS myelin antigens. In the case

of traumatic SCI and stroke, inflammation triggered locally at the site of injury or stroke has also been recognized as contributing to secondary tissue damage and evolving pathology. Studies on neuroinflammation in MS, SCI, and stroke have a long history, but several recent advances have begun to shed new light that is worth taking note of. In contrast, the involvement of neuroinflammation has not been widely appreciated in aging and obesity. In these areas, neuroinflammation can impact on learning and memory, as well as on mood and cognitive function. With the increase in wealth in formerly developing countries, obesity is increasing worldwide at a shocking rate in children and adults and has an impact not only on cardiovascular health and the development of type 2 diabetes but also on the brain. In HIV/acquired immunodeficiency syndrome (AIDS), despite the effectiveness of combined antiretroviral therapy to markedly improve survival of people with HIV/AIDS, the CNS remains a major reservoir of the virus. About a third of patients on antiretroviral therapy have a spectrum of neurocognitive disorders that contributes significantly to morbidity and mortality and remains an important therapeutic target. Inflammation in peripheral nerves also contributes to pathology as seen in its involvement in neuropathic pain. Interestingly, this involves not only macrophage and cytokine responses locally in the injured nerve but also injury-induced microglia/macrophage and cytokine responses in the spinal cord, which provides multiple novel therapeutic targets for the management of pain. Recent work on inherited peripheral neuropathies, such as Charcot-Marie-Tooth disease, has also shown the involvement of the innate and adaptive immune response in the pathogenesis. Such work has led to the identification of immune cells as mediators and amplifiers of the demyelinating and axonal pathology.

Not too long ago there were long and heated debates on whether inflammation in conditions such as CNS injury is good or bad. One exciting development in other fields of immunology in the past decade that has now trickled into neuroscience, shows that the immune response can be good or bad depending on the state of activation of macrophages and microglia, which is influenced by the tissue environment. The idea that macrophages and microglia are very plastic cells that change their phenotype or polarization state along a continuum from proinflammatory, cytotoxic M1 phenotype at one extreme to an anti-inflammatory, pro-repair M2 phenotype at the other extreme with stages in-between is an important conceptual model with increasing supportive evidence. These cells can be polarized differently in different conditions and can also change their polarization state at different times during the evolving pathology. Macrophage and microglial polarization therefore has wide-ranging implications for neurological conditions. This includes neuroinflammation in SCI and stroke, as well as diverse phenomenon such as remyelination in the CNS, and neuronal survival in neurodegenerative diseases such as ALS. A characteristic feature of the adult mammalian CNS is that axons damaged by injury or disease fail to regenerate in situ. Work done on the optic nerve show that induction of an inflammatory response in the eye triggers long-distance axon regeneration of retinal ganglion cells through the optic nerve, showing how some aspects of neuroinflammation can indeed be beneficial to recovery. In another striking discovery, the transplantation of wild type microglia-like cells into the brains of *Mecp2*-null mice (a model of Rett syndrome) improved survival and motor function. Genetic targeting of microglia to express wild type Mecp2 in *Mecp2*-null mice also improved outcome, showing that CNS resident immune cells can be selectively targeted to improve neuronal survival in certain conditions. Another surprising recent discovery is the finding that the proinflammatory cytokine TNF can have profound effects on synaptic plasticity and neuronal function, in particular, the compensatory synaptic adaption in response to prolonged changes in neuronal activity. This has

implications for neuronal function in CNS injury and disease in which increases in TNF occur. Finally, no discussion on inflammation would be complete without a section on the active resolution of inflammation and the pro-resolution bioactive lipid mediators such as resolvins and protectins that attenuate inflammation and improve outcome. There is excitement and hope that these pro-resolution mediators will become important therapeutics to treat a variety of neuroinflammatory conditions.

The reader will find differences but also many commonalities in the inflammatory responses in the various neurological conditions covered in this book. This implies that development of treatments against particular neuroinflammation targets for one neurological condition is likely to also be useful for other conditions. Many of us focus our work in our own particular areas of interest and tend to keep to our own silos. My aim is to bring such diverse areas together in one book and to break down these barriers and foster cross-talk and understanding of neuroinflammation in various fields. There is much we can learn from each other.

I want to thank all the authors for taking the time to contribute to this book. I know how much demand there is on their time and am truly appreciative of their efforts. I am indebted to Dr. Antje Kroner, a senior postdoctoral fellow in my laboratory for so generously helping me in editing the chapters and for her keen attention to detail. I could not have done it as easily without her help. I also want to thank Justin Jeffryes, Editorial Director at Wiley for seeking me out for this project. I thank him for his help, advice, and encouragement in taking this project through to completion. I am also grateful to Stephanie Dollan, Senior Editorial Assistant, for making sure I kept on track, for corresponding with the authors, and for making it all so easy.

Samuel David, PhD
Montreal, Canada

PART I

Introduction

1 Immune Response in the Human Central Nervous System in Multiple Sclerosis and Stroke

Hans Lassmann

Division of Neuroimmunology, Center for Brain Research, Medical University of Vienna, Wien, Austria

Introduction

Traditional pathology provides a clear distinction between inflammatory and neurodegenerative disorders. Inflammatory diseases comprise a large spectrum of infectious and autoimmune diseases. In these conditions, a specific immune response against autoantigens or infectious agents is present, which induces inflammation and specific destruction of cells, which contain the inciting agent or autoantigen. In addition, cells and tissue components, which are present in the vicinity of the specific targets of the immune response, also get injured or destroyed by toxic products or mediators of the immune response, a process termed "bystander damage" (Wisniewski and Bloom, 1975). In contrast, in conditions of neurodegeneration or brain ischemia, the primary cause of cell and tissue injury is due to primary metabolic changes. Also, in these conditions, immune mediators, such as cytokines or activated cells of the immune system, as for instance granulocytes or activated macrophages and microglia, are involved in cell and tissue degeneration. This lead to the broad concept of "neuroinflammation" playing a major role in the pathogenesis of a wide spectrum of brain diseases and being a potential target for neuroprotective treatments (Craft *et al.*, 2005, Ransohoff and Liu, 2007).

The Concept of Neuroinflammation

Any type of tissue injury in the central nervous system (CNS) is associated with local changes in the microenvironment, which are in part similar to those seen in inflammatory conditions. Cell injury in the CNS results in activation of microglia and astrocytes (Ransohoff and Brown, 2012). Furthermore, a similar activation of microglia can be induced even by functional changes in neuronal networks, such as for instance sustained overactivation of neuronal circuits in epileptic seizures (Xanthos and Sandkühler, 2013). Activation of glia is induced by different signals, including release of adenosine triphosphate (ATP) and its signaling through G-protein-coupled

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receptors, by direct neurotransmitter signaling or by the liberation of intracellular components from damaged cells, resulting in the activation of pattern recognition receptors (Iadecola and Anrather, 2011). An important consequence of astrocyte and microglia activation is the production of a wide spectrum of pro- and anti-inflammatory cytokines and growth factors. Thus, microglia and astroglia activation as a reflection of an inflammatory response to tissue injury may have beneficial as well as detrimental consequences for adjacent neurons and glia, depending on the type of the primary tissue injury and on the properties of the environment where it takes place (Griffiths *et al.*, 2007).

In addition, tissue injury and the induction of proinflammatory cytokines and chemokines may lead to disturbance of vascular integrity at the blood–brain barrier, resulting in brain edema and the penetration of serum components into the CNS (Takeshita and Ransohoff, 2012; Erickson *et al.*, 2012). Besides leakage of various additional proinflammatory factors such as complement components, the leakage of fibrin and its coagulation within the perivascular compartment plays an important role in this process. It has been shown in experimental studies that fibrin deposition in the brain augments the inflammatory process and/or the subsequent tissue injury. Thus, inflammatory processes in the brain are much milder in fibrinogen-deficient animals or in conditions of fibrin depletion in the plasma. Fibrin can activate microglia and macrophages through toll-like receptor signaling. In addition, fibrin interacts with microglia through specific binding to the integrin receptor CD11b/CD18, which amplifies the inflammatory process and its associated tissue damage (Davalos *et al.*, 2012). Such a vascular inflammatory process may also recruit inflammatory cells from the circulation. Depending on the type of tissue injury and its local induction of different spectra of adhesion molecules and chemokines, different leukocyte populations will be recruited, such as granulocytes and monocytes, but also different subpopulations of T- and B-lymphocytes (Gorina *et al.*, 2014; Ransohoff and Engelhardt, 2012). This vascular injury is an important component of the inflammatory reaction, giving rise to the cardinal features of an inflammatory response, which have been defined as tissue swelling due to edema (tumor), vasodilatation, and hyperemia (rubor and calor) and activation of sensory receptors (dolor). In the CNS, edema and tissue swelling are the most important consequence, as the brain can swell only to a limited degree due to restrains by the bony skull. Thus, edema results in increased intracranial pressure leading to disturbance of microcirculation and amplification of tissue damage by ischemia (Bor Seng Shu *et al.*, 2013).

Finally, inflammation may also be induced or augmented by specific mechanisms of adaptive immune responses. The prerequisite for such a scenario is that the organism has earlier mounted a specific response of T-lymphocytes or antibodies, which are directed against an antigen that is present within the CNS (Wekerle *et al.*, 1986; Flügel *et al.*, 2001). Inflammation, which is mediated by adaptive immune responses, is especially important in infectious and autoimmune diseases of the nervous system. The diverse patterns of neuroinflammation are summarized in Fig. 1.1.

Basic Principles of Immune Surveillance and Inflammation by Adaptive Immune Responses

The CNS has for long been viewed as an immune-privileged organ, which is shielded from the peripheral immune system by the blood–brain barrier and which does not express major histocompatibility complex (MHC) antigens required for antigen recognition by T-lymphocytes. This

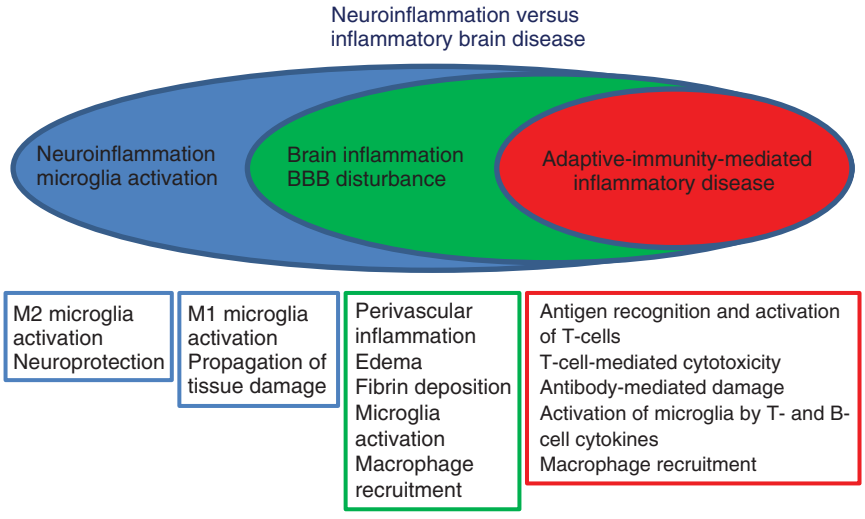

Figure 1.1 Inflammation of the CNS comprises a broad spectrum of tissue alterations including microglia activation, vascular inflammation with blood–brain barrier damage, and inflammation mediated by adaptive immunity. (*See insert for color representation of this figure.*)

concept, however, has been modified during recent years. T-lymphocytes can enter the normal brain through an intact blood–brain barrier in the course of immune surveillance (Wekerle *et al.*, 1986). However, it is only the activated T-cell population, which is able to enter the normal CNS tissue. This implies that a small fraction of T-cells, when activated in the course of an infection, migrates into the brain or spinal cord in search for their specific antigen. When they do not find their cognate antigen, they quickly disappear from the brain tissue due to local destruction by programmed cell death (apoptosis; Bauer *et al.*, 1998). Whether some of these T-cells can also migrate back into the blood stream or into the lymphatic system is currently unresolved. However, when the specific antigen is present in the CNS and is presented in the perivascular or meningeal space by a macrophage population with features of dendritic cells, T-cells receive a further activation signal (Flügel *et al.*, 2001, Mues *et al.*, 2013). They then proliferate and expand clonally and produce additional proinflammatory cytokines and chemokines, which act on endothelia and promote the secondary recruitment of other leukocytes from the bloodstream, such as other T-cells, B-cells, and monocytes (Ransohoff and Engelhardt, 2012). This results in a first stage of perivascular and meningeal inflammation. Proinflammatory cytokines in this condition also activate local microglia and astrocytes, which amplify the inflammatory response through additional production of cytokines, chemokines, and proteases. This further amplifies the perivascular inflammatory response and allows the inflammatory cells to pass the subpial and perivascular astrocytic glia limitans and spread into the CNS parenchyme. Tissue injury can be induced directly by cytotoxic MHC Class I antigen-dependent cytotoxic T-cells (Saxena *et al.*, 2008). These cells can recognize their specific antigen on all cells of the CNS, such as astrocytes, oligodendrocytes, and neurons, as all these cells express MHC Class I antigens in an inflammatory environment (Höftberger *et al.*, 2004). The expression of MHC Class II antigens, which are necessary for antigen recognition by CD4 positive T-cells, is more restricted, being mainly

present on macrophages, microglia, and occasionally on astrocytes. Thus, $CD4^+$ T-cell-mediated inflammation mainly leads to the activation of macrophages and microglia, which are then responsible for the induction of tissue injury through the liberation of toxic immune mediators, such as reactive oxygen or nitrogen species, of cytotoxic cytokines, such as tumor necrosis factor alpha (TNF-α), or of proteases and lipases (Jack *et al.*, 2005). However, direct cytotoxicity can even be mediated by $CD4^+$ T-cells. When highly activated, they may mediate cytotoxicity in a manner that does not depend on specific antigen recognition on the target cell (Nitsch *et al.*, 2004).

Downregulation of the inflammatory response is of critical importance for protecting the CNS against uncontrolled immune-mediated damage. This is in part achieved by highly efficient destruction of T-cells within the brain and spinal cord through apoptosis (Bauer *et al.*, 1998, Flügel *et al.*, 2001). This process eliminates both antigen-specific T-lymphocytes and secondarily recruited T-cells. It is highly efficient in conditions of acute T-cell-mediated brain inflammation and allows persistence of the inflammatory process only as long as there is a continuous influx of T-cells from the circulation into the lesions. The molecules involved in the induction of T-cell apoptosis are currently not well defined. In addition, brain inflammation is further controlled by the recruitment of regulatory T-cells (Tregs) into the inflamed CNS tissue (O'Connor and Anderton, 2008; Fransson *et al.*, 2012) as well as by downregulation of immune response due to activation of the pituitary/adrenal axis (MacPhee *et al.*, 1989). Clearance of the inciting trigger, such as the infectious agent, also terminates inflammation by the lack of further antigen presentation and T-cell activation. Taken together, these mechanisms grant that the brain is controlled by a strictly regulated immune response, which keeps collateral damage as small as possible.

Immune-mediated damage of the brain by specific antibodies in general is low or absent, as they penetrate the normal blood–brain barrier only to a very limited degree. In addition, antibodies require interaction with complement or activated effector cells such as granulocytes or macrophages to induce tissue damage, factors that are not present in the normal CNS (Vass *et al.*, 1992). However, circulating autoantibodies, directed against foreign or self-antigens, which are present on the extracellular surface of cells, may become pathogenic, when they reach the brain in an inflammatory environment, for instance induced by a T-cell-mediated inflammatory response (Linington *et al.*, 1988). T-cell-mediated inflammation not only opens the blood–brain barrier, but also activates local macrophages and microglia and induces granulocyte and macrophage recruitment from the circulation by inducing local chemokine secretion. It further stimulates the production of complement and inhibits the production of complement-inhibitory proteins. These additional factors allow efficient antibody-mediated cell destruction (Pohl *et al.*, 2013).

Data obtained in diseases, which are mediated by autoantibodies against neurotransmitter receptors or cell surface channels, suggest that even antibodies alone may induce disease and damage in the CNS. In this case, high titers of autoantibodies are present in the circulation, and the antibodies exert their pathogenic role by direct binding to the channels or receptors, thus acting more analogous to a pharmacological agonist or antagonist than as an immunological tool (Hughes *et al.*, 2010). In this situation, massive functional disturbances are seen despite only sparse or absent inflammation and structural tissue damage (Bien *et al.*, 2012).

As mentioned previously, recruitment of T- and B-lymphocytes into the CNS may also occur secondarily to tissue injury, for instance in ischemia or in neurodegenerative diseases

(Gelderblom *et al.*, 2009). However, the mere presence of lymphocytes within a brain lesion does not necessarily imply that they are pathogenic. When there is no activation signal within the CNS tissue, such cells are inert bystanders. Such T-cell infiltrates in brain lesions are potentially pathogenic when they are locally activated, for instance by recognizing specific autoantigens. This is indicated, when the respective T-cells locally proliferate, show clonal expansion, or express activation markers (Liesz *et al.*, 2013a).

Inflammation in the Central Nervous System of Patients with Multiple Sclerosis

Multiple sclerosis (MS) has originally been defined as an inflammatory demyelinating disease, suggesting that the formation of brain and spinal cord lesions in this disease is driven by the inflammatory process (Lassmann *et al.*, 2007). Focal plaques of primary demyelination, reflected by complete loss of myelin, but partial preservation of axons and neurons, are the hallmark of MS pathology. Demyelinated plaques are present in the white matter as well as in the grey matter, such as the cerebral and cerebellar cortex (Peterson *et al.*, 2001) and the deep grey matter nuclei (Vercellino *et al.*, 2009), including the basal ganglia, the thalamus, and hypothalamus (Huitinga *et al.*, 2004). In addition to the focal pathology, reflected by demyelinated plaques, there are also diffuse changes in the brain, consistent of small perivascular demyelinated lesions, diffuse axonal injury and neurodegeneration, generalized microglia activation, and diffuse astrocytic scar formation in the entire white and grey matter of the brain and spinal cord (Kutzelnigg *et al.*, 2005). This finally leads to profound tissue loss and atrophy in the entire CNS. While focal demyelinated lesions in the white matter dominate the pathology of patients with early stages of relapsing remitting MS, cortical demyelination and diffuse damage of the white and grey matter are most prominent in patients during the later progressive stage of the disease (Kutzelnigg *et al.*, 2005).

Active demyelination and neurodegeneration in the MS brain are invariably associated with inflammation, consistent of infiltrates of the tissue by T- and B-lymphocytes and by macrophages (Fig. 1.2; Frischer *et al.*, 2009). Most prominent, however, is the profound

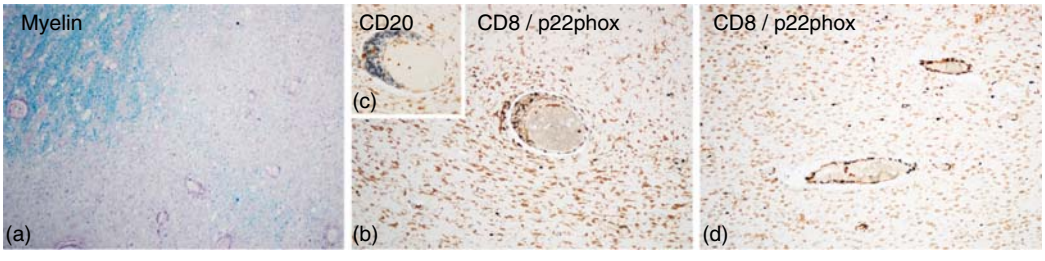

Figure 1.2 Inflammation in multiple sclerosis. (a) Inflammation in active multiple sclerosis lesions is associated with demyelination, reflected by the loss of blue myelin staining in the lesion. (b, c) Infiltration of the tissue with $CD8^+$ T-lymphocytes (b) and $CD20^+$ B-lymphocytes (c) (black cells) in the active lesion edge. The zone of initial demyelination at the lesions edge shows profound microglia activation with intense expression of NADPH oxidase (brown cells; p22phox). (d) CD8+ T-lymphocytes are also present in the lesion center (black cells). Most inflammatory cells are macrophages with low expression of NADPH oxidase (brown cells). (*See insert for color representation of this figure.*)

activation of the local microglia population (Jack *et al.*, 2005). Several arguments speak in favor for a pathogenic role of this inflammatory response. T- and B-cells in the lesions show an activated phenotype and clonal expansion (Babbe *et al.*, 2000; Obermeier *et al.*, 2011), most likely due to proliferation following the encounter with their cognate antigen in the lesions. Furthermore, active lesions in the white matter of patients with MS show contrast enhancement as a consequence of inflammation-induced blood–brain barrier damage (Miller *et al.*, 1988; Gaitan *et al.*, 2011). Most importantly, anti-inflammatory or immunomodulatory treatments have a beneficial effect, in particular in patients in the early relapsing stage of their disease (Wiendl and Hohlfeld, 2009).

There is still some debate, whether inflammation also drives neurodegeneration in the progressive stage of the disease, because in such patients, lesions with contrast enhancement are rare and current anti-inflammatory treatments are no longer effective. Thus, a widely proposed concept for the pathogenesis of the progressive stage of MS is that inflammation in early relapsing disease initiates a cascade of events, which leads to demyelination and neurodegeneration in the progressive stage and becomes independent of the original inflammatory response (Trapp and Nave, 2008). However, detailed pathological studies showed that active tissue injury in the progressive stage is associated with T- and B-cell infiltrates in the CNS and that in patients in whom lymphocyte infiltration in the brain has declined to levels seen in age-matched controls, no active demyelination is found and neurodegeneration also is reduced to levels seen in the respective controls (Frischer *et al.*, 2009). Yet, the nature of the inflammatory response appears to be different between early and late stages of MS. While in new active lesions in acute and relapsing/remitting MS, inflammation is associated with massive blood–brain barrier damage, inflammation in the progressive stages occurs at least in part behind a closed or repaired blood–brain barrier (Hochmeister *et al.*, 2006). Furthermore, aggregates of inflammatory infiltrates, which consist of T-cells, B-cells, and plasma cells and may even form lymph follicle-like structures, are present in the meninges and perivascular spaces (Serafini *et al.*, 2004). The extent of meningeal inflammation and the formation of inflammatory aggregates correlate well with the extent of active cortical demyelination and neurodegeneration (Magliozzi *et al.*, 2007).

The Nature of the Inflammatory Response in Actively Demyelinating Lesions in MS

Profound inflammation, consisting of T-cells and B-cells, is a characteristic feature of actively demyelinating MS lesions. Active demyelination and neurodegeneration are associated with the presence of activated macrophages and microglia, which are present in close contact with degenerating myelin sheaths and axons (Prineas and Graham, 1981; Ferguson *et al.*, 1997; Trapp *et al.*, 1998). However, inflammation in active lesions appears to occur as a two-step phenomenon. In the initial stage, termed initial (Marik *et al.*, 2007) or pre-phagocytic lesions (Barnett and Prineas, 2004), lymphocyte infiltration is moderate or sparse and the lymphocytic population mainly consists of MHC Class-I-restricted $CD8^+$ T-cells. In contrast, when myelin sheaths have been destroyed and taken up by microglia and macrophages, inflammatory infiltration is much higher and a wide spectrum of different leukocyte populations is found, including $CD4^+$ and $CD8^+$ positive T-cells, B-cells, hematogeneous macrophages, and a variable number of plasma cells (Marik *et al.*, 2007; Henderson *et al.*, 2009). Thus, a small number of T-cells (mainly $CD8^+$

cells) appear to enter the brain in the course of immune surveillance, encounter their specific antigen, and start the lesions through microglia activation. However, when myelin and oligodendrocytes get destroyed, intracellular and myelin components are liberated into the extracellular space and provide an additional proinflammatory stimulus. This, then, leads to secondary amplification of the inflammatory process in the lesions.

It has, however, been questioned, whether the mild T-cell infiltration in initial lesions is sufficient to drive the demyelinating process. The alternative interpretation of these findings is that initial demyelination occurs independently from adaptive T- and B-cell responses and that inflammatory cells are secondarily recruited into sites of pre-existing tissue injury, where they then may amplify demyelination and neurodegeneration (Barnett and Prineas, 2004; Henderson *et al.*, 2009).

Both T- and B-cells show clonal expansion in the MS brain, and for B-cells, this is reflected by the presence of oligoclonal intrathecal antibody synthesis (Skulina *et al.*, 2004; Obermeier *et al.*, 2008). Regarding T-cells, the most pronounced clonal expansion is seen for MHC Class-I-restricted $CD8^+$ cells, which also dominate in initial lesions stages. Genome-wide association studies in patients with MS versus controls identified a large number of different genes to be associated with MS susceptibility (Sawcer *et al.*, 2011). Most of them have putative functions in the immune system. Finally, anti-inflammatory or immunomodulatory treatments are beneficial at least in early disease stages (Wiendl and Hohlfeld, 2009).

Studies on local cytokine and chemokine expression in MS lesions are limited but consistent with an inflammatory response, which is driven by T- and possibly B-lymphocytes. Active lesions show the expression of various adhesion molecules (Washington *et al.*, 1994; Allavena *et al.*, 2010; Cavrol *et al.*, 2008; Ifergan *et al.*, 2011; Larochelle *et al.*, 2012) and chemokines (Trebst *et al.*, 2001; Kivisakk *et al.*, 2004), which are instrumental for leukocyte migration through the blood–brain barrier (Steiner *et al.*, 2010). Antigen-specific activation of T-cells in MS lesions is also indicated by the expression of activation antigens (Pohl *et al.*, 2013; Annibali *et al.*, 2010), the presence of costimulatory molecules (Windhagen *et al.*, 1995; Gerritse *et al.*, 1996) or autoantigen and MHC complexes (Krogsgaard *et al.*, 2000), and by the local expression of various pro- and anti-inflammatory cytokines (Mycko *et al.*, 2003; Tzartos *et al.*, 2008, 2011). This has so far been mainly described in classical active lesions. Detailed studies on the phenotype of lymphocytes in different stages of the disease and in relation to the activity of inflammation and neurodegeneration are still missing.

Macrophages and Microglia in MS Lesions

Much of our knowledge on the role of microglia and macrophages comes from experimental studies performed in rodents. However, there are species-related functional differences between rodent and human microglia, which involve cytokine signaling, response to innate immunity stimuli, and effector functions (Smith and Dragunow, 2014). Thus, to understand microglia function in brain disease, analysis of their function in human disorders is important.

There is good agreement that active demyelination and axonal injury in MS occurs in close apposition with activated microglia and macrophages (Prineas *et al.*, 2001; Lassmann, 2011). In the normal-appearing white matter around actively demyelinating lesions, microglia nodules are

seen, which are in close contact with myelinated nerve fibers. In initial lesions, massive microglia activation is associated with oligodendrocyte apoptosis and initial changes of myelin disintegration. Myelin is then taken up by phagocytic cells. Toward the lesion center, there is a continuous transition between cells with microglia and macrophage phenotype, suggesting that most of the phagocytic cells in the lesions come from the microglia cell pool. Furthermore, dystrophic axons within and around active MS lesions are seen in close contact with microglia or macrophages (Ferguson *et al.*, 1997). Activated microglia and macrophages in initial and active MS lesions express a variety of markers, including MHC class I and Class II molecules (Höftberger *et al.*, 2004), Fc-receptors (Ulvestad *et al.*, 1994), and markers associated with phagocytosis, such as CD68 (Brück *et al.*, 1995). Most importantly, they highly express components of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX1 and NOX2) complexes, suggesting high oxidative burst activation. In contrast, expression of inducible nitric oxide synthase (iNOS) is sparse or absent in initial lesions, but it is upregulated on a subset of macrophages in established lesions. When myelin has been taken up in macrophage-like cells, they lose their expression of NADPH oxidase but retain the expression of phagocytosis-related molecules, such as CD68 or CD163 (Fig. 1.2; Fischer *et al.*, 2012, 2013). As, however, shown in spinal cord injury, this conversion from an M1 to an M2 phenotype of macrophages in response to myelin phagocytosis can be counteracted by the presence of TNF and by iron loading of macrophages in the lesions (Kroner *et al.*, 2014).

Unrelated to the presence of demyelinated lesions, there is also a general activation of microglia in the normal-appearing white matter in patients with MS and to a lower extent also in the white matter of age-matched controls (Lassmann, 2011). Recent studies suggest that microglia in the normal-appearing white matter of patients with MS are in an alerted state, which may be transformed into a cytotoxic state by the additional presence of proinflammatory cytokines (Vogel *et al.*, 2013). Global microglia activation may in part reflect anterograde and retrograde neuronal and axonal degeneration due to lesions in other brain areas. This may explain why new MS lesions are more frequently seen in areas, which receive axonal input from distant regions, affected by other MS-related pathology (Kolasinski *et al.*, 2012). In addition, global microglia activation in the MS brain also reflects diffuse neurodegeneration in the normal-appearing white and grey matter.

From all these data, it is assumed that activated microglia and macrophages play a major role in the induction of demyelination and tissue degeneration in the brain of patients with MS. However, astrocytes take part in this process as well. As reviewed recently, they are involved in propagating and controlling inflammation. Furthermore, functional impairment of astrocytes in active lesions augments demyelination, oligodendrocyte death, and axonal injury (Brosnan and Raine, 2013). Reactive astrocytes in MS lesions lose their cell polarity, which results in the loss of connexins, the excitatory amino acid transporter EAAT2, and the water channel aquaporin 4 (Masaki *et al.*, 2013). This impairs energy supply to oligodendrocytes and axons and increased excitotoxicity and may also propagate brain edema.

Mechanisms of Demyelination and Neurodegeneration in MS

There are many different acute and chronic inflammatory diseases of the CNS, but the widespread primary demyelination leading to large plaques of myelin destruction with axonal preservation