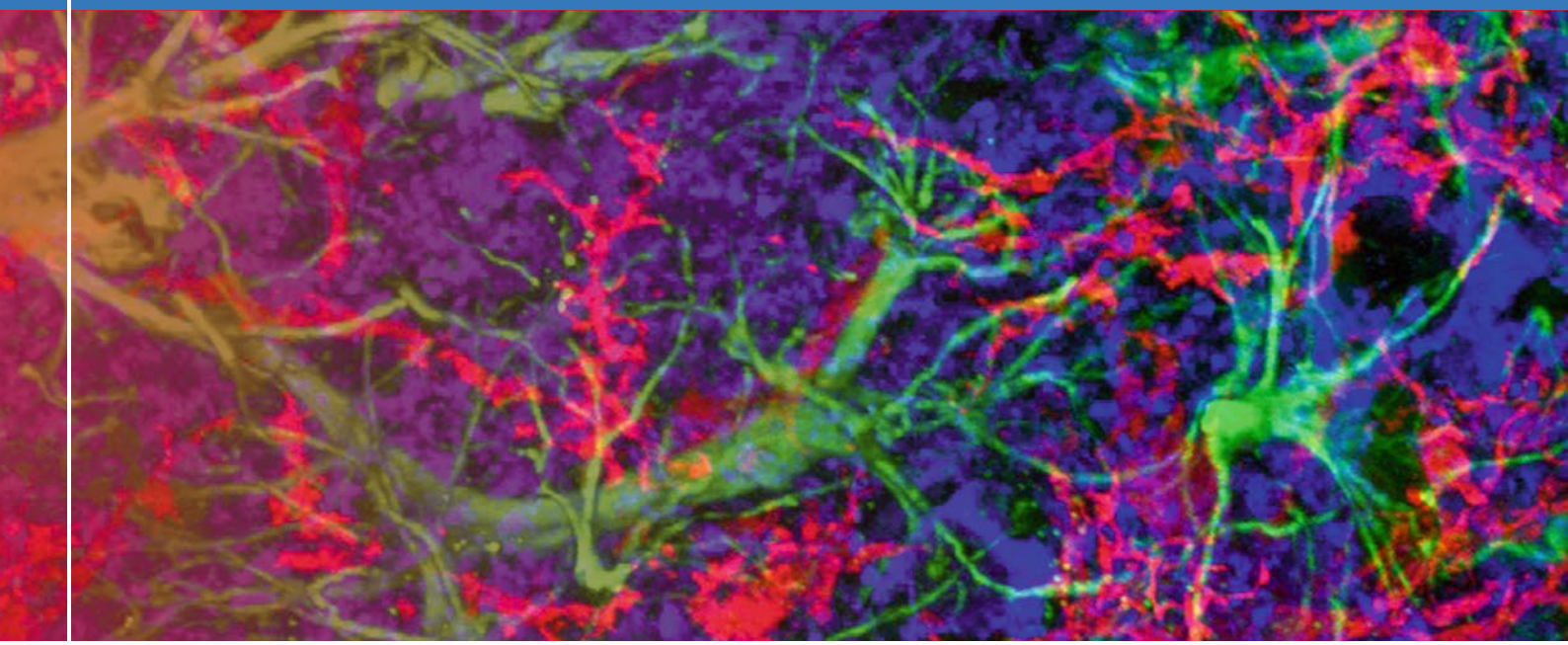
Tsuneya Ikezu
Howard E. Gendelman *Editors*



# Neuroimmune Pharmacology

*Second Edition*

Serge Przedborski · Eliezer Masliah · Marco Cosentino
*Associate Editors*

Springer

Tsuneya Ikezu • Howard E. Gendelman
Editors

# Neuroimmune Pharmacology

Springer

*Editors*
Tsuneya Ikezu
Departments of Pharmacology and Experimental Therapeutics and Neurology
Boston University School of Medicine
Boston, MA, USA

Howard E. Gendelman
Department of Pharmacology and Experimental Neuroscience
University of Nebraska Medical Center
Nebraska Medical Center
Omaha, NE, USA

ISBN 978-3-319-44020-0 ISBN 978-3-319-44022-4 (eBook)
DOI 10.1007/978-3-319-44022-4

Library of Congress Control Number: 2016954435



Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

# Preface

In the past three decades, enormous strides have been made in our understanding of the relationships between inflammation, immune responses, and degenerative human diseases. Neuroinflammation has grown from a tiny beginning to its current status as the largest field of brain research. Its growth continues to be rapid so this dominance will continue to expand. The field commenced with the obscure identification of activated microglia in the brains of Alzheimer's disease cases. A grant application of ours to the Canadian government to explore the ramifications of this finding was turned down with the terse comment: "This hypothesis is ridiculous!"

To grasp the dimensions of growth in tis field, and to gain a perspective of future opportunities, today's neuroscientists only need to read this volume from cover to cover. The developing information has mostly appeared in specialty journals that have dealt only with isolated aspects of these tightly related fields. As a result, contemporary scientists have had a difficult time finding sources, even in review articles, that provide an integrated picture. This updated volume, by assembling chapters that demonstrate the relationship between these historically separated fields, overcome that difficulty. There are 56 chapters which cover a broad spectrum of topics on immunology of the nervous system. Included are diseases that result from immunological dysfunction, current therapeutic approaches, and prospects for the future. Overall, it integrates cutting-edge neuroscience, immunology, pharmacology, neurogenetics, neurogenesis, gene therapy, adjuvant therapy, nanomedicine, pharmacogenetics, biomarkers, proteomics, and magnetic resonance imaging. It is a rich harvest and readers will gain a perspective that has not previously been so readily available. Exposure to such a wealth of ideas is bound to inspire readers to undertake new and productive research initiatives.

The modern era of research into neuroinflammation and its relationship to neurodegenerative diseases began in the 1960s with the elaboration by Ralph van Furth of the monocyte phagocytic system. He injected labeled monocytes into animals and followed their migration and maturation into resident phagocytes in all body tissues. This provided a closure between Metchnikoff's 1882 discovery of mesodermal attack cells in starfish larvae, which he named phagocytes, and del Rio Hortega's 1919 discovery of phagocytic mesodermal cells entering the brain, which he named microglia. Hortega's results had always been questioned, and for more than two further decades, the controversy continued as to whether microglia were truly phagocytes of mesodermal origin or were merely typical brain cells of epidermal origin. Resolving the controversy required development of the techniques of immunohistochemistry and monoclonal antibody production. These tools for exploring brain biochemistry at the cellular level opened new vistas for understanding brain functioning and the pathogenesis of human disease. Using these tools, our laboratory and that of Joseph Rogers in Sun City at that time demonstrated that HLA-DR was strongly expressed on activated microglia. The identification of HLA-DR, a well-known leukocyte marker displayed by antigen-presenting cells, on these cells vindicated both Hortega and van Furth. The way was paved for many productive investigations exploring the properties of microglial cells and their relationship to inflammation and immune responses. This example of a conjunction between a fundamental concept and technical advances to establish its validity has been repeated many times since, as the chapters in this volume illustrate.

For a time, the concept that the brain is immunologically privileged held sway among neuroscientists. This was based on a narrow view that only the invasion of brain by lymphocytes could be taken as evidence of an inflammatory response. But immunohistochemistry, coupled with newly developed molecular biological techniques, revealed that a spectrum of inflammatory mediators, including many known to cause tissue damage, was produced within the brain by resident brain cells. These discoveries required entirely new interpretations as to the nature of neuroinflammation and its relationship to immune responses. The innate immune system, operating at the local level in brain, has clearly proved to be the first line of defense. Indeed, the basic discoveries from studying the response of the brain in a variety of neurological diseases are causing a reevaluation of a number of peripheral degenerative disorders where innate immune responses, which had previously been ignored, have been shown to play a critical role in their pathogenesis. In other words, those studying the brain are providing immunologists with revolutionary new concepts regarding classical peripheral diseases. The insights of this volume need to be interpreted in this broader context.

Major neurologic disorders and details of their pathobiology are presented as individual chapters. They involve disorders where innate immune responses predominate, as in Alzheimer's disease, to others such as multiple sclerosis, where adaptive immune responses predominate, and others which seem to involve both. We have suggested that diseases involving self-damage generated by innate immune responses be defined as autotoxic to differentiate them from classical autoimmune diseases where self-damage is generated by adaptive immune responses. The common theme, however, is the involvement of microglia as the effector cells.

Genetics is well covered. It is a rapidly moving field. The methodology for linking familial disease to DNA mutations commenced in the late 1970s through identification of restriction fragment-linked polymorphisms. By 1983, when James Gusella and his colleagues demonstrated a linkage of the G8 fragment to Huntington disease, only about 18 markers were known. Now over 1,500,000 single-nucleotide polymorphisms have been localized so that every centimorgan of the human genome can be explored. This advance has been coupled with rapid methods for sequencing DNA. The report on genetics must be regarded as the tiny tip of a giant iceberg where much below the surface will soon be revealed.

The ultimate objective of neuroscientists studying human disease is to find more effective treatments. Part 3 covers the pharmacology of existing drugs, as well as describing approaches now in clinical evaluation, and those still at the bench level. Some of these include concepts that depart from established therapeutic approaches, giving the reader much food for thought.

There is an important chapter on the new field of biomarkers. Biomarkers have established that neurodegenerative disorders such as Alzheimer's disease commence at least a decade before clinical signs develop. A window of opportunity is opened up where early anti-inflammatory intervention can arrest disease progression and abort disease development. This can be combined with brain imaging to measure objectively the effects of therapeutic agents in diseases where progressive brain degeneration occurs.

In summary, this is a volume not to be put on the shelf as a reference text, but to be read cover to cover by aspiring neuroscientists.

Vancouver, BC Patrick L. McGeer

# Introducing Neuroimmune Pharmacology

Neuroscience, immunology, and pharmacology are each of and by themselves broad disciplines that, without argument, impact upon a large component of what we come to know as biomedical science (Elenkov et al. 2000; Gendelman 2002; Holzer et al. 2015; McGeer and McGeer 2004). Each, by themselves and even more so when put together, is multidisciplinary and require, for the student, both a broad knowledge and deep understanding of molecular and cellular biology, neuroimmunity, the functional blood–brain barrier, neurochemistry, neuroinfectious and neurodegenerative disorders, cancer, and neurodevelopment. For many, it is considered a branch of immunology, but that is a start point as the field bridges investigations of drug action and development with nervous system biology and disease pathogenesis. To those engaged in this field, linking of the disciplines is ever more challenging as when they are joined, they come interactive. The bridges between disciplines are what we now call multidisciplinary science and require another level of insight. However, we posit that each need be first understood as a single entity. To this end, we forged chapters that cover the structure, function, and biology of each of the fields independent of one another. Then step-by-step they are each combined one with the other to form the basis of our engagement with the environment, to disease and a means to restore homeostasis by protective immune and pharmacologic means. Indeed, drugs that include immune modulators can certainly influence organ function, aging, and tissue homeostasis and improve clinical outcomes.

Indeed, a special feature of "humankind" among other species is the presence of an extraordinary complex immune system that can be used to protect against a plethora of harmful microbial pathogens, including viruses, bacteria, and parasites, as well as abnormal cells and proteins (Petranyi 2002; Obermeier et al. 2016). This underlies the complexity of the human genome which encodes expansive immune-related genes not found in lower species (Hughes 2002). When the immune system is compromised, disease occurs and often does with ferocity; a wide range of clinical manifestations ensue that follows as a consequence of neurodegenerative, psychiatric, cancer, and infectious diseases, or those elicited by the immune system's attack on itself. The latter is commonly referred to as "destructive" autoimmunity (Christen and von Herrath 2004). Interestingly enough, the immune system may sometimes be an impediment to therapy. Indeed, modulating its function is required for long-term and successful organ transplantation (Samaniego et al. 2006; Horst et al. 2016). On balance, modulation of the immune system can affect "neuroprotective" responses for certain diseases (Anderson et al. 2014).

Like the immune system, the nervous system contains surveillance functions and also possesses a number of functional roles that include mentation, movement, reasoning, sensation, vision, hearing, learning, breathing, and most behaviors. The nervous system includes defined tissue structures such as the brain, spinal cord, and peripheral nerves. On the cellular level, it includes networks of nerve cells with a variety of functional activities; complex networks and communications; supportive and regulatory cells, called glia; and a protective barrier that precludes the entry of a variety of macromolecules, cells, and proteins. It also possesses connections throughout the body that permits it function. Neuroscience is the discipline used to explore each of the nervous system regions and cells that include their networks and modes of communication in health and disease. As humans, we have ~100 billion neurons that are each

functional units contained within the nervous system. Molecular and biochemical studies along with cell and animal systems were each used alone and together to explore and define neural biology. The task of neurosciences is to better understand the brain's function in the context of ontogeny, organism development, and aberrations during disease. Neuroscience is an interdisciplinary field and evolved as such during the past quarter-century. It includes neurobiology, neurochemistry, neurophysiology, mathematics, psychology, computer neuroscience, and learning and behavior. It also included the field of immunology (Sehgal and Berger 2000). In a historical context, the brain, for a long time, was considered an immune-privileged organ, meaning that its protective shield or barrier, commonly termed the blood–brain barrier, served to protect, defend, and as a consequence exclude ingress of toxins, cells, and pathogens (Streilein 1993; Becher et al. 2000). This has undergone a reassessment of purpose (Louveau 2015). Nonetheless, this is balanced by the fact that resident inflammatory cells do exist inside the brain and are capable of producing robust immune responses. In recent years, we have come to accept that it is the mononuclear phagocytes (MP; perivascular macrophage and microglia) that are disease perpetrators, while the astrocyte serves as "supportive" and "homeostatic" in nurturing neurons and in protection against the ravages of disease (Gendelman 2002; Simard and Nedergaard 2004; Trendelenburg and Dirnagl 2005). The neuron in this neuroimmune model is the passive recipient of the battles that rage between the MP and the astrocyte. Findings that have emerged over the past half-decade have challenged this model. We now know that dependent upon environmental cues and disease, microglia, astrocytes, and other neural cell elements including endothelial cells and oligodendrocytes possess immunoregulatory functions. We also know that microglia and astrocytes dependent upon the environment and stimuli can be supportive, destructive, or both. Even more importantly, neurons can secrete immunoregulatory factors and engage directly into cell–cell–environmental cue stimulations. To make the system perhaps even more complex, local neuroimmune processes can result in the recruitment of T cells and enticement of the adaptive immune response, significantly affecting disease outcomes (Olson and Gendelman 2016). All in all, things appear more complex than once thought even in the past decade.

These three disciplines and the complexities inherent in each academic field is perhaps the most multidisciplinary, serving to bring scientists and clinicians together with knowledge of neurobiology, immunology, pharmacology, biochemistry, cellular and molecular biology, virology, genetics, gene therapy, medicinal chemistry, nanomedicine, proteomics, pathology, and physiology. Even more than immunology and neuroscience, pharmacology integrates a broad knowledge in scientific disciplines, enabling the pharmacologist a unique perspective to tackle drug-, hormone-, immune-, and chemical-related pathways as they affect human health and behavior. Drug actions and therapeutic developments form the basis of such discoveries, but the central understanding of how they act provides vision for further research to improve human well-being and health.

This textbook is unique in scope by serving to investigate the intersection of this new discipline. Neuropharmacologists study drug actions including neurochemical disorders underlying a broad range of diseases such as psychiatric disorders (e.g., schizophrenia and depression) and neurodegenerative diseases (e.g., Alzheimer's and Parkinson's diseases). Drugs can also be used to examine neurophysiological or neurobiochemical changes as they affect brain, behavior, movement, and mental status. Immunopharmacology seeks to control the immune response in the treatment and prevention of disease. Research does include immunosuppressant agents used in organ transplant as well as developing agents that affect bone marrow function and cell differentiation in cancer therapies.

What then defines the field of *neuroimmune pharmacology*? Is it simply a field that intersects the three disciplines of neuroscience, immunology, and pharmacology in seeking to better define the epidemiology, prevention, and treatment of immune disorders of the nervous systems (Fig. 1)? Are these disorders limited in their scope in affecting behavior, cognition, motor, and sensory symptoms, or do they also involve developmental and degenerative disorders? It is clear that the immune system is linked, in whole or in part, to diseases that develop

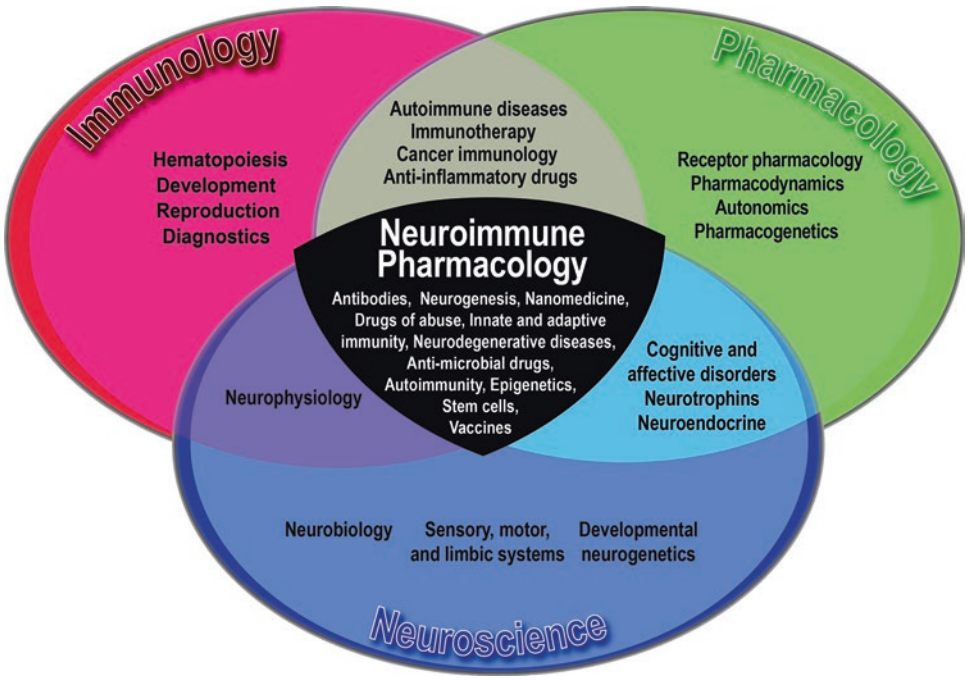



**Fig. 1** This Venn diagram pictorially represents the fields of immunology, pharmacology and neuroscience with common elements overlapping into the discipline of neuroimmune pharmacology. The blackened area depicts the diseases and research areas covered in this book and integral to the field of study

as a consequence of genetic abnormalities and a broad range of environmental cues (including microbial infections and abused drugs) and to toxins. So, where does neuroimmune pharmacology find its niche? These can occur, in part, as a consequence of neuropeptides, neurotransmitters, cytokines, chemokines, and abused drugs. Like much in science, we are left with more questions than answers. In the end, we seek avenues for translational research and better understanding of disease mechanisms. Diseases are together linked to microbial agents, by inflammatory processes, by emergence of cancerous cells or tumors, by stress, by environmental cues, and by genetic disturbances. No matter the cause, harnessing the immune processes for pharmacological benefit will, at days end, provide "real" solutions to positively affect some of the most significant and feared disorders of our century.

What do we seek to accomplish by editing such a textbook? First, we would be remiss in not acknowledging the pivotal discoveries made by others when research fields intersect. These include the discovery and characterization of the guanosine triphosphate (GTP) binding and prion proteins (Gilman 1995; Rodbell 1995; Prusiner 1998, 2001), neurotransmission and memory functions (Carlsson 2001; Greengard 2001; Kandel 2001), and odorant receptors (Buck 2000; Axel 2005). We posit that new discoveries can and will be made through the intersections of neuroscience, immunology, and pharmacology and as such sought to define it for the student. The notion that inflammation contributes in significant manner to neurodegeneration and significantly beyond autoimmune diseases is brought front and center and demonstrated without ambiguity for multiple sclerosis, peripheral neuropathies, Alzheimer's and Parkinson's disease, and amyotrophic lateral sclerosis as well as for microglial infections of the nervous system including NeuroAIDS where microglial activation is central to disease processes (Hooten et al. 2015; Appel et al. 1995; Toyka and Gold 2003; McGeer and McGeer 2004; Ercolini and Miller 2005; Gendelman 2002; Gendelman and Mosley 2015). Perhaps most importantly, we have laid the groundwork for how the immune system can be harnessed either through its modulation, through altering blood–brain barrier integrity and function, or by drug-delivery strategies that target the brain. This second edition will add on to the success

of the first edition with additional chapters on emerging topics, including but not limited to enteric nervous system, microbiota, innate immunity signaling, exosomes, stress granules, microRNA, autism spectrum disorders, traumatic brain injury, biomarkers, macromolecular therapeutics, and "omics" pharmacology.

No doubt this textbook is an expansive read for the student and scholar alike. To this end, we are humbled by its realization and even more so during this second edition. These words lay only the beginnings to what we believe will be a significant future footprint into the integration between neuroscience, immunology, and pharmacology.

Tsuneya Ikezu
Department of Pharmacology and Experimental
Therapeutics and Neurology
Boston University School of Medicine
Boston, MA, USA

Howard E. Gendelman
Department of Pharmacology and Experimental Neuroscience
University of Nebraska Medical Center
Nebraska Medical Center
Omaha, NE, USA

## References

Anderson KM, Olson KE, Estes KA, Flanagan K, Gendelman HE, Mosley RL (2014) Dual destructive and protective roles of adaptive immunity in neurodegenerative disorders. Transl Neurodegener 3(1):25

Appel SH, Smith RG, Alexianu M, Siklos L, Engelhardt J, Colom LV, Stefani E (1995) Increased intracellular calcium triggered by immune mechanisms in amyotrophic lateral sclerosis. Clin Neurosci 3:368–374

Axel R (2005) Scents and sensibility: a molecular logic of olfactory perception (Nobel lecture). Angew Chem Int Ed Engl 44:6110–6127

Becher B, Prat A, Antel JP (2000) Brain-immune connection: immuno-regulatory properties of CNS-resident cells. Glia 29:293–304

Buck LB (2000) The molecular architecture of odor and pheromone sensing in mammals. Cell 100:611–618

Carlsson A (2001) A half-century of neurotransmitter research: impact on neurology and psychiatry. Nobel lecture. Biosci Rep 21:691–710

Christen U, von Herrath MG (2004) Initiation of autoimmunity. Curr Opin Immunol 16:759–767

Elenkov IJ, Wilder RL, Chrousos GP, Vizi ES (2000) The sympathetic nerve—an integrative interface between two supersystems: the brain and the immune system. Pharmacol Rev 52:595–638

Ercolini AM, Miller SD (2005) Role of immunologic cross-reactivity in neurological diseases. Neurol Res 27:726–733

Gendelman HE (2002) Neural immunity: friend or foe? J Neurovirol 8:474–479

Gendelman HE, Persidsky Y (2005) Infections of the nervous system. Lancet Neurol 4:12–13

Gendelman HE, Mosley RL (2015) A perspective on roles played by innate and adaptive immunity in the pathobiology of neurodegenerative disorders. J Neuroimmune Pharmacol 10(4):645–650

Gilman AG (1995) Nobel lecture. G proteins and regulation of adenylyl cyclase. Biosci Rep 15:65–97

Greengard P (2001) The neurobiology of dopamine signaling. Biosci Rep 21:247–269

Holzer P, Hassan AM, Jain P, Reichmann F, Farzi A (2015) Neuroimmune pharmacological approaches. Curr Opin Pharmacol 25:13–22

Hooten KG, Beers DR, Zhao W, Appel SH (2015) Protective and toxic neuroinflammation in amyotrophic lateral sclerosis. Neurotherapeutics 12(2):364–375

Horst AK, Neumann K, Diehl L, Tiegs G (2016) Modulation of liver tolerance by conventional and nonconventional antigen-presenting cells and regulatory immune cells. Cell Mol Immunol 13(3):277–292

Hughes AL (2002) Natural selection and the diversification of vertebrate immune effectors. Immunol Rev 190:161–168

Kandel ER (2001) The molecular biology of memory storage: a dialogue between genes and synapses. Science 294:1030–1038

Louveau A, Smirnov I, Keyes TJ, Eccles JD, Rouhani SJ, Peske JD, Derecki NC, Castle D, Mandell JW, Lee KS, Harris TH, Kipnis J (2015) Structural and functional features of central nervous system lymphatic vessels. Nature 523(7560):337–341

McGeer PL, McGeer EG (2004) Inflammation and the degenerative diseases of aging. Ann N Y Acad Sci 1035:104–116
Obermeier B, Verma A, Ransohoff RM (2016) The blood–brain barrier. Handb Clin Neurol 133:39–59
Olson KE, Gendelman HE (2016) Immunomodulation as a neuroprotective and therapeutic strategy for Parkinson's disease. Curr Opin Pharmacol 26:87–95
Petranyi GG (2002) The complexity of immune and alloimmune response. Transpl Immunol 10:91–100
Prusiner SB (1998) Prions. Proc Natl Acad Sci U S A 95:13363–13383
Prusiner SB (2001) Shattuck lecture—neurodegenerative diseases and prions. N Engl J Med 344:1516–1526
Rodbell M (1995) Nobel lecture. Signal transduction: evolution of an idea. Biosci Rep 15:117–133
Samaniego M, Becker BN, Djamali A (2006) Drug insight: maintenance immunosuppression in kidney transplant recipients. Nat Clin Pract Nephrol 2:688–699
Sehgal A, Berger MS (2000) Basic concepts of immunology and neuroimmunology. Neurosurg Focus 9:e1
Simard M, Nedergaard M (2004) The neurobiology of glia in the context of water and ion homeostasis. Neuroscience 129:877–896
Streilein JW (1993) Immune privilege as the result of local tissue barriers and immunosuppressive microenvironments. Curr Opin Immunol 5:428–432
Toyka KV, Gold R (2003) The pathogenesis of CIDP: rationale for treatment with immunomodulatory agents. Neurology 60:S2–S7
Trendelenburg G, Dirnagl U (2005) Neuroprotective role of astrocytes in cerebral ischemia: focus on ischemic preconditioning. Glia 50:307–320

# Acknowledgments

These comprehensive volumes would not have been possible without the tireless, dedicated, and determined effort of one very special person, Reed Felderman. Reed is skillful, resourceful, and enthusiastic. Any task offered was never too minor and never left unattended. The linkages between editors, authors, administrators, and publishers flowed seamlessly. He quickly rose to the task of managing editor with aplomb, and it was his attention to detail that was singularly responsible for the success of this text.

Gregory Baer and his staff at Springer USA are already trusted friends, colleagues, and publishing visionaries. Their leadership together with Ms. Robin Taylor and our administrative staff at our own University of Nebraska Medical Center and Boston University School of Medicine has and continues to be a foundation for all we've done and for this textbook, for our *Journal of Neuroimmune Pharmacology* and beyond. We are lucky to have them as partners in this endeavor and so appreciate being able to continue to work with such talented individuals who are so dedicated to the pursuit of excellence.

To our leaders and visionaries, Jeffrey Gold, Harold Maurer, Donald Leuenberger, Dele Davies, and Deb Thomas, a simple thank you is clearly inadequate for all you have done to ensure the success of our mission and so many of our endeavors. To Carol Swarts, a friend, a colleague, a confidant, and a soul mate whose gifts of time and person ensure our sustained academic successes: Carol, you have no parallel. To Susan Leeman, the pioneer of molecular biology in neuroinflammation, our mentor and a friend who always navigate our neuroinflammation research. Your contribution to science is immeasurable. Thank you all for keeping the fire burning very bright for all we do. To Harriet Singer, Frani Blumkin and Jim Roubal who have been steadfast in support for so many years. Your faith in our research and unquestioned faith strengthens us each and every day.

To our chapter contributors and reviewers whose expertise, knowledge, intellect, and dedications proved invaluable in completing the tasks.

To Seiko Ikezu and Bonnie Bloch, our partners in life, life journeys, and always best friends.

To Yohei and Michiko Ikezu and Soffia Gendelman, our parents, navigators, and role models.

To Yumiko Aoyama, for her continuous support of family and friendship, a simple thank you is nearly inadequate.

To our children and grandchildren who are all our life treasures: Clark and David Ikezu and Sierra, Jason and Sacha Tobias and Adam and Jen Wolf-Gendelman and Lesley Gendelman and Emma Ehrenkranz.

We salute *the Journal of Neuroimmune Pharmacology* that provided and continues to be the source of inspiration for this work. To a special friend, Joel Alperson, for the gift of his ears and to our patients, students, mentors, and colleagues who inspire and guide us always, thank you all so much.

Tsuneya Ikezu
Howard E. Gendelman

# Contents

# Abbreviations

| | |
|---|---|
| $^{1}$H | Proton |
| $^{1}$H-MRSI | Proton magnetic resonance spectroscopic imaging |
| 2D SDS-PAGE | Two-dimensional polyacrylamide gel electrophoresis |
| 3'UTR | 3'-Untranslated region |
| 3HK | 3-Hydroxykynurenine |
| 5-ASA | 5-Aminosalicylic acid |
| 5-HIAA | 5-Hydroxyindole acetic acid |
| 5-HT | 5-Hydroxy tryptophan |
| 5-HT | 5-Hydroxytryptamine |
| 6-OHDA | 6-Hydroxydopamine |
| 8-OHdG | 8-Hydroxy-2'-deoxyguanosine |
| γc | Common γ-chain |
| AAAD | Aromatic L-amino acid decarboxylase |
| AAV | Adeno-associated virus |
| Ab | Antibody |
| Aβ | Amyloid-β |
| ABD | Adamantiades-Behçet's disease |
| ABP | Actin-binding protein |
| AC | Anterior chamber |
| ACAID | Anterior chamber-associated immune deviation |
| αCamKII | α-Calcium/calmodulin-dependent protein kinase II |
| ACh | Acetylcholine |
| AChE | Acetylcholinesterase |
| ACTH | Adrenocorticotrophic hormone (pro-opiomelanocortin; POMC) |
| AD | Alzheimer's disease |
| ADHD | Attention deficit hypersensitivity disorder |
| ADI | Acceleration/deceleration injury |
| ADAM | A disintegrin and metalloprotease |
| ADCC | Antibody-dependent cellular cytotoxicity |
| ADEM | Acute disseminated encephalomyelitis |
| AF | Activation factor |
| Ag | Antigens |
| AGE | Advanced glycation end products |
| AGM | Aorta-gonad mesonephros |
| AHSCT | Autologous hematopoietic stem cell transplantation |
| AICA | Anterior inferior cerebellar artery |
| AID | Activation-induced (cytidine) deaminase |
| AIDP | Acute inflammatory demyelinating polyradiculoneuropathy |
| AIDS | Acquired immunodeficiency syndrome |
| AIF | Apoptosis inducing factor |
| AIR | Autoimmune retinopathy |
| AIRE | Autoimmune regulator |

| | |
|---|---|
| AIS | Anterior chamber-associated immune deviation (ACAID)-inducing signal |
| ALR | AIM2-like receptors |
| ALS | Amyotrophic lateral sclerosis |
| AMAN | Acute motor axonal neuropathy |
| AML | Acute myeloid leukemia |
| AMN | Adrenomyeloneuropathy |
| AMPA | α-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid |
| AMSAN | Acute motor-sensory axonal neuropathy |
| ANG | Angiotensin II |
| ANI | Asymptomatic neurocognitive impairment |
| ANS | Autonomic nervous system |
| AP | Area postrema |
| AP-1 | Activating protein-1 |
| APAF 1 | Apoptotic peptidase activating factor 1 |
| APC | Antigen-presenting cells |
| aPKC | Atypical protein kinase C |
| aPL | Antiphospholipid antibody |
| APO1 | Apoptosis antigen 1 (Fas/CD95) |
| apoE | Apolipoprotein E |
| APP | Amyloid precursor protein |
| APPs | Acute phase proteins |
| AQP4 | Aquaporin-4 |
| ARE | AU-rich response element |
| ARMD | Age-related macular degeneration |
| ART | Antiretroviral therapy |
| ASD | Autism spectrum disorders |
| ASL | Arterial spin-labeled |
| ASTIN | Acute stroke therapy by inhibition of neutrophils |
| AT | Adoptive transfer |
| ATL | Adult T cell leukemia |
| ATON | Atacicept in optic neuritis |
| ATP | Adenosine triphosphate |
| AVE | Anterior visceral endoderm |
| AVG | Anti-viral granule |
| AVP | Arginine vasopressin |
| AV3V | Anteroventral third ventricular |
| AZT | 3'-Azidothymidine/zidovudine |
| BACE | Beta-site amyloid precursor protein cleaving enzyme |
| BBB | Blood–brain barrier |
| BBMEC | Bovine brain microvessel endothelial cells |
| BCR | B-cell receptor |
| BCRP | Breast cancer resistance protein |
| BCSFB | Blood-CSF barrier |
| BD | Bipolar disorder |
| BDNF | Brain-derived neurotrophic factor |
| bFGF | Basic fibroblast growth factor (FGF2) |
| bHLH | Basic helix-loop-helix |
| BHV-1 | Bovine herpesvirus-1 |
| BLBP | Brain lipid binding protein |
| BLIMP-1 | B lymphocyte-induced maturation protein-1 |
| BLV | Bovine leukemia virus |
| BM | Bone marrow |
| BMDM | Bone marrow-derived macrophages |

| | |
|---|---|
| BMP | Bone morphogenetic protein |
| BMVEC | Brain microvascular endothelial cell |
| BP | Biological processes |
| BrdU | Bromodeoxyuridine |
| BRMs | Biological response modifiers |
| BSE | Bovine spongiform encephalopathy |
| BTLA | B and T lymphocyte attenuator |
| C/EBP | CCAAT box/enhancer binding protein |
| C3d | Complement C3 fragment d |
| C4d | Complement C4 fragment d |
| CA | Cornu ammonis |
| CA II | Carbonic anhydrase II |
| CaMKII | Calcium/calmodulin-dependent protein kinase II |
| CAMs | Cell adhesion molecules |
| CAPS | Cryopyrin-associated periodic syndromes |
| CAR | Cancer-associated retinopathy |
| CARD | Caspase recruitment domain |
| CARD15 | NOD2/caspase recruitment domain 15 |
| CB | Cannabinoid |
| CB1 | Cannabinoid receptor 1 |
| CB2 | Cannabinoid receptor 2 |
| CBA | Cytokine bead arrays |
| CBF | Cerebral blood flow |
| CBF1 | C promoter binding factor 1 |
| CBP | cAMP-response element binding protein (CREB)-binding protein |
| CBV | Cerebral blood volume |
| CCI | Controlled cortical impact |
| CCK | Cholecystokinin |
| CCT | Central corneal thickness |
| CD | Cluster of differentiation |
| CD11b | Complement component 3 receptor 3 subunit (integrin alpha M; ITGAM) |
| CD40L | CD40 ligand (TNFSF5) |
| CDP | Common DC progenitor |
| CDR | Complementarity-determining region |
| CDV | Canine distemper virus |
| CEP | Carboxyethylpyrrole |
| CFT | 2-β-Carbomethoxy-3β-(4-fluorophenyl) tropane |
| cGMP | Cyclic guanosine 5’-monophosphate |
| CGRP | Calcitonin gene-related peptide |
| CHAT | Choline acetyltransferase |
| CHN | Congenital hypomyelinating neuropathy |
| Cho | Choline |
| CIDP | Chronic inflammatory demyelinating polyradiculoneuropathy |
| CINC1 | Cytokine-induced neutrophil chemoattractant-1 |
| CIS | Clinically isolated syndrome |
| CJD | Creutzfeldt-Jakob disease |
| CLA | Cutaneous leukocyte antigen |
| CLL | Chronic lymphocytic leukemia |
| CLP | Common lymphoid progenitor |
| CMAP | Compound motor action potential |
| CMC | Critical micelle concentration |
| CMP | Common myeloid precursor |
| CMT | Charcot-Marie-Tooth (disease) |

| | |
|---|---|
| CMV | Cytomegalovirus |
| CNG | cGMP-gated |
| CNPase | Cyclic nucleotide 3' phospohydrolase |
| CNS | Central nervous system |
| CNTF | Ciliary neurotrophic factor |
| CO | Cytochrome oxidase |
| COMT | Catechol-*O*-methyltransferase |
| Con A | Concanavalin A |
| COP-1 | Copolymer-1 |
| COX | Cyclooxygenase (prostaglandin-endoperoxide synthase; PTGS) |
| CP | Choroid plexus |
| CR | Complement receptor |
| CRalBP | Cellular retinal binding protein (retinaldehyde binding protein 1; RLBP1) |
| CRD | Carbohydrate-recognition domains |
| CRE | cAMP-responsive element |
| Cre | Creatine |
| CREB | cAMP-response element binding protein |
| CRH | Corticotrophin-releasing hormone |
| CRID | Cytokine release inhibitory drugs |
| CRP | C-reactive protein |
| CRPM | Collapsing response mediator protein |
| CRVO | Central retinal vein occlusion |
| CSF | Cerebrospinal fluid |
| CSF-1R | Colony-stimulating factor receptor |
| CSPG | Chondroitin sulfate proteoglycans |
| CT | Computed tomography |
| CTA | Computed tomographic angiography |
| CTE | Chronic traumatic encephalopathy |
| cTEC | Cortical thymic epithelial cell (TEC) |
| CTL | Cytotoxic T lymphocyte |
| CTLA-4 | Cytotoxic T lymphocyte antigen-4 |
| CTP | Circulating T-cell progenitors |
| CVF | Cobra venom factor |
| CVO | Circumventricular organ |
| Cx | Connexin |
| cyto c | Cytochrome c |
| D1R | Type-1 family of dopamine receptors |
| D9-THC | D9-tetrahydrocannabinol |
| DA | Daniel's strain of Theiler's virus |
| DA | Dopamine |
| DAF | Decay-accelerating factor |
| DAG | Diacylglycerol |
| DAMP | Danger-associated molecular patterns |
| DAT | Dopamine transporter (solute carrier family 6A3; SLC6A3) |
| DβH | Dopamine-β-hydroxylase |
| DC | Dendritic cells |
| DCX | Doublecortin |
| ddC | Dideoxycytidine |
| ddI | Dideoxyinosine |
| DG | Dentate gyrus |
| dGTP | Deoxyguanosine triphosphate |
| DHA | Docosahexaenoic acid |
| Dhh | Desert hedgehog |

| | |
|---|---|
| DHP | Dihydropyridine |
| DIGE | Difference gel electrophoresis |
| DIRA | Deficiency of IL-1 receptor antagonist |
| DISC | Death-inducing signaling complex |
| DM | Diabetes mellitus |
| DN | Double/dominant negative |
| DNA | Deoxyribonucleic acids |
| Doc2 | Double C2 protein |
| DOR | Delta-opioid receptor |
| Dox | Doxorubicin |
| Dox | Doxycycline |
| DR | Dopamine receptors |
| DRPLA | Dentatorubral-pallidoluysian atrophy |
| DSI | Depolarization induced suppression of inhibition |
| DSPN | Distal sensory peripheral neuropathy |
| DSS | Dejerine-Sottas syndrome |
| DTH | Delayed-type hypersensitivity |
| DTI | Diffusion tensor imaging |
| DTR | Diphtheria toxin receptor |
| DWI | Diffusion weighted imaging |
| E2F | Early-region-2 transcription factor |
| EAE | Experimental allergic/autoimmune encephalomyelitis |
| EAN | Experimental allergic/autoimmune neuritis |
| EAU | Experimental autoimmune uveitis |
| EBV | Epstein-Barr virus |
| ECT | Electroconvulsive therapy |
| EDSS | Expanded disability status scale |
| EEG | Electroencephalography |
| EGC | Embryonic germ cell |
| EGF | Epidermal growth factor |
| EGFR | Epidermal growth factor receptor |
| EIAV | Equine infectious anemia virus |
| ELAVL4 | Embryonic lethal, abnormal vision, Drosophila-like 4 |
| ELP | Early lymphoid progenitors |
| eLTP | Early long-term potentiation |
| ELVIS | Extravasation through Leaky Vasculature and subsequent Inflammatory cell-mediated Sequestration |
| EMG | Electromyography |
| EMP | Erythromyeloid progenitors |
| EndoG | Endonuclease G |
| EOAD | Early-onset Alzheimer's disease |
| EPI | Echo-planar imaging |
| EPR | Enhanced permeability and retention |
| EPS | Extrapyramidal symptoms |
| EPSC | Excitatory postsynaptic currents |
| EPSP | Excitatory postsynaptic potential |
| ER | Endoplasmic reticulum |
| ERK2 | Extracellular signal-regulated kinase 2 |
| ESC | Embryonic stem cell |
| ESCRT | Endosomal-sorting complex required for transport |
| ESI-MS/MS | Electrospray ionization-mass spectrometry/mass spectrometry |
| ET | Endothelin |
| ETP | Early T lineage progenitor |

| | |
|---|---|
| EV | Extracellular vesicles |
| FA | Fractional anisotropy |
| FAD | Familial Alzheimer's disease |
| fALS | Familial amyotrophic lateral sclerosis |
| FasL | Fas ligand (FASLG) |
| FcgR-1 | Fc receptor IgG, high affinity-1 |
| fCJD | Familial Creutzfeldt-Jakob disease |
| FcR | Fc receptor |
| FDA | Food and Drug Administration |
| FDC | Follicular dendritic cell |
| FDG | Fluorodeoxyglucose |
| FDOPA | 6-[(18)F]fluoro-L-dopa |
| FFI | Fatal familial insomnia |
| FGF | Fibroblast growth factor |
| FID | Free induction decay |
| FIRE | Febrile infection-related epilepsy syndrome |
| FIV | Feline immunodeficiency virus |
| FKN | Fractalkine (CX3CL1) |
| fMRI | Functional magnetic resonance imaging |
| Foxp3 | Forkhead box P3 transcription factor |
| FPI | Fluid percussion injury |
| FR | Folate receptor |
| FRC | Fibroblastic reticular cell |
| FRS2 | Fibroblast growth factor receptor substrate 2 |
| FS | Fisher syndrome |
| FSH | Follicle-stimulating hormone |
| FTD | Frontotemporal dementia |
| FT-ICR | Fourier transformed ion cyclotron resonance mass spectrometry |
| FTLD | Frontotemporal lobar dementia |
| FUS | Fused in sarcoma |
| Fz/PCP | Frizzled/planar cell polarity |
| GA | Glatiramer acetate |
| GABA | Gamma-aminobutyric acid |
| GAD | Glutamate decarboxylase |
| GalC | Galactocerebroside |
| GALT | Gut-associated lymphoid tissue |
| GAP | GTPase activating protein |
| GAPDH | Glyceraldehyde 3 phosphate dehydrogenase |
| GBM | Glioblastoma multiforme |
| GBS | Guillain-Barré syndrome |
| GC | Germinal center |
| GCDC | Germinal center dendritic cell |
| GCL | Ganglion cell layer |
| GEF | Guanine nucleotide exchange factor |
| GL | Granular cell layer |
| GC-MS | Gas chromatography combined with mass spectrometry |
| G-CSF | Granulocyte colony-stimulating factor |
| Gd | Gadolinium |
| GDF | Growth and differentiation factor |
| GDNF | Glial-derived neurotrophic factor |
| GDP | Guanosine diphosphate |
| GEF | GDP-GTP exchange factor |
| GFAP | Glial fibrillary acidic protein |

| | |
|---|---|
| GFP | Green fluorescent protein |
| GI | Gastrointestinal |
| GKAP | Guanylate kinase-associated protein |
| GLC1A | Chromosome 1 open-angle glaucoma gene |
| Gln | Glutamine |
| GLP-1 | Glucagon-like peptide-1 |
| GM-CSF | Granulocyte-macrophage colony-stimulating factor |
| GMP | Granulocyte monocyte precursor |
| GnRH | Gonadotropin-releasing hormone |
| GO | Gene ontology |
| GO | Graves' ophthalmopathy |
| GPCR | G-protein-coupled receptor |
| GPI | Glycosylphosphatidylinositol |
| GR | Glucocorticoid receptor |
| GRIP | Glucocorticoid receptor-interacting protein |
| GRO-α | Growth-related oncogene alpha |
| GSH | Glutathione |
| GSS | Gerstmann-Straussler-Scheinker disease |
| GSTO1 | Glutathione *s*-transferase omega-1 |
| GT | G-protein transducing |
| GTP | Guanosine triphosphate |
| GUCY | Guanylate cyclase |
| GWAS | Meta-genome-wide association study |
| HAART | Highly active antiretroviral therapy |
| HAD | HIV-associated dementia |
| HAM/TSP | HTLV-I associated myelopathy/tropical spastic paraparesis |
| HAND | HIV-associated neurocognitive disorder |
| HAT | Histone acetyltransferase |
| HAV | Hepatitis A virus |
| HBsAg | Hepatitis B surface antigen |
| HCV | Hepatitis C virus |
| HCMV | Human cytomegalovirus |
| HD | Huntington's disease |
| HDAC | Histone deacetylase |
| HDLS | Hereditary diffuse leukoencephalopathy with spheroids |
| HES | Hairy and enhancer of split homolog |
| HEV | High endothelial venule |
| HFS | High frequency stimulation |
| Hh | Hedgehog |
| HHH | Hypervolemic-hemodilution and hypertensive |
| HHV-6 | Human herpes virus-6 |
| HIV-1 | Human immunodeficiency virus type 1 |
| HIVE | Human immunodeficiency virus encephalitis |
| HLA | Human leukocyte antigen |
| HLH | Helix-loop-helix |
| HMG-CoA | 3-Hydroxy-3-methylglutaryl coenzyme A |
| HNE | 4-Hydroxy-2-nonenal |
| hnRNP-A1 | Heterogeneous nuclear ribonuclear protein-A1 |
| HPA | Hypothalamic-pituitary-adrenal |
| HPMA | *N*-(2-Hydroxypropyl) methacrylamide |
| HRP | Horseradish peroxidase |
| HSC | Hematopoietic stem cell |
| HSC70 | Heat shock cognate protein 70 |

| | |
|---|---|
| Hsp | Heat shock protein |
| HSV | Herpes simplex virus |
| HSVE | Herpes simplex virus-mediated encephalitis |
| HT | Huntington's disease |
| HTLV | Human T-cell lymphotropic virus type |
| HTRA2 | High temperature requirement serine protease 2 |
| Htt | Huntingtin |
| HUVEC | Human umbilical vein endothelial cells |
| HveA | Herpesvirus entry mediator A |
| I-1 | Regulatory protein inhibitor-1 |
| IBS | Inflammatory bowel syndrome |
| ICAM | Intracellular adhesion molecule |
| ICAT | Isotope-coded affinity tags |
| ICE | IL-1β-converting enzyme |
| ICGA | Indocyanine green angiography |
| ICH | Intracerebral hemorrhage |
| iCJD | Iatrogenic Creutzfeldt-Jakob disease |
| ICOS | Inducible co-stimulatory molecule |
| ICV | Intra-cerebro-ventricular |
| Id | Inhibitor of differentiation |
| IDE | Insulin degrading enzyme |
| IDO | Indoleamine 2,3-dioxygenase |
| IE | Immediate early |
| IF | Intermediate filament |
| IFN | Interferon |
| Ig | Immunoglobulin |
| IGF | Insulin-like growth factor |
| IgG | Immunoglobulin G |
| IGIV | Immunoglobulin intravenous therapy |
| Ihh | Indian hedgehog |
| IIDD | Idiopathic inflammatory demyelinating disease |
| IkB | Inhibitory kappa B |
| IKK | IkB kinase |
| IL | Interleukin |
| IL1RA | IL-1 receptor antagonist |
| ILBD | Incidental Lewy body disease |
| ILK | Integrin-linked kinase |
| ILM | Inner limiting membrane |
| ILV | Intraluminal vesicles |
| IM | Intramuscular |
| IMAC | Immobilized metal affinity chromatography |
| IMPDH | Inosine monophosphate dehydrogenase |
| iNKR | Inhibitory natural killer cell receptor |
| INL | Inner nuclear layer |
| INO | Internuclear ophthalmoplegia |
| iNOS | Inducible nitric oxide synthase (NOS2A) |
| IOP | Intraocular pressure |
| IP | Interferon-inducible protein |
| IPC | Intermediate progenitor cells |
| InsP3R | Inositol 1,4,5-triphosphate receptor |
| IPL | Inner plexiform layer |
| IRAK | IL-1 receptor-associated protein kinase |
| IRBP | Interphotoreceptor retinoid-binding protein |

| | |
|---|---|
| IRF | Interferon regulatory factor |
| ISCOM | Immunostimulating complexes |
| ISH | In situ hybridization |
| IT15 | Interesting transcript 15 |
| ITAM | Immunoreceptor tyrosine-based activation motif |
| ITGAM | Integrin, alpha M |
| ITR | Inverted terminal repeat |
| IVDU | Intravenous drug use |
| IVIg | Intravenous immunoglobulin |
| JAK | Janus kinase |
| JEV | Japanese encephalitis virus |
| JNK | c-Jun N-terminal kinase |
| KLH | Keyhole limpet hemocyanin |
| KO | Knockout |
| KOR | Kappa opioid peptide receptor |
| KYN | Kynurenine |
| KYNA | Kynurenic acid |
| LAK | Lymphokine-activated killer (cell) |
| L-AP4 | L-2-Amino-4-phosphonobutyric acid |
| LAT | Latency-associated transcript |
| LAMP | Lysosome-associated membrane protein |
| LB | Lewy bodies |
| LC | Locus coeruleus |
| LCA | Leukocyte common antigen |
| LC-FTICR MS | Liquid chromatography Fourier transform ion cyclotron resonance mass |
| LC-MS | Liquid chromatography combined with mass spectrometry |
| LC-UV-SPE-NMR | Liquid chromatography, UV detection, solid phase extraction, and nuclear magnetic resonance |
| LD | Linkage disequilibrium |
| LDL | Low density lipoprotein |
| LFA-1 | Leukocyte function-associated antigen-1 (integrin beta 2; ITGB2) |
| LGN | Lateral geniculate nucleus |
| LH | Luteinizing hormone |
| LIF | Leukemia inhibitory factor |
| Lingo-1 | Leucine-rich repeat and Ig domain containing Nogo receptor-interacting protein-1 |
| LMN | Lower motor neuron |
| LOAD | Late-onset Alzheimer's disease |
| LOS | Lipooligosaccharide |
| LPA | Lysophosphatidic acid |
| LPBN | Lateral parabrachial nucleus of the pons |
| LPS | Lipopolysaccharide |
| LRP-1 | Lipoprotein receptor-related protein-1 |
| LRR | Leucine-rich repeat |
| LRRK2 | Leucine-rich repeat kinase 2 |
| LT | Lymphotoxins |
| LTD | Long-term depression |
| LTNP | Long-term nonprogressors |
| LTP | Long-term potentiation |
| LTR | Long-terminal repeat |
| LT-bR | Lymphotoxin beta receptor |
| MCAO | Middle cerebral arterial occlusion |
| MCI | Mild cognitive impairment |

| | |
|---|---|
| MG | Myasthenia gravis |
| MHV | Mouse hepatitis virus |
| mI | Myoinositol |
| MIF | Migration inhibitory factor |
| Mint1 | Munc-18 interacting protein 1 |
| MIP | Macrophage inflammatory protein |
| MJO | Machado-Joseph disease |
| MME | Membrane metalloendopeptidase (neprilysin) |
| MMP | Matrix metalloproteinase |
| MMSE | Mini-Mental State Examination |
| MNGC | Multinucleated giant cell |
| MnPO | Median preoptic nucleus |
| Mn-SOD | Manganese superoxide dismutase |
| MOAT | Multispecific organic anion transporter |
| MOBP | Myelin-associated/oligodendrocyte basic protein |
| MOG | Myelin oligodendrocyte glycoprotein |
| MOI | Multiplicity of infection |
| MOR | mu opioid receptor |
| MOSP | Myelin/oligodendrocyte-specific protein |
| MP | Mononuclear phagocytes |
| MPA | Mycophenolic acid |
| MPL | Monophosphoryl lipid A |
| MPO | Myeloperoxidase |
| MPO | Medial preoptic area |
| MAC | Membrane attack complex |
| MPP | Multipotent progenitors |
| MPP+ | 1-Methyl-4-phenylpyridinium |
| MAdCAM-1 | Mucosal addressin cell adhesion molecule-1 |
| MAG | Myelin-associated glycoprotein |
| MAML | Mammalian mastermind-like |
| MAO | Monoamino-oxidase |
| MAP | Microtubule-associated protein |
| MAPK | Mitogen-activated protein kinases |
| Mash1 | Mammalian achaete-scute homologue 1 |
| MBGI | Myelin-based growth inhibitor |
| MBP | Myelin basic protein |
| MC | Mast cell |
| MC-1R | Melanocortin-1 receptor |
| MCMD | Minor cognitive motor disorder |
| MCP | Membrane cofactor protein (CD46) |
| MCP-1 | Monocyte chemoattractant protein-1 (CCL2) |
| M-CSF | Macrophage colony-stimulating factor (CSF1) |
| MD | Major depression |
| MDA | Malondialdehyde |
| MDD | Major depressive disorder |
| MDM | Monocyte-derived macrophages |
| MDP | Muramyl-dipeptide |
| MDP | Monocytes and dendritic cell progenitor |
| MDR | Multidrug resistant |
| MDSC | Myeloid-derived suppressor cells |
| ME | Median eminence |
| MEG | Magnetoencephalography |
| MEPP | Miniature end-plate potential |

| | |
|---|---|
| MFS | Miller Fisher syndrome |
| MHC | Major histocompatibility complex |
| MHC-II | Class II major histocompatibility complex |
| MHPG | Methoxy-hydroxy-phenylethanolamine |
| MLR | Mixed lymphocyte reaction |
| MLV | Murine leukemia virus |
| MND | Mild neurocognitive disorder |
| MP | Mononuclear phagocyte |
| MPMV | Mason-Pfizer monkey virus |
| MPTP | 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine |
| MR | Mineralocorticoid receptors |
| MRA | Magnetic resonance angiography |
| MRI | Magnetic resonance imaging |
| MRP | Multidrug resistance protein |
| MRS | Magnetic resonance spectroscopy |
| MRSI | Magnetic resonance spectroscopic imaging |
| MS | Multiple sclerosis |
| MSA | Multisystem atrophy |
| MSCs | Myelinating Schwann cells |
| MSH | Melanocyte-stimulating hormone |
| MSN | Medium spiny neuron |
| mSOD1 | Mutant $Cu^{2+}/Zn^{2+}$ superoxide dismutase 1 |
| MSRV | Multiple sclerosis retrovirus |
| MT | Magnetization transfer |
| Mtb | Mycobacterium tuberculosis |
| MTI | Magnetization transfer imaging |
| mTEC | Medullary thymic epithelial cell |
| mTOR | Mammalian target of rapamycin |
| MTR | Magnetization transfer ratio |
| MUC1 | Mucin type 1 glycoprotein |
| MuLV | Murine leukemia virus |
| Munc-18 | Mammalian homologue of unc-18 |
| MV | Microvesicles |
| MVB | Multivesicular bodies |
| MVE | Murray Valley encephalitis virus |
| MW | Molecular weight |
| MZ | Marginal zone |
| NAA | *N*-Acetyl-aspartate |
| NAC | *N*-Acetyl cysteine |
| NADPH | Nicotinamide adenine dinucleotide phosphate |
| NB | Nucleotide-binding domain |
| NCAM | Neural cell adhesion molecule |
| NCC | Neural crest cell |
| NE | Norepinephrine |
| NEP | Neutral endopeptidase metalloendopeptidase |
| NET | Norepinephrine transporter |
| NeuN | Neuronal nuclei |
| NF | Neurofilament |
| NF-kB | Nuclear factor-k-B |
| NFAT | Nuclear factor of activated T lymphocytes |
| NFL | Nerve fiber layer |
| NFT | Neurofibrillary tangles |
| Ng-CAM | Neuronal-glial cell adhesion molecule (L1/NILE) |

| | |
|---|---|
| NGF | Nerve growth factor |
| NgR | Nogo-66 receptor |
| NICD | Notch intracellular domain |
| NK | Natural killer (cells) |
| NKT | Natural killer T (cells) |
| NLR | Nod-like receptors |
| NMDA | *N*-methyl-D-aspartate |
| NMDAR | *N*-methyl-D-aspartate receptors |
| NMJ | Neuromuscular junction |
| NMO | Neuromyelitis optica |
| NMR | Nuclear magnetic resonance |
| NMSCs | Nonmyelinating Schwann cells |
| nNOS | Neuronal nitric oxide synthase |
| NNRTIs | Nonnucleoside analogue reverse transcriptase inhibitors |
| NO | Nitric oxide |
| NOD | Nucleotide oligomerization domain |
| NOD | Non-obese diabetic mice |
| NOS | Nitric oxide synthase |
| NOT | Nucleus of the optic tract |
| NP | Nanoparticle |
| NPC | Neural progenitor cell |
| NPY | Neuropeptide Y |
| NPZ-8 | Neuropsychological Z score for 8 tests |
| NR | Nuclear receptor |
| NRG | Neuregulin |
| NRL | Nuclear receptor ligand |
| NRTI | Nucleoside analogue reverse transcriptase inhibitors |
| NSAID | Nonsteroidal anti-inflammatory drug |
| NSC | Neural stem cell |
| NSE | Neuron specific enolase |
| NSF | *N*-ethylmaleimide sensitive factor |
| NT | 3-Nitrotyrosine |
| NTF | Neurotrophin |
| NVU | Neurovascular unit |
| OB | Olfactory bulb |
| OCB | Oligoclonal band |
| OCD | Obsessive compulsive disorder |
| ODN | Oligonucleotides |
| OE | Olfactory epithelium |
| OHT | Ocular hypertension |
| OL | Oligodendrocyte |
| OLM | Outer limiting membrane |
| OMgp | Oligodendrocyte-myelin glycoprotein |
| OMP | Olfactory marker protein |
| ONH | Optic nerve head |
| ONL | Outer nuclear layer |
| OP | Oligodendrocyte progenitors |
| OPC | Oligodendrocyte progenitor cell |
| OPCA | Olivopontocerebellar atrophy |
| OPL | Outer plexiform layer |
| ORF | Open reading frame |
| ORN | Olfactory response neuron |
| OSP | Oligodendrocyte-specific protein |

| | |
|---|---|
| OVA | Ovalbumin |
| OVLT | Organum vasculosum of the lamina terminalis |
| P0 | Myelin protein zero |
| p75NTR | p75 neurotrophin receptor (nerve growth factor receptor; NGFR) |
| PACAP | Pituitary adenylate cyclase-activating polypeptide |
| PACT | Protein activator of the interferon-induced protein kinase |
| PAF | Platelet-activating factor |
| PAG | Periaqueductal gray |
| PAMP | Pathogen-associated molecular pattern |
| PANDAS | Pediatric autoimmune neuropsychiatric disorders associated with Streptococcus |
| PANSS | Positive and negative syndrome scale |
| PARP | Poly(ADP-ribose) polymerase |
| PASAT | Paced Auditory Serial Addition Test |
| PBBS | Peripheral benzodiazepine binding sites |
| PBL | Peripheral blood lymphocyte |
| PBMC | Peripheral blood mononuclear cell |
| PBR | Peripheral benzodiazepine receptor |
| PCP | Phencyclidine |
| PCR | Polymerase chain reaction |
| PD | Parkinson's disease |
| pDC | Plasmacytoid dendritic cells |
| PD1 | Program death-1 |
| PDE | Phosphodiesterase |
| PDGF | Platelet-derived growth factor |
| PDTC | Pyrrolidine dithiocarbamate |
| PE | Plasma exchange |
| Pe | Periventricular |
| PEG | Polyethylene glycol |
| PEI | Polyethyleneimine |
| PENK | Proenkephalin |
| PERG | Pattern electroretinogram |
| PET | Positron emission tomography |
| PFS | Periodic fever syndromes |
| PG | Prostaglandin |
| Pgp | P-glycoprotein |
| PHF | Paired helical filament |
| PI | Phosphatidylinositol |
| PI3K | Phosphatidylinositol-3-kinase |
| PICA | Posterior inferior cerebellar artery |
| PICK1 | Protein interacting with C kinase 1 |
| PKA | cAMP-dependent protein kinase |
| PKG | Protein kinase G |
| PLGA | Poly(D,L-lactide-co-glycolide) |
| PLOSL | Polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy |
| PLP | Proteolipid protein |
| PMCA | Plasma membrane bound $Ca^{2+}$-ATPase |
| PMD | Pelizaeus-Merzbacher disease |
| PML | Progressive multifocal leukoencephalopathy |
| PMN | Polymorphonuclear (leukocyte) |
| PMP22 | Peripheral myelin protein 22 |
| PNS | Peripheral nervous system |
| POAG | Primary open-angle glaucoma |
| polyQ | Polyglutamine |

| | |
|---|---|
| POMC | Pro-opiomelanocortin |
| POU3F2 | POU class 3 homeobox 2 |
| PP | Protein phosphatase |
| PPAR | Peroxisome proliferator activated receptor |
| PPF | Paired-pulse facilitation |
| PPG | Poly(propylene glycol) |
| PP-MS | Primary progressive multiple sclerosis |
| PR | Photoreceptor |
| PrPc | Cellular prion protein |
| PrPres | Protease resistance prion |
| PrPsc | Disease-associated prion protein |
| PRR | Pattern recognition receptor |
| PS | Presenilin (PSEN) |
| PSA-NCAM | Poly-sialylated form of the neural cell adhesion molecule |
| PSCs | Perisynaptic Schwann cells |
| PSD | Postsynaptic density |
| PSP | Progressive supranuclear palsy |
| PSW | Periodic sharp wave |
| Ptc | Patched, a hedgehog receptor |
| PTM | Post-translational modifications |
| PTP | Post-tetanic potentiation |
| PTSD | Post-traumatic stress disorder |
| PTZ | Pentylenetetrazol |
| PVL | Periventricular leukomalacia |
| PVM | Perivascular macrophages |
| PVN | Paraventricular nucleus |
| PYD | Pyrin domain |
| PYY | Peptide YY |
| RA | Rheumatoid arthritis |
| Rag | Recombination-activating gene |
| RAGE | Receptor for advanced glycation end product |
| RANTES | Regulated upon activation normal T-cell expressed and secreted (CCL5) |
| RAS | Renin-angiotensin system |
| Rb | Retinoblastoma |
| RBP | RNA-binding proteins |
| REM | Rapid eye movement |
| RER | Rough endoplasmic reticulum |
| RF | Radiofrequency |
| RFLPs | Restriction fragment length polymorphisms |
| RGC | Retinal ganglion cell |
| RISC | RNA-induced silencing complex |
| RIG1 | Retinoic acid-inducible gene 1 |
| RIM | Rab3-interacting molecule |
| RIP | Receptor-interacting protein |
| RMS | Rostral migratory stream |
| RNAi | RNA interference |
| RNI | Reactive nitrogen intermediates |
| RNP | Ribonucleoprotein |
| RNS | Reactive nitrogen species |
| ROCK | Rho kinase |
| ROHHAD | Rapid-onset obesity, hypoventilation, hypothalamic dysfunction, and autonomic dysregulation |
| ROI | Reactive oxygen intermediate |

| | |
|---|---|
| RORγ | Retinoic-acid-receptor-related orphan receptor-γ |
| ROS | Reactive oxygen species |
| RP | Relapsing polychondritis |
| RPE | Retinal pigment epithelial (cells) |
| RRM | RNA recognition motifs |
| RRMS | Relapsing and remitting multiple sclerosis |
| RSV | Rous sarcoma virus |
| RTK | Receptor tyrosine kinase |
| rt-PA | Recombinant tissue plasminogen activator |
| RT-PCR | Reverse transcription polymerase chain reaction |
| RyR | Ryanodine receptor |
| sALS | Sporadic amyotrophic lateral sclerosis |
| SAP | Synapse-associated protein |
| SAPAP | SAP-associated protein (discs, large homolog-associated protein-1; DLGAP1) |
| SAPK | Stress-activated protein kinase (JNK, MAPK8) |
| sAPP | Secreted β-amyloid precursor protein |
| SBMA | Spinobulbar muscular atrophy |
| SCs | Schwann cells |
| SC | Superior colliculus |
| SCA-3 | Spinocerebellar ataxia-3 |
| scFv | Single-chain Fv antibodies |
| SCI | Spinal cord injury |
| SCID | Severe combined immunodeficiency |
| sCJD | Sporadic Creutzfeldt-Jakob disease |
| SCPs | Schwann cell precursor |
| sCrry | Soluble complement receptor-related protein y |
| SDF-1 | Stromal cell-derived factor 1 (CXCL12) |
| SEC | Sinus endothelial cell |
| SELDI-TOF | Surface enhanced laser desorption ionization time-of-flight |
| SER | Smooth endoplasmic reticulum |
| SERCA | Sarco(endo)plasmic reticulum $Ca^{2+}$-ATPase |
| SERT | Serotonin transporter |
| sFI | Sporadic fatal insomnia |
| SFO | Subfornical organ |
| SG | Stress granule |
| SGLPG | Sulfated glucuronyl lactosaminyl paragloboside |
| SGZ | Subgranular zone |
| Shh | Sonic hedgehog |
| sIg | Surface immunoglobulin |
| sIL-2R | Soluble IL-2 receptor |
| SITA | Swedish interactive thresholding algorithm |
| SIV | Simian immunodeficiency virus |
| SIVE | Simian immunodeficiency virus encephalitis |
| SLE | Systemic lupus erythematosus |
| SMA | Spinal muscular atrophy |
| SMAC | Second mitochondrial-derived activator of caspase |
| SMase | Sphingomyelinase |
| SMN | Survival motor neuron gene |
| SN | Substantia nigra |
| SNAP | Sensory nerve action potential |
| SNAP-25 | Synaptosome-associated protein of 25,000 daltons |
| SNARE | NSF attachment receptor |
| SNpc | Substantia nigra pars compacta |

| | |
|---|---|
| SNPs | Single-nucleotide polymorphisms |
| SNS | Sympathetic nervous system |
| SOCS | Suppressors of cytokine signaling |
| SOD1 | Superoxide dismutase 1 |
| SON | Supraoptic nuclei |
| SP1 | Specificity protein 1 |
| SPARC | Secreted protein acidic and rich in cysteine |
| SPECT | Single photon emission computed tomography |
| SPG-II | Spastic paraplegia type II |
| SR-A | Scavenger receptor type A |
| SRBCs | Sheep red blood cells |
| SREBP | Sterol regulatory element-binding protein |
| SRF | Serum response factor |
| SSRI | Selective serotonin reuptake inhibitors |
| STAT | Signal transducers and activators of transcription |
| STP | Short-term potentiation |
| SV5 | Simian virus 5 |
| SVZ | Subventricular zone |
| SWAP | Short wavelength automated perimetry |
| SWATH | Sequential window acquisition of all theoretical mass spectra |
| SWI | Susceptibility weighted imaging |
| SYN | α-Synuclein |
| SZ | Schizophrenia |
| T | Tesla |
| Tat | Trans-activator of transcription |
| TBE | Tick-borne encephalitis virus |
| TBI | Traumatic brain injury |
| TBP | TATA-binding protein |
| TCA | Tricarboxylic acid |
| TCR | T-cell receptor |
| TCV | T-cell vaccination |
| TDO | Tryptophan 2,3-dioxygenase |
| TE | Echo time |
| TEC | Thymic epithelial cell |
| TES | Traumatic encephalopathy syndrome |
| Teff | T effector cells |
| TF | Transcription factor |
| TG | Trigeminal ganglia |
| TGF | Transforming growth factor |
| TH | Thyroid hormone |
| TH | Tyrosine hydroxylase |
| Th1 | T helper type 1 cell |
| Th2 | T helper type 2 cell |
| TIA | Transient ischemic attack |
| TIR | Toll/IL-1 receptor |
| TJ | Tight junction |
| TLR | Toll-like receptor |
| TMEV | Theiler's mouse encephalomyelitis virus |
| TMT | Trimethyltin |
| TMZ | Temozolomide |
| TN | Terminal nuclei |
| TNF | Tumor necrosis factor |
| TNFR | Tumor necrosis factor receptor |

| | |
|---|---|
| TOR1 | Target of rapamycin 1 |
| TRAF | TNF-receptor mediated factor |
| TRAIL | TNF-related apoptosis-inducing ligand (TNFSF10) |
| TRANCE | TNF-related activation-induced cytokine (TNFSF11) |
| TRANCER | TRANCE receptor (TNFRSF11A) |
| TRE | Tax responsive element |
| Treg | T regulatory cells |
| TREM | Triggering receptor expressed on myeloid cells |
| Trk | Receptor tyrosine kinase |
| TRP | Transient receptor potential |
| TRPC | Transient receptor potential canonical channels |
| TSA | Tissue-specific antigen |
| TSE | Transmissible spongiform encephalopathies |
| TSP | Thrombospondins |
| TULP-1 | Tubby-lie protein 1 |
| Tyk2 | Protein tyrosine kinase 2 |
| UACA | Uveal autoantigen with coiled domains and ankyrin repeats |
| UMN | Upper motor neuron |
| V1 | Primary visual cortex |
| VAMP | Vesicle-associated membrane protein |
| VCAM | Vascular cell adhesion molecule |
| VEGF | Vascular endothelial growth factor |
| VEP | Visual evoked potential |
| VIP | Vasoactive intestinal peptide |
| VMAT-2 | Vesicular monoamine transporter-2 (solute carrier family 18; SLC18A2) |
| VZV | Varicella-zoster virus |
| WDM | Welander distal myopathy |
| WKAH | Wistar-King-Aptekman-Hokudai |
| WNV | West Nile virus |
| X-ALD | X-adrenoleukodystrophy |
| XIAP | X-linked inhibitor of apoptosis protein |
| ZO-1 | Zonula occludens-1 (TJP1) |

## References

HUGO Gene Nomenclature Committee: http://www.genenames.org/index.html
"Abbreviations and Symbols for Chemical Names": www.blackwell-symergy.com/doi/pdf/10.1111/j.1432.1033.1967.tb00070.x

# Part I

# Immunology of the Nervous System

# Innate and Adaptive Immunity in Health and Disease

1

Howard E. Gendelman and Eliezer Masliah

**Abstract**

Neuroinflammatory processes play a significant role in health and disease of the nervous system. These regulate development, maintenance and sustenance of brain cells and their connections. Linked to aging, epidemiologic, animal, human, and therapeutic studies all support the presence of a neuroinflammatory cascade in disease. This is highlighted by the neurotoxic potential of microglia. In steady state, microglia serve to protect the nervous system by acting as debris scavengers, killers of microbial pathogens, and regulators of innate and adaptive immune responses. In neurodegenerative diseases, activated microglia affect neuronal injury and death through production of glutamate, pro-inflammatory factors, reactive oxygen species, quinolinic acid amongst others and by mobilization of adaptive immune responses and cell chemotaxis leading to transendothelial migration of immunocytes across the blood-brain barrier and perpetuation of neural damage. As disease progresses, inflammatory secretions engage neighboring glial cells, including astrocytes and endothelial cells, resulting in a vicious cycle of autocrine and paracrine amplification of inflammation perpetuating tissue injury. Such pathogenic processes contribute to neurodegeneration. Research from others and our own laboratories seek to harness such inflammatory processes with the singular goal of developing therapeutic interventions that positively affect the tempo and progression of human disease.

**Key words**

Alzheimer's disease • Microglia • Neurodegenerative disorders • Neuroinflammatory processes • Parkinson's disease

Neuroinflammatory processes play a significant role in health and disease of the nervous system. These regulate development, maintenance and sustenance of brain cells and their connections. Linked to aging, epidemiologic, animal, human, and therapeutic studies all support the presence of a neuroinflammatory cascade in disease. This is highlighted by the neurotoxic potential of microglia. In steady state, microglia serve to protect the nervous system by acting as debris scavengers, killers of microbial pathogens, and regulators of innate and adaptive immune responses. In neurodegenerative diseases, activated microglia affect neuronal injury and death through production of glutamate, pro-inflammatory factors, reactive oxygen species, quinolinic acid amongst others and by mobilization of adaptive immune responses and cell chemotaxis leading to transendothelial migration of immunocytes across the blood-brain barrier and perpetuation of neural damage. As disease progresses, inflammatory secretions engage neighboring glial cells, including astrocytes and endothelial cells, resulting in a vicious cycle of autocrine and

H.E. Gendelman (✉)
Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68198, USA
e-mail: hegendel@unmc.edu

E. Masliah
Departments of Neurosciences and Pathology, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA



T. Ikezu, H.E. Gendelman (eds.), *Neuroimmune Pharmacology*, DOI 10.1007/978-3-319-44022-4_1

paracrine amplification of inflammation perpetuating tissue injury. Such pathogenic processes contribute to neurodegeneration. Research from others and our own laboratories seek to harness such inflammatory processes with the singular goal of developing therapeutic interventions that positively affect the tempo and progression of human disease.

As the life expectancy of the human population continues to increase, the possibility of developing neuroinflammatory and neurodegenerative diseases have increased considerably during the past 50 years. Of the neurodegenerative disorders Alzheimer's disease continued to be the leading cause of dementia in the aging population. Traditionally, neuro degenerative disorders have been define as conditions where there is selective loss of neurons within specific region of the brain accompanied by astrogliosis. However, in the past 20 years, we have learned that the pathological process leading to the dysfunction of selected circuitries in the brain initiates with damage to the synapses rather than with the loss of neurons. In fact, neuronal loss is a late event that is probably preceded by damage to axons and dendrites followed by shrinkage of the neuronal cell body and abnormal accumulation of filamentous proteins.

Therefore, the revised concept of neurodegeneration suggest that neuronal injury initiates at the synaptic junction and propagates throughout selected circuitries leading to neuronal dysfunction which resolves in the classical clinical symptoms characteristic to each of the neurodegenerative disorders (Hashimoto and Masliah 2003). So for example in Alzheimer's disease early damage to the synapses between the entorhinal cortex and the molecular layer of the dentate gyrus (perforant pathway) resolves in the short term memory deficits characteristic of this dementing disorder. Later on disconnection of the cortico-cortico fibers in the frontal, parietal, and temporal cortex resolved in more severe memory deficits, and alterations in executive functions and abstraction. Degeneration of connections between the nucleus basalis of Meynert and the neocortex resolves in attention and memory deficits that usually associated with loss of cholinergic neurons. Other circuitries and neuronal populations are also affected in Alzheimer's disease illustrating the complexity of these disorders and the fact that the concept of single population is affected is limited. That is the case with several other disorders including Parkinson's disease where degeneration is not limited to the dopaminergic system, but also involves the limbic system, the raphe nucleus, the insula, and other systems.

In response to the injury neurons produce adhesions molecules and trophic factors that recruit astroglial and microglial cells to participate in the process of repair of the damage. In addition the microvasculature and other glial systems might also participate in the process. Thus, neurodegeneration is accompanied by astrogliosis, microgliosis, and microvascular remodeling. While astroglial cells initially produce trophic factors and cytokines that aid in the tissue repair, eventually these factors could amplify the inflammatory response, increase vascular permeability and result in microglial activation, which in turn might lead to the production of more proinflammatory cytokines and chemokines. A critical balance between the repair and proinflammatory factors often determines the future rate and progression of the degenerative process.

The understanding of the mechanisms of neurodegeneration and inflammatory response in these neurological conditions has seen a tremendous progress in the past 10 years. It is now recognized that probably small soluble misfolded protein aggregates denominated oligomers are responsible for the injury. So for example in Alzheimer's disease Aβ protein oligomers might damage the synapses in neocortical regions and the limbic system while in Parkinson's disease α-synuclein oligomers may damage the axons in the striatum, brainstem and cortical regions. While significant progress has been made in understanding the fundamental mechanisms for the neuronal injury, less is known about the reasons for the selective neuronal vulnerability characteristic to these neurological conditions and the role of the innate immune system in this process.

## Reference

Hashimoto M, Masliah E (2003) Cycles of aberrant synaptic sprouting and neurodegeneration in Alzheimer's and dementia with Lewy bodies. Neurochem Res 28(11):1743–1756

# The Blood-Brain Barriers

2

William A. Banks

**Abstract**

The blood-brain barriers (BBBs) are mainly located at the levels of the vasculature, choroid plexus, and the circumventricular organs and play multiple roles in neuroimmunology. The ability of the BBBs to separate the blood and its contents from the central nervous system (CNS) is largely responsible for the CNS being an immune-privileged region. However, the BBB then revises this separation in a regulated way by a variety of mechanisms, including the ability to transport cytokines, regulate the entry of immune cells into the brain, and to itself secrete into the blood and into the CNS immunoactive substances. The BBB thereby participates in a number of neuroimmune axes that allow communication between the CNS and the peripheral immune cells. Failure of the highly regulated activities of the BBBs can be both a cause and consequence of immune diseases.

**Keywords**

Active transport • Adsorptive endocytosis • Adsorptive transcytosis • AIDS • Blood-brain barrier • Brain • Brain endothelial cell • Central nervous system • Cytokine • Endothelin • Facilitated diffusion • Immune cell • Interleukin • Neuroimmune • Neurovascular unit • Opiate • Transmembrane diffusion • Transport • Tumor necrosis factor • Virus

## 2.1 Introduction

The roles played by the blood-brain barrier (BBB) in neuroimmunology are diverse and ever expanding. Conceptually, the BBB is those processes that restrict, control, or otherwise influence the exchange of substances between the peripheral circulation and the brain interstitial fluid and cerebrospinal fluid (CSF). More concretely, there are multiple BBBs: the barrier formed by monolayers of endothelial cells (the vascular BBB), the barrier formed by ependymal/epithelial cells (the blood-CSF barrier) and a barrier formed by tanycytes interposed between the circumventricular organs and the adjacent brain tissue. It is the restrictive properties of the BBB that limit and control the trafficking of immune cells and prevent the unrestricted leakage of immune active substances from the blood into the brain that renders the central nervous system (CNS) an immune-privileged area. But the BBB also transports immunoactive substances between the blood and CNS, responds to immunoactive substances secreted into the blood or CNS fluids, and secretes substances into those compartments. These last three processes of transport, responsiveness, and secretion allow the BBB to be in constant cross talk with other cells in both the CNS and periphery in a formation referred to as the neurovascular unit (NVU). The BBB both responds to and influences the CNS and peripheral microenvironment and does so through reacting to immunoactive substances, including cytokines, chemokines, prostaglandins, and nitric oxide. Several consequences arise from this cross talk, including that under physiological conditions the metabolic needs of the brain are met by the BBB. The BBB helps to keep the CNS informed of

W.A. Banks (✉)
Division of Gerontology and Geriatric Medicine, Department of Medicine, University of Washington School of Medicine, 1660 S. Columbian Way, Seattle, WA 98108, USA
e-mail: wabanks1@uw.edu



T. Ikezu, H.E. Gendelman (eds.), *Neuroimmune Pharmacology*, DOI 10.1007/978-3-319-44022-4_2

peripheral events including immune events and is central to the formation and functioning of neuroimmune axes. When any of these physiological consequences are violated, disease can arise; and the BBB can be a target, a cause, or a conduit to treatment of those diseases.

### 2.1.1 Structures and Functions of the BBB

Evidence for an interface between the circulation and the CNS dates back to the end of the nineteenth century (Bradbury 1979). The best known of those early studies were done by a young Paul Erlich who found that some dyes did not stain the brain after their peripheral injection. Erlich concluded erroneously that the lack of staining was because these dyes did not bind to brain tissue. Several decades later, Goldmann, a student of Erlich's, found that these dyes could stain the brain when injected intravenously. Thus, these studies were reinterpreted as evidence in favor of some sort of barrier between the CNS and blood.

The location and nature of that barrier was controversial through much of the twentieth century. Elegant studies by Davson and colleagues identified the barrier at the vascular level. However, alternative opinions were held until Reese and coworkers conducted classic studies with the electron microscope in the late 1960s (Brightman and Reese 1969; Reese and Karnovsky 1967). Previous work had shown no difference between vascular beds of peripheral tissues and the CNS when studied grossly or at the light microscope level. However, Reese and coworkers found numerous differences at the ultrastructural level. These included a much-reduced rate of pinocytosis and an absence of intracellular fenestrations. Currently, the most widely discussed finding is the presence of tight junctions between adjacent endothelial cells. The tight junctions, low rate of pinocytosis, and low number of intracellular fenestrations effectively eliminate intercellular gaps and pores. This, in turn, essentially eliminates the production of a plasma-derived ultrafiltrate and hence the leakage of serum proteins into the brain.

From this single change, the lack of a production of an ultrafiltrate, evolves a large number of consequences for CNS function. Obviously, it is the basis of the restriction of protein access, which first defined the BBB in late nineteenth century. The need for an efficient lymphatic system is eliminated, but the lack of a classic lymphatic system means that the CNS needs other methods to rid itself of the free water and wastes produced by metabolism and the secretions of the choroid plexus. Without production of an ultrafiltrate, the CNS depends on other methods to extract nourishment from the blood. The BBB addresses this need with a large number of selective transporters for substances from electrolytes to regulatory proteins (Davson and Segal 1996b, c). Because the CNS is not equipped to handle an ultrafiltrate, the reintroduction of a leaky BBB, as with hypertensive crisis, can result in increased intracranial pressure and encephalopathy (Al-Sarraf and Phillip 2003; Johansson 1989; Mayhan and Heistad 1985).

### 2.1.2 The Various BBBs

The BBB is not a single barrier but several barriers, which are in parallel. This contrasts with the testis-blood barrier, which consists of several barriers in series (Holash et al. 1993; Neaves 1977). The most studied of these barriers are the vascular barrier and the choroid plexus. Often, the terms BBB and blood-cerebrospinal (CSF) fluid barrier are used to refer specifically to the vascular barrier and the barrier formed at the choroid plexus, respectively. The least studied barriers are the barriers formed by tanycytes at the circumventricular organs (CVO) and other specialized neural barriers, such as the blood-retinal barrier (Neuwelt et al. 2008).

#### 2.1.2.1 Vascular BBB

The vascular BBB occurs because of the modifications, noted by Reese and co-workers, in the endothelial cells that comprise the capillary bed and line the venules and arterioles of the CNS (upper panel Fig. 2.1). It is likely that these three regions are highly specialized. For example, immune cells primarily cross at the venules, and most of the classic transporters are located at the capillaries (Engelhardt and Wolburg 2004). No CNS cell is more than about 40 μm from a capillary. This means that a substance that can cross the vascular BBB can immediately access the entire CNS. Substances that cross the vascular BBB can be either flow-dependent or not dependent on flow rate. A flow-dependent substance is one in which the BBB extracts from the blood nearly the maximal amount possible (Kety 1987). The only way to increase the amount of a flow-dependent substance entering the brain is to increase the flow rate to the brain. Glucose is an example of a flow-dependent substance (Rapoport et al. 1981). A brain region that is particularly active has its increased demand for glucose met by an increase in regional blood flow. In contrast, transport of a cytokine such as tumor necrosis factor-alpha (TNF-α) is not flow dependent. Only a small percent of the TNF-α in blood is extracted by the brain via the saturable transporter for TNF-α located at the BBB (Gutierrez et al. 1993). Alterations of blood flow within physiological limits do not alter the uptake of TNF-α from blood by brain. However, extreme changes in the rate of blood flow or capillary tortuosity can result in rheological changes, such as the loss of laminar flow. Such alterations likely occur in stroke, AIDS, and Alzheimer's disease (de la Torre and Mussivand 1993; Nelson et al. 1999). This may result in impaired permeation of flow-dependent and non-flow dependent substances.

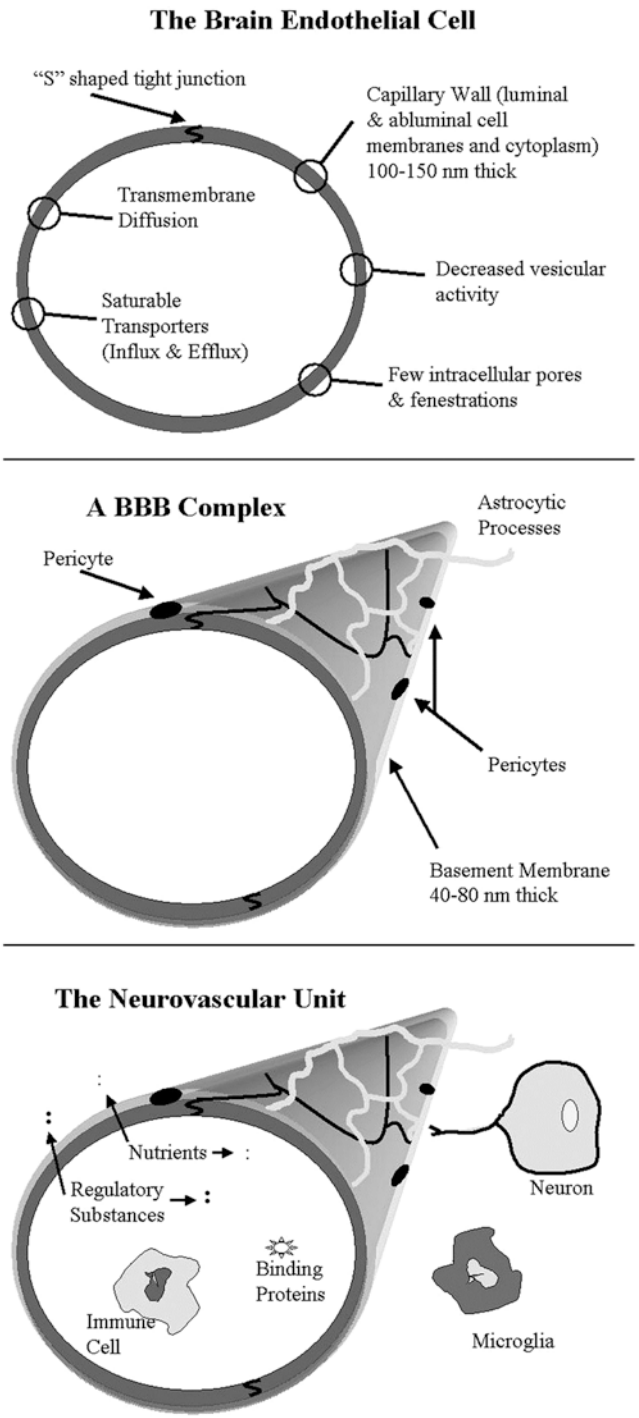



**Fig. 2.1** The vascular blood-brain barrier: the *upper panel* illustrates the brain endothelial cell. This is the functional and anatomical site of both barrier function and of saturable and non-saturable mechanisms of passage. The major modifications allowing both barrier function and selective penetration of substances are indicated. The *middle panel* illustrates other cell types and structures important in BBB function. Pericytes are embedded in a basement membrane and astrocytes form a structure over the capillary bed above the pericytes and basement membrane. Both cell types are in paracellular communication with the brain endothelial cells. The *lower panel* illustrates the neurovascular unit, a concept that emphasizes integration of peripheral, BBB, and central interactions

The vascular BBB has regional variations in terms of function and susceptibilities to disease. Huber et al. noted that some regions of the brain suffer larger and earlier disruptions to their barriers during diabetes mellitus than others (Huber 2008; Huber et al. 2006). Many peptides and regulatory substances have unique regional variations in their transport rates across the BBB (Banks et al. 1996; Banks and Kastin 1998). It is assumed that these reflect on brain function. For example, the brain region with the highest rate of transport of leptin is the region of the arcuate nucleus, an area important in leptin-mediated control of feeding (Banks et al. 1996; Schwartz et al. 2000).

#### 2.1.2.2 Choroid Plexus

The choroid plexus are bags composed of monolayers of epithelial cells that project into the ventricles and contain a capillary plexus (Johanson 1988). The capillaries do not have barrier function and so produce an ultrafiltrate that fills the bag. The epithelial cells have tight junctions and so prevent the ultrafiltrate from entering the ventricular space. Unlike the capillaries, the epithelial cells of the choroid plexus have a high rate of vesicular turnover that is responsible for the production of the CSF. However, the CSF is not an ultrafiltrate but a secreted substance. The choroid plexus also has many selective transport systems, some of which are specific to it or are enriched in comparison to the vascular BBB. In some cases, the choroid plexus seems not to be a system complementary to the vascular BBB, but one that is contrary to it. For example, efflux of amyloid beta peptide by the vascular BBB is impaired with aging but is increased with aging at the choroid plexus (Pascale et al. 2011). Thus, the choroid plexus should not be thought of as a secondary barrier but as an independent system with its own unique characteristics.

#### 2.1.2.3 Barriers at Circumventricular Organs

The CNS of mammals contains seven regions of the brain where the vasculature does not fully participate in a BBB (Gross et al. 1987). These regions have at least one side that faces a ventricle and so are termed circumventricular organs (CVOs). Together, they comprise about 0.5 % of the brain by weight. Their capillaries allow the production of an ultrafiltrate and so their cells are in more intimate contact with the circulation. They are known to play vital roles as sensing organs for critical peripheral events; for example, they act as emetic centers and are important in blood pressure modulation (Johnson and Deckwerth 1993; Ferguson 1991). They can relay their signals to the rest of the brain by neurons that project from them to distant brain regions or project to them from other brain regions. However, the mixing of their interstitial fluids with that of adjacent brain tissue or CSF has been shown to be limited in most studies (Peruzzo et al. 2000; Plotkin et al. 1996; Rethelyi 1984). Diffusion through brain tissue is poor, and this alone would tend to produce a

limit to mixing within a few hundred microns of the CVO (Cserr and Berman 1978; de Lange et al. 1995). However, the major factor preventing leakage of substances from the CVO into the adjacent CSF and brain tissue is a physical barrier to diffusion. The epithelial cells that line the ventricles form tight junctions when they are next to CVOs, thus limiting CVO-to-CSF diffusion. A functional barrier formed by bands of tanycytes also exists for the diffusion of substances from the CVO to the adjacent brain region (Peruzzo et al. 2000; Plotkin et al. 1996; Rethelyi 1984). These tanycytes themselves can project to the cells and vessels within the CVO and can respond dynamically to relay information to adjacent regions of the brain that have BBBs (Langlet et al. 2013). A recent review by Rodriguez (Rodriguez et al. 2010) has explored the various aspects of the CVO barriers.

#### 2.1.2.4 Other Specialized Neural Barriers

These barriers include the blood-retinal barrier, blood-spinal cord barrier, and blood-nerve barriers (for a full listing, see Neuwelt et al. (2008)). These barriers generally include tight junctions participating in a vascular barrier but exhibit varying degrees of leakiness. They can also vary markedly in transporter activity from the vascular BBB during both health and in response to disease (Pan et al. 2008a; Pan and Kastin 2003; Prockop et al. 1996).

### 2.1.3 Concept of the Neurovascular Unit and Comparison to Peripheral Vascular Beds

The endothelial cell is the anatomical location of the barrier aspect of the vascular BBB and of its various saturable transporters (Fig. 2.1). Capillary beds from peripheral tissues have numerous intracellular and intercellular pores and fenestrations and high rates of pinocytosis that account for their leakiness. The brain endothelial cell engages in comparatively little macropinocytosis, has few intracellular pores or fenestrations, and intercellular pores or gaps are eliminated because of tight junctions.

However, the brain endothelial cell does not function in isolation. The abluminal (brain side) of the capillary is encased in a basement membrane 40–80 nm thick. This membrane does not act as a barrier to molecules but may restrict viral-sized particles (Muldoon et al. 1999). It also holds pericytes in close approximation to the endothelial cell (Balabanov and Dore-Duffy 1998). The pericyte is a pluripotent cell and a modulator of BBB function (Dore-Duffy et al. 2000; Deli et al. 2005; Dore-Duffy 2008). It, like astroctyes and microglia, secretes cytokines and other immune active substances both constitutively and when induced (Kovac et al. 2011). In the rat hippocampus, astrocytes project endfeet that surround the capillary in what looks at the ultrastructural level like a complete covering, albeit without intercellular tight junctions (Mathiisen et al. 2010). Astrocytes and pericytes both secrete substances that induce tight junction formation in endothelial cells (Deli et al. 2005; Daneman et al. 2010). All the major cell types of the NVU (pericytes, astrocytes, microglia, and endothelial cells) secrete a variety of substances, including cytokines, into their local environment (Fabry et al. 1993; Nath et al. 1999; Banks 2014). Pericytes and astrocytes play interrelated but distinct roles in BBB function. For example, pericytes are the primary protectors of the blood-retinal and blood-brain barriers during glycemic stress (Romeo et al. 2002; Nakaoke et al. 2007).

It is clear that immune cell trafficking occurs across the normal BBB (Greenwood et al. 2011). One study found that about one in 5000 intravenously injected lymphocytes resided in the brain at any given time and that uptake was affected by strain and immune activation (Banks et al. 2012). The microglia may be at equilibrium with circulating macrophages/monocytes, although whether monocytes cross the BBB of the adult healthy animal to become microglia seems to still be unresolved (Williams and Hickey 1995). Other immune cells also enter and exit the CNS at unknown rates and frequencies as influenced by yet to be determined factors. Clearly, secretions of prostaglandins, nitric oxide, and cytokines from each of these cells are important for intercellular communication and can influence endothelial cell permeability (Chao et al. 1994; Nath et al. 1999; Shafer and Murphy 1997).

The concept of the neurovascular unit (NVU) emphasizes the interactive role that cells and events within the CNS and in the circulation play on BBB permeability as well as the consequences of the permeability itself. The NVU includes other factors long known to influence the penetration of substances across the BBB, such as degradation, sequestration, and serum protein binding. The encompassing concept of the NVU is particularly useful when considering the next section, the mechanisms of transport across the BBB.

## 2.2 Mechanisms of Transport Across the BBB

Substances can enter or exit the CNS by a variety of mechanisms. Some of these mechanisms are operational in both the blood-to-brain (influx) and the brain-to-blood (efflux) directions; whereas, others are unidirectional.

### 2.2.1 Blood to CNS

Saturable and nonsaturable modes predominate influx. Within each of these categories are a diverse number of mechanisms. These different mechanisms tend to favor certain groups or types of substances.

#### 2.2.1.1 Nonsaturable Passage

A hallmark of nonsaturable passage is that the percent of material crossing into the CNS is not affected by the amount of material available for transport. The two main mechanisms of nonsaturable passage are transmembrane or transcellular diffusion and the extracellular pathways. The former is much better studied and its principles are widely applied by industry for the development of CNS drugs; the latter has received much less attention.

##### Transcellular Diffusion

The most studied nonsaturable mechanism by which small molecules cross the BBB is by transmembrane or transcellular diffusion (Rapoport 1976). The major determinant of passage is the degree to which the substance is lipid soluble. A substance that is too lipid soluble will be unable to repartition into the brain's interstitial fluid and so will become trapped in the cell membranes of the BBB. A ratio of about 10:1 in favor of lipid versus aqueous solubility is near ideal for maximal passage across the BBB. The second most important determinant is molecular weight with passage being favored for smaller molecules. Other physicochemical determinants, such as charge, can occasionally become dominant for specific compounds. Work by Lipinksi and colleagues in Caco-2 cells, an immortalized cell line derived from a gastrointestinal cancer, clearly shows that smaller, less charged, more lipid soluble drugs are favored in transmembrane diffusion (Lipinski et al. 1997). Many exogenous substances, including many drugs with CNS activity, enter the brain predominantly by way of transmembrane diffusion. Morphine and ethanol are prime examples of common substances that cross the BBB by this mechanism (Oldendorf 1974).

Although higher molecular weight (MW) is an impedance to transmembrane diffusion at the BBB, there seems to be no absolute molecular weight cut-off. A previous study which had thought to define such an absolute limit had discovered, in retrospect, early evidence for an efflux system (Levin 1980). The largest substance to date noted to have a measurable uptake by brain by transcellular diffusion is cytokine-induced neutrophil chemoattractant-1 (CINC1), with a MW of about 7.8 kDa (Pan and Kastin 2001a). A surprisingly large number of small, lipid soluble compounds cross the BBB at a rate considerably greater or lesser than that predicted by their physicochemical characteristics (Oldendorf 1971, 1974). Binding to serum proteins and efflux systems are major factors decreasing influx, and the presence of a saturable blood-to-brain transporter is a major factor increasing influx.

##### Extracellular Pathways

Albumin derived from serum is present in small amounts in the CSF, showing that the BBB is not absolute. The amount of protein in CSF, however, is very small, being about 0.5 %, or 1/200th, of that in plasma. The CSF is not an ultrafiltrate but a secreted fluid. This means that the relative and absolute concentrations of proteins, electrolytes, minerals, and other substances can differ tremendously to that of plasma. The extracelluar pathways are another avenue by which substances can enter the CNS (Balin et al. 1986; Broadwell 1993). These represent what have sometimes been termed "functional leaks" at discreet areas of the brain, including the large vessels of the pial surface and subarachnoid space, the circumventricular organs, the nasal epithelium, the sensory ganglia of spinal and cranial nerves, and some deep brain regions, such as the nucleus tractus solitarius (Broadwell and Banks 1993).

The amount of a substance that enters the brain by the extracellular pathways is small. However, this route may be therapeutically relevant for compounds which have favorable peripheral pharmacokinetics, such as a long serum half-life and a small volume of distribution (Banks 2004). Antibodies, erythropoietin, and enzymes can access the brain by way of the extracellular pathways (Banks et al. 2004a, 2005a, 2007; Kozlowski et al. 1992; Grubb et al. 2008), and this may underlie their therapeutic benefits when given in high doses (Grubb et al. 2008; Alafaci et al. 2000; Ehrenreich et al. 2002; Erbyraktar et al. 2003; Morgan et al. 2000; Janus et al. 2000; Hock et al. 2003; Farr et al. 2003).

#### 2.2.1.2 Receptor-Mediated and Saturable Transports

Saturable processes represent a diverse group of mechanisms. Included in this group are diapedesis and adsorptive endocytosis/transcytosis that share characteristics with the saturable systems.

##### Active Transport Versus Facilitated Diffusion

Saturable transporters (Yeagle 1987) can be divided into those which require energy (active transport) and those which do not (facilitated diffusion). Both are dependent on a protein which acts as the transporter, may have co-factors, and be modulated by physiologic and disease processes. Energy requiring systems can be unidirectional; that is, they may have only an influx or efflux component. Non-energy requiring saturable transport (facilitated diffusion) is bidirectional; that is, it transports substances in both directions with net flux being from the side of higher concentration to the side of lower concentration.

Most of the classic saturable transporters at the BBB are facilitated diffusion systems (Kaur et al. 1992). For example, GLUT-1, the transporter for glucose, is a facilitated diffusion transporter. If the level of glucose is artificially raised above that of serum (or if radioactive glucose is introduced into the CNS, but not the serum), efflux of glucose can be shown.

##### Transcytotic Versus Transmembrane Transport

Saturable transporters can also be categorized based on whether they use pores/channels or vesicles to transport their ligands across the BBB. In the pore system, the molecule

crosses from one side of the cell membrane to the other by passing through a cavity in the transporter protein. The substance is thus transported either into or out of the cytoplasm of the BBB cell; a second set of transporters on the opposing cell membrane completes the transfer across the BBB or the substance can rely on transmembrane diffusion. With vesicular transport, the transported substance adheres to a binding site, usually a glycoprotein. Invagination then produces a vesicle that is then routed to the opposite membrane, and the contents of the vesicle are released from the cell surface. A specificity of transport distinguishes these vesicles from the macropinocytosis whose reduction is a defining characteristic of the BBB (Reese and Karnovsky 1967).

Most small molecules, such as glucose, electrolytes, and amino acids, use pores or channels. Pore systems may be either active or facilitated diffusion systems. Vesicular transporters, on the other hand, are energy requiring and so are characterized by unidirectional transport. The best described of these vesicular dependent systems is receptor-mediated transcytosis and is characterized by clathrin- and transglutaminase-dependence (Davies et al. 1980). However, non-clathrin dependent vesicles, such as podocytes, are also likely active at the BBB.

It is reasonable to assume that very large molecules would be required to use vesicles rather than pores and channels to cross, but the molecular weight at which vesicles would be requisite is not known. It has been proposed that interleukin-2 (IL-2) is transported (Drach et al. 1996) by p-glycoprotein (P-gp). As P-gp is a pore system (Begley 2004), IL-2 would be the largest substance currently known to be transported by a pore system. Peptides much smaller than IL-2 are known to cross by vesicular dependent pathways (Shimura et al. 1991; Terasaki et al. 1992). It is clear, then, that the size of the ligand alone does not dictate the need for vesicular transport.

### Diapedesis of Immune Cells

A major shift in thinking about the relation of immune cells to the CNS and BBB has occurred over the last few decades. The CNS was once viewed as separate from the immune system and sterile in terms of immune cell occupancy except under conditions of brain infection. As reviewed above, it is now clear that immune cells patrol the normal CNS, although many important questions remain. For example, a major type of brain cell, the microglia, is known to be derived from peripheral macrophages, although the extent to which the pools of peripheral macrophages and microglia mix in the normal postnatal condition is unknown.

### Adsorptive Endo- and Trans-cytosis

Adsorptive endocytosis occurs when a glycoprotein on the brain's endothelial surface binds another glycoprotein in ligand like fashion (Broadwell et al. 1988; Broadwell 1989). This second glycoprotein (the ligand) may be free or attached to the surface of a virus or immune cell (Mellman et al. 1986). The binding can initiate endocytosis with the subsequent vesicle having several potential fates (Banks and Broadwell 1994). In some cases, the vesicle is routed to lysosomes, the glycoprotein destroyed, and the vesicle rerouted to the endothelial cell surface for discharge of contents. In other cases, the vesicle can be routed to the Golgi complex and endoplasmic reticulum. In other cases still, the vesicle can be discharged at the endothelial cell surface opposite to that of uptake. In this case, the vesicle has crossed the width of the endothelial cell, and hence crossed the BBB, in a transcytotic event. What determines the fate of these vesicles is largely unknown, but at least some vesicles can engage in more than one fate (Broadwell 1993). It may be that binding of a large amount of glycoprotein to the endothelial cell can overwhelm the lysosomal pathway and result in the vesicles being routed to the trancytotic or Golgi complex pathways.

Several principles of adsorptive endocytosis and transcytosis (also termed adsorptive-mediated transcytosis) are clear. Many of the glycoprotein ligands are toxic, and endocytosis may represent a mechanism to rejuvenate or repair the membrane (Raub and Audus 1990; Vorbrodt 1994; Westergren and Johansson 1993). Viruses and other pathogens that can infect or cross the BBB have often co-opted adsorptive endocytosis/transcytosis mechanisms (Marsh 1984; Chou and Dix 1989; Schweighardt and Atwood 2001). These processes may also be related to diapedesis as many of the events of immune cell passage across the BBB resemble these endocytic mechanisms. For example, both LFA-1 (leukocyte function-associated antigen-1) and ICAM (intercellular adhesion molecule), important to immune cell passage across the BBB, are glycoproteins. Although adsorptive endocytosis is in some sense saturable because of a finite amount of any single glycoprotein on a cell surface, it is not easy to demonstrate classical saturable kinetics for this process. In fact, excess glycoprotein can sometimes further stimulate endocytosis and so lead to a paradoxical increase, rather than decrease, in the rate of passage across the BBB (Banks et al. 1997). Glycoprotein distribution on brain endothelial cells is polarized; that is, a glycoprotein may be enriched on either the luminal or abluminal membranes (Vorbrodt 1994; Zambenedetti et al. 1996). The tight junctions act as a "fence" to keep the glycoproteins confined to their respective sides of the endothelial cell (Deli et al. 2005). This means that the movement of a glycoprotein molecule (or a virus whose coat displays that glycoprotein) can be unidirectional as its transcytosis can only be initiated from the side of the brain endothelial cell that contains the ligand's complementary glycoprotein (Villegas and Broadwell 1993; Broadwell 1989). The possession and distribution of glycoproteins similarly dictate which viruses can invade the brain; neurovirulent viruses that invade the brain as free virus (as opposed to entering in Trojan horse fashion inside an infected

immune cell) can do so because they possess a glycoprotein ligand capable of binding to the BBB.

Other molecules besides glycoproteins can also induce adsorptive endocytosis/transcytosis type mechanisms. A classic example is polycationic molecules such as the poly-L-lysines and the protamines. Protamines are peptides of about 30 amino acids that contain an abundance of arginine molecules. They can induce adsorptive transcytosis so vigorously as to result in BBB disruption (Vorbrodt et al. 1995; Hardebo and Kahrstrom 1985). One of the proofs that viruses co-opt adsorptive trancytosis like mechanisms is that they, like protamine, bind to heparins and heparans; indeed, protamine sulfate blocks viruses such as HIV-1 from binding to the BBB (Banks et al. 2004c; Ramos-Kuri et al. 1996; Bobardt et al. 2004). Many of the highly charged penetrating peptides, such as those derived from Tat, and many of the antibodies that target receptor-mediated transporters likely are taken up by adsorptive endocytosis/transcytosis related mechanisms (Niewoehner et al. 2014; Weissmann 1976; Herve et al. 2008).

### 2.2.2 CNS to Blood

Traditionally, passage in the brain-to-blood direction (efflux) has been neglected. However, efflux often accounts for the inability of otherwise effective drugs to accumulate in the CNS. Pharmacogenomic studies have suggested that the individual variation in efflux mechanisms may explain why some individuals are less sensitive to the CNS effects of drugs or more sensitive to their toxicities (Loscher and Potschka 2002). Efflux mechanisms are important to the homeostasis of the CNS, ridding the brain of toxins (Taylor 2002). The rate of efflux can be, in addition to synthesis and degradation, an important determinant of the level of a substance produced within the CNS (Chen et al. 1997; Chen and Reichlin 1998; Maness et al. 1998).

#### 2.2.2.1 Nonsaturable

Efflux, like influx, has both saturable and non-saturable mechanisms of entry. Transmembrane diffusion occurs for both influx and efflux. Other mechanisms, such as bulk flow, are unique for efflux.

##### Transmembrane Diffusion

Many of the principles that govern influx by transmembrane diffusion are also important in efflux. The dramatic role that efflux by transmembrane diffusion can play can be illustrated by comparing the fate of small, lipid soluble molecules to that of a protein after intrathecal administration. Intrathecal application of small, lipid soluble molecules, such as anesthetics, can have a local effect on spinal cord function but have little or no effect on the brain (Bernards 1999). These substances readily cross the brain endothelial cell by transmembrane diffusion and do this as easily in the brain-to-blood direction as in the blood-to-brain direction. Therefore, they are cleared from the CSF before they are able to reach the brain (McQuay et al. 1989). In contrast, proteins such as leptin and lysosomal enzymes are too large and water soluble to undergo significant transmembrane diffusion (McCarthy et al. 2002; LeBel et al. 1999). Leptin, tetanus antitoxin, and the lysosomal enzyme idursulfase can reach the brain after intrathecal administration in amounts sufficient to produce CNS effects (Calias et al. 2012; McCarthy et al. 2002; Kabura et al. 2006; LeBel et al. 1999).

Efflux by transmembrane diffusion can also contribute to the poor diffusion of substances within brain parenchyma. Diffusion within the interstitial space of the brain is dependent on Brownian motion and the production of metabolic free water as driving forces and so is very slow (Cserr 1984; Cserr and Berman 1978). However, efflux by non-saturable (and saturable) mechanisms can further reduce the distance a substance will ultimately diffuse. For example, the less lipid soluble drug atenolol can diffuse about three times further into brain tissue than can the more lipid soluble drug acetaminophen (de Lange et al. 1993).

##### Bulk Flow and the Glymphatic System

Bulk flow refers to the reabsorption of CSF into the blood, which occurs at the level of the arachnoid villi (Davson and Segal 1996a) and cribriform plate (Widner et al. 1987; Yamada et al. 1991). Any substance dissolved in CSF will enter the blood by this mechanism (Pollay and Davson 1963; Jones and Robinson 1982). The glymphatic system provides an important mechanism for the mixing of the CSF and brain interstitial fluid, with aquaporin-dependent fluid production at the astrocytes providing the circulant and arteriole pulsations providing the directionality (Iliff et al. 2012). Thus, the glymphatics are important not only for bulk flow, but also for the extracellular pathways, and possibly for the movement of CSF from the spinal cord into the cranium.

The characteristics of this system result in several surprising but important phenomena. For example, CSF drained at the cribriform plate, which is likely the dominant route for CSF drainage at normal CSF pressures (Boulton et al. 1999), can enter into the cervical lymphatic system. This can provide a direct route from the CNS to the cervical lymphatics (Oehmichen et al. 1979), as has been illustrated for gp120, the glycoprotein of the human immunodeficiency virus, HIV-1 (Cashion et al. 1999). This route to the lymphatics may explain why substances injected into the brain can produce a different immune response than when the substance is injected peripherally (Knopf et al. 1995; Cserr and Knopf 1992). Another example is that in some cases, the levels of a substance in blood achieved after injection into the CSF can be sustained longer and at higher levels than after an intravenous bolus (Maness et al. 1998; Chen

et al. 1997; Chen and Reichlin 1998) This is because the central injection acts similarly to an intravenous infusion, slowly delivering drug to the blood. Impairment in glymphatic circulation can result in decreased bulk flow and so may contribute to increased levels of protein and toxins in the CSF (Iliff et al. 2013).

#### 2.2.2.2 Saturable Transport

The last decade has seen a huge increase in the interest of efflux by saturable mechanisms. Just as efflux by transmembrane diffusion can limit diffusion of a substance within the CNS, so can the presence of a saturable efflux transporter (Blasberg 1977). Much of this interest centers on the multidrug efflux transport systems (Begley 2004), most notably p-glycoprotein (P-gp). However, other efflux transporters for peptides, proteins, endogenous substances, and drugs are known to play important roles in physiology and disease (Martins et al. 1997; Taylor 2002; Drion et al. 1996; Mealey et al. 2001). For example, peptide transport system-1 is a major regulator of brain levels of methionine enkephalin, an endogenous opiate which suppresses voluntary ethanol drinking (Plotkin et al. 1998). Depression and recovery of peptide transport systems-1 with ethanol drinking may relate to alcohol withdrawal seizures (Banks and Kastin 1989, 1994). IL-2 is currently the only cytokine known to be transported by a saturable efflux system (Banks et al. 2004b); some have postulated this transporter may be P-gp. Poor accumulation of protease inhibitors, antibiotics, AZT, anti-cancer drugs, and many other substances occurs because of efflux systems (Glynn and Yazdanian 1998; King et al. 2001; Lee et al. 1998; Loscher and Potschka 2002; Masereeuw et al. 1994; Spector and Lorenzo 1974). P-gp plays a major role in the efflux of intrathecally administered opiate analgesics (Thompson et al. 2000). Brain-to-blood transport of a corticotropin-releasing hormone is sufficient to influence spleenic levels of beta-endorphin (Martins et al. 1997). Impaired efflux of amyloid ß peptide, the peptide believed to cause Alzheimer's disease, develops with aging in mice which overexpress amyloid precursor protein, thus promoting further accumulation within brain of amyloid ß protein (Ghersi-Egea et al. 1996; Banks et al. 2003; Deane et al. 2004). Evidence suggests that impaired transport develops in humans as well and so may be a major mechanism for induction of Alzheimer's disease (Tanzi et al. 2004; Shibata et al. 2000).

## 2.3 Neuroimmune Interactions

The above discussion of BBB fundamentals is tailored towards understanding the role of the BBB in neuroimmune interactions. Below are specific examples of how the BBB is involved in neuroimmune interactions.

### 2.3.1 Binding Sites at the BBB: Receptors and Transporters

An important distinction for understanding the function of the BBB is that of receptors vs transporters. The term "receptor" has undergone a transformation of its usage since its introduction in the late nineteenth century when it was first used to denote some physiological function. Eventually, the term receptor was used to denote a physical binding site through which a drug or hormone could exert its effects on a cell. In the 1980s, a distinction was made between "receptor" and "binding site", the former being coupled to intracellular machinery that translated its binding into a cellular effect. Binding sites on the brain endothelial cell can represent transporters, but they can also represent traditional receptors, that is, binding sites coupled to intracellular machinery. For example, brain endothelial cells have both insulin receptors and transporters. As a result, insulin is transported across the BBB to exert effects inside the CNS, but insulin also alters a number of functions of the brain endothelial cell. As examples of the latter, insulin alters the BBB transport of zidovudine (AZT (Ayre et al. 1989), tryptophan (Cangiano et al. 1983), and leptin (Kastin and Akerstrom 2001) and alters brain endothelial cell alkaline phosphatase activity (Catalan et al. 1988). BBB studies have assumed that a binding site represents transporter function and are so designed as to not consider whether receptors as well as transporters may exist at the BBB. However, a great deal of indirect evidence and some direct evidence indicates that the vascular BBB and the choroid plexus probably possess a large variety of receptors that can alter BBB functions. Besides insulin, substances which bind to and alter the function of brain endothelial cells include mu opiate receptor ligands (Baba et al. 1988; Vidal et al. 1998; Chang et al. 2001), cytokines (Ban et al. 1991; Cunningham et al. 1992; van Dam et al. 1996; Vidal et al. 1998; Moser et al. 2004; Khan et al. 2003), leptin (Kastin et al. 2000; Bjorbaek et al. 1998; Hsuchou et al. 2013a), acetylcholine (Grammas and Caspers 1991), adrenergics (Walsh et al. 1987; Kalaria and Harik 1989), glutamate (Koenig et al. 1992; Krizbai et al. 1998), and chemokines (Sanders et al. 1998).

### 2.3.2 Permeability to Cytokines and Related Substances

The BBB is known to transport several cytokines in the blood-to-brain direction. For example, the BBB transports the IL-1s, IL-6, and TNF-α by three separate transport systems. Additionally, nerve growth factor, brain derived neurotrophic factor, interferons, neurotrophins, eotaxin, fibroblast factor 19, and leukemia inhibitory factor (Poduslo

and Curran 1996; Erickson et al. 2014; Hsuchou et al. 2013b; Pan et al. 1997b, 1998a, b) are also transported across the BBB. In some cases, the same gene which gives rise to a cytokine's receptor also produces the cytokine's transporter; whereas in other cases, the receptor and transporter are different proteins (Pan and Kastin 2002; Banks et al. 2002). Recently, a BBB transporter for pituitary adenylate cyclase activating polypeptide (PACAP) was found to be the same protein which acts as a neuronal receptor for enterostatin, but not for PACAP, and acts as a lipid transporter in the liver (Martinez et al. 2003; Park et al. 2004; Dogrukol-Ak et al. 2009). In general, BBB transporters occur throughout the CNS, including the spinal cord, although the transport rate across the BBB can vary greatly among CNS regions (McLay et al. 1997; Pan et al. 1997b, 1998b; Banks et al. 1994). Enough cytokine is transported into the brain to affect CNS function. For example, IL-1 alpha crosses the BBB at the posterior division of the septum where it mediates cognitive impairments (Banks et al. 2001). Similarly, serum TNF-α crosses the BBB to induce CNS release of TNF-α, which in turn can induce apoptosis in the substantia nigra (Qin et al. 2007).

The cytokine transporters are not static but respond to physiological and pathological events. The transport rates of IL-1 and TNF-α each show diurnal variations (Pan et al. 2002; Banks et al. 1998b). The transport rate of TNF-α is altered in animals with experimental allergic encephalomyelitis (EAE), spinal cord injury, or blunt trauma to the brain (Pan et al. 1996, 1997a, 2003b; Pan and Kastin 2001b; Pearse et al. 2003).

### 2.3.3 Permeability to Other Neuroimmune Substances

Other substances with neuroimmune actions are handled by the BBB in a variety of ways. Monoamines are largely excluded by the BBB (Hardebo and Owman 1990; Kalaria et al. 1987), and opiates and opiate peptides as a rule enter the brain by transmembrane diffusion but are transported by saturable systems in the brain-to-blood direction (King et al. 2001; Banks and Kastin 1990; Elferink and Zadina 2001). Pituitary adenylate cyclase activating peptide, a member of the VIP/secretin/PACAP family, has immune functions (Arimura 1992). Transport of its two major forms across the BBB is complex, involving both brain-to-blood and blood-to-brain components (Banks et al. 1993). Its blood-to-brain transport is altered with CNS injury (Somogyvari-Vigh et al. 2000). Some of the other immune active substances whose passage across the BBB has been investigated are melanocyte stimulating hormone (Martins et al. 1996; Wilson et al. 1984), corticotrophin releasing hormone (Martins et al. 1996), and enkephalins (Banks et al. 1986; Elferink and Zadina 2001).

### 2.3.4 Permeability to Immune Cells

As discussed above, immune cells cross the BBB by the highly regulated process of diapedesis. The mechanism by which immune cells cross the BBB has also been greatly clarified by recent work. Two major assumptions about how immune cells would enter the CNS has not withstood investigation. The first assumption was that immune cells would enter the CNS by leaking across a disrupted BBB. However, disruptions to the BBB are usually mediated by increased vesicular activity in the endothelial cells (Vorbrodt et al. 1995; Lossinsky et al. 1983; Mayhan and Heistad 1985). These vesicles of 100 nm or so could not accommodate the passage of an immune cell 10,000 nm in diameter. Even in diseases where there is both increased immune cell trafficking into the CNS and a disrupted BBB, there is often a mismatch between the site of immune cell entry and BBB disruption (Engelhardt and Wolburg 2004).

The second major assumption is that immune cells would cross between opposing endothelial cells taking the "paracellular route." However, evidence suggests that many immune cells favor a transcellular route and that cells can cross both the vascular BBB and the choroid plexus (Kivisakk et al. 2003; Wolburg et al. 2005). In brief, immune cells tunnel through venular endothelial cells leaving the intercellular tight junctions intact (Engelhardt 2008; Wolburg et al. 2005). This tunneling process is complex and is initiated when LFA-1 on an immune cell binds to ICAM on the brain endothelial cell. Other paracellular messengers, which likely include cytokines, are then released (Male 1995; Persidsky et al. 1997). Protrusions and invaginations of the endothelial cell and protrusions of the immune cell occur, with the immune cell possibly using the tight junction as an initial anchoring site (Lossinsky et al. 1991). Other ligands which have been postulated to play a role in this transcytotic process include PECAM, VE-cadherin, members of the JAM family and CD99 (Engelhardt and Wolburg 2004). Some plasma inevitably accompanies the passage of the immune cells, which can give the appearance of a disrupted BBB (Greenwood et al. 1995; Avison et al. 2004; Persidsky et al. 2000).

The immune processes that induce an immune cell to cross the BBB are complex. Quan has shown that injection of interleukin-1 into brain tissue induces immune cell trafficking, but that such induction can be blocked by injection of lipopolysaccharide (LPS) into the periphery (Ching et al. 2005, 2006; Quan et al. 1994). Although trafficking is a product of a complex interaction between the immune cell and the BBB, the degree to which trafficking occurs can reside primarily with the immune cell or the BBB and genetics or immune events can shift that dominance (Banks et al. 2012). Such events may underlie euflammation, the phenomenon by which subclinical activation of the innate immune system renders it increasingly resistant to such activation by increasingly stronger stimuli (Tarr et al. 2014).