INFLAMMATION, LIFESTYLE, AND CHRONIC DISEASE

THE SILENT LINK

EDITED BY

BHARAT B. AGGARWAL • SUNIL KRISHNAN • SUSHOVAN GUHA

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OXIDATIVE STRESS AND DISEASE

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Dedicated to:

Our Sages, Rishis, Saints, Acharyas, Scientists, Gurus, and Parents whose wisdom continues to inspire and guide us!

"Gururbrahma Gururvishnuh Gururdevo Maheshwarah,

Guru Sakshat Parambrahma Tasmai Shree Gurave Namah"

(Salutations are to that Guru who is the creator, sustainer and the destroyer, the limitless one!)

"Yatkaromi Yatashnami Yajjuhomi Dadami Yat

Yatpsyami Kountiya Tatkromi Tavarpanam"

(modified from Gita 9-27)

(Whatever I do, whatever I eat, whatever I offer in sacrifice, whatever I give as charity, whatever austerity I perform. I do that as offering unto you, O lord supreme!)

Contents

Preface

Inflammation has been described for thousands of years by many names: in Indian Ayurvedic medicine it is *Sooj*; in traditional Chinese medicine it is *Qi*. It was, however, Cornelius Celsus from first-century Rome who first described inflammation as consisting of heat, pain, redness, and swelling (i.e., calor, dolor, rubor, and tumor). The link between inflammation and various chronic diseases was first suggested by the German physician Rudolf Virchow in the nineteenth century.

The word *inflammation* is derived from the word for *flame* or *fire*. Just as controlled fires can be harnessed for societal benefit in multiple ways, inflammation is an evolutionarily conserved defense mechanism that is essential for diverse human bodily functions.

However, when these flames flare out of control, they can trigger a plethora of unwanted phenomena that eventually culminate in chronic disease. Whereas acute inflammation generated by the immune system serves a therapeutic role, chronic low-level inflammation that may persist "silently" for decades is responsible for chronic diseases. Dysregulated or excessive inflammation, induced by lifestyle factors such as psychological stress, grilled meat, radiation, tobacco, infections, and environmental pollution, is emerging as a fundamental initiator of most chronic human diseases, including cancer, diabetes, obesity, Alzheimer's disease, arthritis, and cardiovascular diseases. Since most of these are diseases of old age, inflammation appears to be linked to the aging process as well.

The current monograph is an attempt to describe the essential role of dysregulated inflammation in various chronic diseases. In many instances, these chronic diseases are preventable, provided major lifestyle changes are made. However, once these diseases manifest themselves, their treatment with steroids and nonsteroidal anti-inflammatory drugs (NSAIDs), the traditional treatments for acute inflammatory diseases, is fraught with devastating side effects that preclude their long-term use. Because chronic diseases caused by chronic inflammation require chronic treatment, many of the chapters in this monograph also address the role of dietary agents, such as fruits, vegetables, legumes, pulses, nuts, and spices, as ideal anti-inflammatory agents that can be consumed chronically. This supports the aphorism by Hippocrates recorded almost 25 centuries ago: "Let food be thy medicine and medicine be thy food."

We first thank all the authors for their exciting contributions to this book. We also thank Dr. Chitra Sundaram for her help in assembling the whole manuscript. We hope that our readers find this book informative and useful.

Bharat Bhushan Aggarwal, PhD

Sunil Krishnan, MD

Sushovan Guha, MD, PhD

Editors

Dr. Bharat Bhushan Aggarwal is the Ransom Home, Jr., Distinguished Professor of Cancer Research in the University of Texas MD Anderson Cancer Center Division of Cancer Medicine's Department of Experimental Therapeutics, and chief of the Cytokine Research Laboratory, in Houston, Texas. He earned a PhD in biochemistry at the University of California in Berkeley, then underwent postdoctoral training at the University of California Medical Center in San Francisco. Afterwards, he worked for 10 years at Genentech, Inc., where he isolated and determined the structure of TNF-α and TNF-β, before returning to a university-based academic position.

The primary focus of Dr. Aggarwal's research has been the role of inflammatory pathways in tumorigenesis, and especially the impact of the transcription factor nuclear factor κB and STAT3 pathways in cancer. His group has also been interested in the use of natural products such as anti-inflammatory nutraceuticals from diet, spices, and traditional medicine, to modulate both inflammatory pathways linked to survival, proliferation, invasion, angiogenesis, and metastasis of tumors, and cancer-induced bone loss. Several of these efforts have led to clinical trials targeting cancer patients using agents that are safe and affordable.

He has published more than 600 papers, has been granted more than a dozen patents, and has been invited to more than 300 national and international conferences to deliver lectures. Dr. Aggarwal's research is currently funded by the National Institutes of Health and by various private organizations. Dr. Aggarwal also cofounded the International Society for Translational Cancer Research. His work has garnered many awards, including the Ranbaxy Award, an Outstanding Scientist Award from the American Association of Indian Scientists in Cancer Research, a McCormick Science Institute Research Award from the American Society of Nutrition, the Kosuna Distinguished Lecture Award from the University of California, Davis, and World Congress on Oxygen Club of California.

Dr. Sunil Krishnan is director of Gastrointestinal Translational Research and associate professor of radiation oncology at the University of Texas MD Anderson Cancer Center in Houston. He received his MD degree at the Christian Medical College in Vellore, India, and then completed an internal medicine residency at Penn State Geisinger Medical Center in Danville, Pennsylvania, and a radiation oncology residency at Mayo Clinic College of Medicine in Rochester, Minnesota, before joining the University of Texas MD Anderson Cancer Center.

The overarching goal of Dr. Krishnan's research is to develop novel strategies to improve radiation treatment outcomes for gastrointestinal malignancies. In the case of incurable malignancies such as locally advanced pancreatic, hepatic, and biliary tract neoplasms, an improvement in efficacy and reduction in toxicity of radiation therapy are likely to translate to an improvement in survival rates and quality of life. In case of resectable malignancies such as rectal, gastric, and gastroesophageal cancers, this approach could potentially result in adoption of organ-sparing alternatives to radical surgery in select subsets of patients. His laboratory's primary focus has been the role of inducible resistance to radiation therapy mediated by inflammatory signaling pathways, especially the impact of the transcription factor nuclear factor kappa B pathway. Although the quest for radiosensitization strategies started with the use of highly targeted pharmaceutical agents, more recently these inquiries have focused on the use of broad-spectrum natural products that simultaneously and seamlessly modulate multiple inflammatory pathways linked to survival, proliferation, DNA repair, invasion, angiogenesis, and metastasis of tumors. Some of these efforts have led to clinical trials in cancer patients.

Dr. Krishnan has published more than 80 papers in peer-reviewed journals, presented numerous seminars and lectures at national and international academic centers and conferences, and is currently funded by the National Institutes of Health and various nonprofit organizations.

Dr. Sushovan Guha is the site director of the Gastroenterology Fellowship Program and assistant professor of gastroenterology, hepatology, and nutrition at the University of Texas MD Anderson Cancer Center in Houston. He earned his MD degree from Jawaharlal Institute of Post-Graduate Medical Education and Research (JIPMER), Pondicherry, India, and subsequently graduated with an MA/MPhil in microbiology and immunology from Columbia University, New York. Next, he completed his internship and residency in internal medicine at the Albert Einstein College of Medicine in Bronx, New York. Dr. Guha then joined the prestigious Specialty Training and Advanced Research (STAR) Fellowship in Gastroenterology and Hepatology at the David Geffen School of Medicine at UCLA, Los Angeles, where he also received his PhD from the Molecular Biology Institute (MBI) under the astute tutelage of Professor Enrique Rozengurt.

Dr. Guha is a board-certified clinical gastroenterologist, internist, and physician-scientist at the University of Texas MD Anderson Cancer Center. The research focus in Dr. Guha's laboratory consists of unraveling signal transduction pathways in pancreatic cancer (PaCa), a devastating disease quite incalcitrant to conventional therapeutic regimens. Thus, there is an urgent need to develop novel therapeutic regimens, which will arise from a better understanding of the genetic and epigenetic changes leading to mitogenic signal transduction pathways. His current focus is to dissect G-protein-coupled receptor (GPCR)-mediated protein kinase D (PKD)-induced mitogenic and angiogenic signaling pathways in PaCa. His group showed that PKC-PKD signaling pathways downstream of GPCRs contribute to both mitogenesis and angiogenesis in PaCa. His laboratory uses various molecular and cellular biological techniques to unravel PKD-dependent critical signaling pathways. His group has developed an orally available specific small-molecule inhibitor of PKD with strong therapeutic potency and is performing preclinical studies in multiple animal models of PaCa. His group is also developing genetically engineered models in mice to study the role of PKD in initiation and progression of PaCa. Finally, his laboratory is characterizing novel downstream targets (substrates) of PKD that modulate key cellular processes, including oncogenic *Ras*-dependent mitogenesis, migration, drug resistance, and epithelial-to-mesenchymal transition in PaCa. He has published more than 75 papers in peer-reviewed journals, presented numerous seminars and talks at national and international academic centers and conferences, and is currently funded by the National Institutes of Health and nonprofit organizations.

Contributors

Ghassan H. Abusaid
Division of Cardiology
Department of Internal Medicine
University of Texas Medical Branch at Galveston
Galveston, Texas

Naveed A. Adoni
Division of Cardiology
Department of Internal Medicine
University of Texas Medical Branch at Galveston
Galveston, Texas

Bharat B. Aggarwal
Cytokine Research Laboratory
Department of Experimental Therapeutics
University of Texas MD Anderson Cancer Center
Houston, Texas

Constance Aldinger
Musculoskeletal Research Group
Institute of Anatomy
Ludwig Maximilians University Munich
Munich, Germany

Lisa M. Alleva
Research School of Biology
Australian National University
Canberra, Australia

Nadir Arber
Integrated Cancer Prevention Center
Tel Aviv Sourasky Medical Center
Tel Aviv, Israel

Jack L. Arbiser
Atlanta Veterans Administration Medical Center
and
Department of Dermatology
Emory University School of Medicine
Atlanta, Georgia

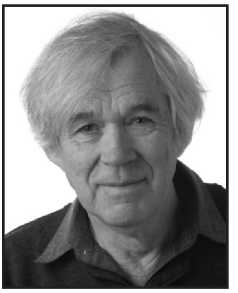
Milan Basta
Biovisions, Inc.
Potomac, Maryland

Ricardo L. Berrios
Department of Dermatology
Emory University School of Medicine
Atlanta, Georgia

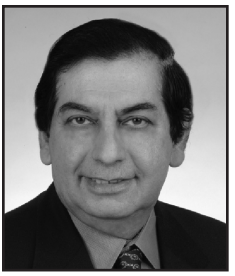
Ajay Chaudhuri
Division of Endocrinology, Diabetes, and Metabolism
State University of New York and Kaleida Health
Buffalo, New York

XinZhi Chen
Laboratory of Neurosciences
National Institute on Aging Intramural Research Program
Biomedical Research Center
Baltimore, Maryland

Chang Cheng
Department of Pharmacology
Yong Loo Lin School of Medicine
National University Health System
Singapore



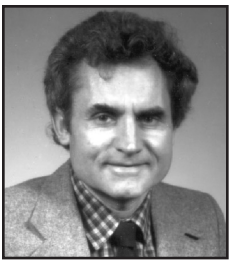
Ian A. Clark
Research School of Biology
Australian National University
Canberra, Australia



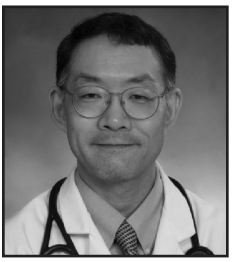
Paresh Dandona
Division of Endocrinology, Diabetes, and Metabolism
State University of New York and Kaleida Health
Buffalo, New York

Amitava Das
Comprehensive Wound Center
Department of Surgery
The Ohio State University
Columbus, Ohio

Sandeep Dhindsa
Division of Endocrinology, Diabetes, and Metabolism
State University of New York and Kaleida Health
Buffalo, New York



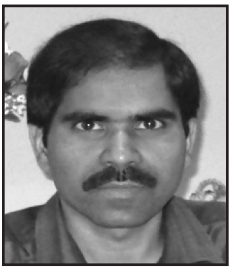
Milan Fiala
VA Greater Los Angeles Healthcare System
and
Department of Medicine
David Geffen School of Medicine at UCLA
Los Angeles, California



Ken Fujise
Division of Cardiology
Departments of Internal Medicine, and Biochemistry and Molecular Biology
University of Texas Medical Branch at Galveston
Galveston, Texas

Kasturi Ganesh
Comprehensive Wound Center
Department of Surgery
The Ohio State University
Columbus, Ohio

Husam Ghanim
Division of Endocrinology, Diabetes, and Metabolism
State University of New York and Kaleida Health
Buffalo, New York



Subash C. Gupta
Department of Experimental Therapeutics
University of Texas MD Anderson Cancer Center
Houston, Texas

Eugene W. X. Ho
Departments of Pharmacology, and Epidemiology and Public Health
Yong Loo Lin School of Medicine
National University Health System
Singapore

Ramaswamy Kannappan
Department of Experimental Therapeutics
University of Texas MD Anderson Cancer Center
Houston, Texas

Elena Kapilevich
Integrated Cancer Prevention Center
Tel Aviv Sourasky Medical Center
Tel Aviv, Israel

Ji Hye Kim
Department of Experimental Therapeutics
University of Texas MD Anderson Cancer Center
Houston, Texas

Sarah Kraus
Integrated Cancer Prevention Center
Tel Aviv Sourasky Medical Center
Tel Aviv, Israel

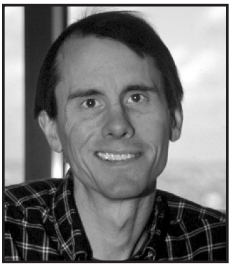
Antonio La Cava
Department of Medicine
David Geffen School of Medicine at UCLA
Los Angeles, California

Khai Pang Leong
Department of Allergy, Immunology, and Rheumatology
Tan Tock Seng Hospital
Singapore

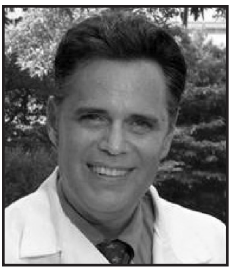
Victoria Lisiansky
Integrated Cancer Prevention Center
Tel Aviv Sourasky Medical Center
Tel Aviv, Israel

Michelle Mahanian
Department of Medicine
David Geffen School of Medicine at UCLA
Los Angeles, California

Antoine Makdissi
Division of Endocrinology, Diabetes, and Metabolism
State University of New York and Kaleida Health
Buffalo, New York



Mark P. Mattson
Laboratory of Neurosciences
National Institute on Aging Intramural Research Program
Biomedical Research Center
Baltimore, Maryland



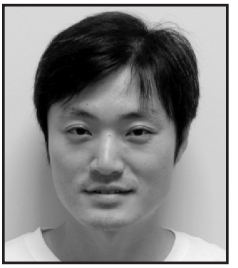
Andrew H. Miller
Department of Psychiatry and Behavioral Sciences
Winship Cancer Institute
Emory University School of Medicine
Atlanta, Georgia

Mathew T. Mizwicki
Department of Biochemistry
University of California, Riverside
Riverside, California

Ali Mobasheri
Musculoskeletal Research Group
School of Veterinary Medicine and Science
University of Nottingham
Leicestershire, United Kingdom

Eitan Okun
Laboratory of Neurosciences
National Institute on Aging Intramural Research Program
Biomedical Research Center
Baltimore, Maryland

Thaddeus W. W. Pace
Department of Psychiatry and Behavioral Sciences
Winship Cancer Institute
Emory University School of Medicine
Atlanta, Georgia



Byoungduck Park
Department of Experimental Therapeutics
University of Texas MD Anderson Cancer Center
Houston, Texas

Jigar R. Patel
Department of Dermatology
Emory University School of Medicine
Atlanta, Georgia

Kanokkarn Phromnoi
Department of Experimental Therapeutics
University of Texas MD Anderson Cancer Center
Houston, Texas

Sahdeo Prasad
Department of Experimental Therapeutics
University of Texas MD Anderson Cancer Center
Houston, Texas

Irfan Rahman
Department of Environmental Medicine
Lung Biology and Disease Program
University of Rochester Medical Center
Rochester, New York

Charles L. Raison
Department of Psychiatry and Behavioral Sciences
Winship Cancer Institute
Emory University School of Medicine
Atlanta, Georgia

Simone Reuter
Department of Experimental Therapeutics
University of Texas MD Anderson Cancer Center
Houston, Texas

Sashwati Roy
Comprehensive Wound Center
Department of Surgery
The Ohio State University
Columbus, Ohio

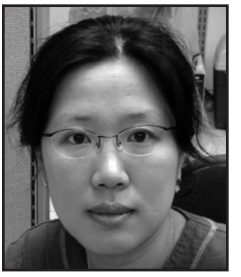
Chandan K. Sen
Comprehensive Wound Center
Department of Surgery
The Ohio State University
Columbus, Ohio

Gautam Sethi
Department of Pharmacology
Yong Loo Lin School of Medicine
National University of Singapore
Singapore

Mehdi Shakibaei
Musculoskeletal Research Group
Institute of Anatomy
Ludwig Maximilians University
Munich
Munich, Germany

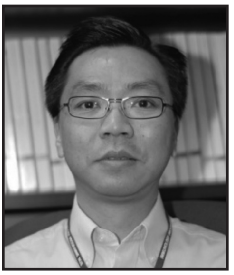
Muthu K. Shanmugam
Department of Pharmacology
Yong Loo Lin School of Medicine
National University of Singapore
Singapore

Chitra Sundaram
Department of Experimental
Therapeutics
University of Texas MD Anderson
Cancer Center
Houston, Texas



Bokyung Sung
Department of Experimental
Therapeutics
University of Texas MD Anderson
Cancer Center
Houston, Texas

Vinay Tergaonkar
Institute of Molecular and Cellular Biology
Singapore



W. S. Fred Wong
Immunology Program
Life Science Institute
National University of Singapore
and
Department of Pharmacology
Yong Loo Lin School of Medicine
National University Health System
Singapore

Vivek R. Yadav
Department of Experimental Therapeutics
University of Texas MD Anderson Cancer Center
Houston, Texas

Hongwei Yao
Department of Environmental Medicine
Lung Biology and Disease Program
University of Rochester Medical Center
Rochester, New York

Jeffrey Zweig
Integrated Cancer Prevention Center
Tel Aviv Sourasky Medical Center
Tel Aviv, Israel

1 Roles of Innate Immunity and Inflammation in the Aging Brain

Eitan Okun, XinZhi Chen, Milan Basta, and Mark P. Mattson

CONTENTS

1.1 INTRODUCTION

Aging is associated with increased incidence of several neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS), as well as stroke (Joseph et al. 2009). All these disorders involve chronic inflammatory changes in the affected regions of the central nervous system (CNS) that contribute to the pathologic process (McGeer et al. 2004). There is evidence that in each of these disorders both the intrinsic (innate) and peripheral (humoral) immune systems are involved, perhaps initially as an adaptive response that subsequently becomes deleterious. While both the innate and peripheral immune processes are interconnected in various ways, we will mostly focus in this chapter on the roles of the innate immune response in the brain during aging and in age-related neurodegenerative conditions. For more information on the involvement of the peripheral immune system in the aging brain, see Kin and Sanders (2006).

Innate immune cells and innate immune receptors are expressed in both non-immune cells (neurons and astrocytes, for example) and classical immune cells, such as microglia. Microglial cells arise from bone marrow/mesenchymal cell-derived monocytes that enter the brain during development and differentiate intraparenchymally; microglia may reside in either a resting (surveying) phenotype with a small soma and highly branched processes, or an activated amoeboid form (Aloisi 2001). There is some evidence that new microglia may also be generated from bone marrow cells during adult life, particularly in response to injury (Guo et al. 2004). Microglia are similar, if not identical, to macrophages; they are considered innate immune cells because of their ability to respond directly to a pathogen without the need to communicate with the humoral immune system. Innate immune receptors, also referred to as pattern recognition receptors (PRRs), bind directly to pathogen-associated molecular patterns (PAMPs) in molecules produced by microbial pathogens. Danger-associated molecular patterns (DAMPs) are altered intrinsic molecules generated in damaged or severely stressed cells; these molecules act as ligands that can activate various innate immune receptors, including Toll-like receptors (TLRs) and RIG1-like receptors (RLRs).

1.2 INNATE IMMUNE EFFECTORS IN THE CENTRAL NERVOUS SYSTEM

The two most studied innate immune protein families in the context of CNS physiology and pathology are the TLRs and the complement system. In the following sections we will briefly describe these two protein families and expand on how they and related innate immune components are involved in molecular, cellular, and functional changes that occur in the brain during aging.

1.2.1 Toll-Like Receptors

TLRs are major PRRs that have a central role in the initiation of innate immunity against invading microbial pathogens. These integral membrane proteins have a single membrane-spanning domain, a leucine-rich extracellular domain, through

which they recognize PAMPs, and a cytoplasmic Toll/IL-1 receptor (TIR) domain similar to that of the interleukin-1 receptor (IL-1R), which initiates downstream signaling (Kawai and Akira 2007). Each TLR by itself or in combination with other TLRs recognizes distinct PAMPs that include lipids, lipoproteins, nucleic acids, and proteins. TLRs are ubiquitous, present in both immune and nonimmune cells, and their expression is rapidly altered in response to pathogens, cytokines, and environmental stressors (Akira et al. 2006).

Thus far, 11 human and 13 mouse TLRs have been identified. TLRs rely on receptor dimerization to achieve specificity in agonist recognition. TLRs may be segregated into groups based on the specific PAMPs they recognize. For example, TLRs 1, 2, 4, and 6 recognize lipids, while TLR4 predominantly recognizes lipopolysaccharides (LPSs) from Gram-negative bacteria. TLR2 dimerizes with TLR1 to recognize triacylated lipopeptides from bacteria, such as Pam3Csk4, or with TLR6 to respond to a variety of PAMPs, including peptidoglycan, diacylated lipopeptides such as Pam2Csk4, lipopolysaccharides of Gram-positive bacteria, fungal zymosan, and mucoplasma lipopeptides. TLR10, which is expressed only in humans, can heterodimerize with TLR2 and TLR1 (Akira et al. 2006). The second class of TLRs includes TLR5 and TLR11, which are activated in response to protein ligation. TLR5 is mainly expressed in the intestine, where it senses bacterial flagellin protein (Uematsu et al. 2008). TLR11 recognizes an unknown ligand of uropathogenic bacteria and a profilin-like molecule of the protozoan parasite *Toxoplasma gondii* (Akira et al. 2006). TLRs 3, 7, 8, and 9 comprise the third group in the TLR family, and are localized intracellularly, where they are ideally positioned for activation by nucleic acids of bacterial and viral origin. TLR3 is activated in response to double-stranded RNA (dsRNA) of viral origin. Human TLR8 and its murine orthologue, TLR7, recognize imidazoquinoline and viral ssRNA. TLR9 recognizes unmethylated CpG dinucleotides found in bacteria as well as viral genomes. An illustration of the different TLRs and their respective cellular localizations is shown in Figure 1.1.

In addition to the numerous exogenous ligands that activate the different TLRs, endogenous TLR ligands (or DAMPs, as defined above) have been identified in recent years. Endogenous TLR ligands include an array of extracellular matrix (ECM) proteins, such as low molecular weight hyaluronic acid (HA), fibrinogen, fibronectin, heparin sulfate proteoglycans, and immune-related proteins such as β-defensins (Pandey and Agrawal 2006). During tissue injury or proteolysis, ECM components undergo cleavage, with one or more of their cleavage products gaining the ability to act as TLR ligands. For example, high molecular weight HA is cleaved to low molecular weight HA, which subsequently binds TLRs 2 and 4 and activates signaling cascades downstream of these TLRs. Heat shock proteins, released from stressed cells, may also activate TLR4 (Lehnardt et al. 2008). In this way innate immune inflammatory responses may be activated without the presence of invading pathogens (Shimada et al. 2008).

Functional TLR signal transduction is complex and relies on receptor dimerization as well as the presence of accessory proteins and coreceptors, which regulate the signaling pathways initiated by each receptor. After recognition of PAMPs, TLRs activate the signaling components that mediate immune responses required for host defense. The cytoplasmic region of TLRs contains a TIR domain, which mediates

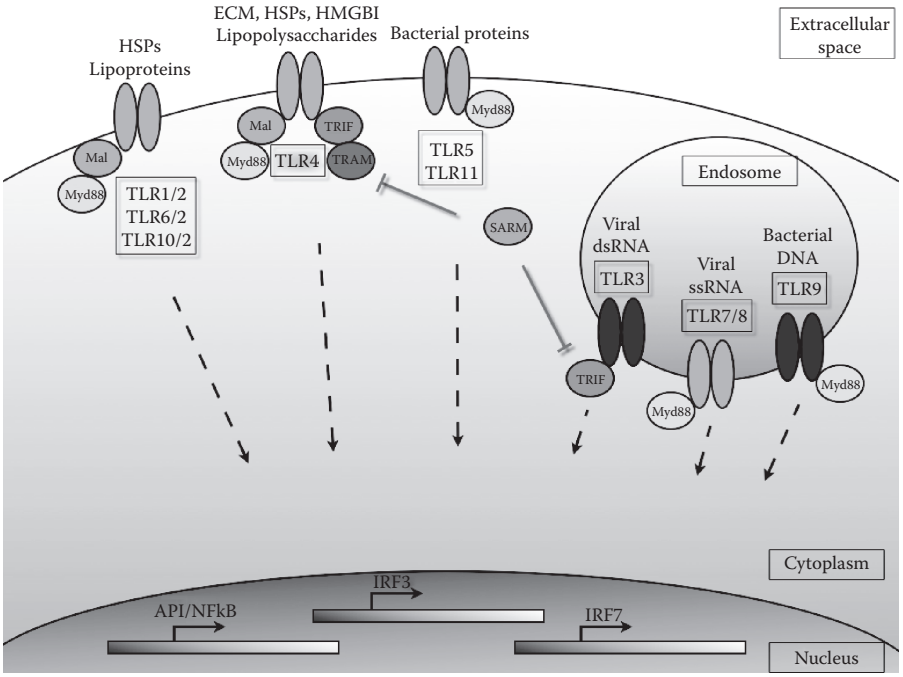


FIGURE 1.1 Illustration of the cellular localizations of the different TLRs. Plasma membrane TLRs: TLR2 can heterodimerize with either TLR1, TLR6, or TLR10. TLR4 forms mostly homodimers that can recognize lipopolysaccharides from Gram-negative bacteria or various DAMPs, such as HSPs, high mobility group B1 (HMGB1) proteins, or different extracellular matrix components, such as low molecular weight hyaluronic acid and fibronectin. TLR5 and TLR11 form mostly homodimers as well, and are thought to recognize bacterial proteins. Endosomal TLRs: TLR3 detects viral dsRNA, but can also detect endogenous RNA from ruptured cells. TLR7 and TLR8 detect ssRNA, whereas TLR9 detects CpG-rich bacterial DNA. Elliptical circles represent the different TIR domain-containing adaptor proteins (Mal, Myd88, TRIF, TRAM, and SARM) used by various TLRs, with SARM the only inhibitor adaptor protein capable of inhibiting TRIF-mediated signals from TLR3 and TLR4. Signaling from all TLRs culminates in activating members of the interferon regulatory factors, AP1, or NF-κB families of transcription factors.

homo- and heterophilic interactions between TLRs and TIR-containing adapters. TLRs recruit a set of adaptor proteins with TIR domains by homophilic interaction of their TIR domains. The signaling pathways activated by TLRs are broadly classified into myeloid differentiation factor 88 (MyD88)-dependent and -independent pathways; MyD88 is the universal adapter protein recruited by all TLRs except TLR3 (Kawai and Akira 2007).

Upon receptor activation and interaction with MyD88, one or more TIR-containing adapter proteins, TIRAP/Mal (TIR-domain-containing adapter/MyD88 adaptor-like), TICAM1/TRIF (TIR-domain-containing adaptor molecule 1/TIR-domain-containing adaptor-inducing interferon-po, and TRAM (TRIF-related adaptor molecule), are recruited along with IL-1R-associated kinases (IRAKs)-1, 2, 3, 4, or the inhibitory

IRAK-M. Once phosphorylated, IRAKs dissociate from MyD88 and interact with TNF receptor-associated factor 6 (TRAF6). TRAF6 forms a complex with Ubc13 and Uev1A to promote the synthesis of lysine 63-linked polyubiquitin chains, which in turn activate transforming growth factor β-activated kinase 1 (TAK1), a mitogen-activated protein kinase kinase kinase (MAPKKK) (Wang et al. 2001). TAK1, in combination with an activator subunit TAB1, TAB2, or TAB3, activates two downstream pathways involving the IKK complex and the MAPK family (ERK, JNK, or p38). The IKK complex, composed of the catalytic subunits IKKα and IKKβ and a regulatory subunit IKKγ, catalyzes the phosphorylation of IκB proteins (Kawai and Akira 2007). This phosphorylation leads to the degradation of IκBs and the subsequent nuclear translocation of the transcription factor nuclear factor κB (NF-κB). Members of the MAPK family phosphorylate and activate the transcription factor activator protein 1 (AP-1). Activation of the transcription factors NF-κB and AP-1 results in expression of pro-inflammatory cytokines such as interleukin (IL)-6, IL-1 and tumor necrosis factor (TNF)-α.

Most of the TLRs seem to be absolutely dependent on the expression of MyD88 for all of their functions, whereas TLR3 and TLR4 are capable of signaling through a MyD88-independent pathway. SARM, the fifth known TIR domain-containing adaptor protein, is the only inhibitory adaptor protein; SARM inhibits TLR3, as well as the MyD88-independent responses to TLR4 activation (Kenny and O'Neill 2008). Both TLR3 and TLR4 differ from other TLRs by their ability to activate interferon regulatory factor 3 (IRF3). Following TLR4 activation, a MyD88-independent pathway can be activated when TRIF is recruited in concert with TRAM. This culminates in MAPK signaling and activation of the transcription factors NF-κB and IRF-3. TRIF-dependent signaling following TLR3 activation acts through recruitment of the IKKs, TBK1, and IKK, which activate IRF3 (Arancibia et al. 2007). Alternatively, TLR3 may activate IRF2 through TRIF-dependent activation of phosphatidylinositol 3-kinase and Akt (Sarkar et al. 2004). Exceptionally, MyD88-dependent signaling of TLR7, TLR8, and TLR9 can also induce type I IFN production (Kawai and Akira 2007).

1.2.2 The Complement System

The complement system has at least 35 circulating and membrane-bound components, factors, regulatory proteins, and inhibitors. The complement system can be viewed as a link between innate immunity and humoral immunity, because its activation results in opsonic, chemotactic, and cytolytic activities to clear invading pathogens as an important component of the immune system. Excessive or unregulated complement activation may exacerbate host tissue injury associated with a variety of pathologic states, including aging and aging-related disease in the CNS (Gasque et al. 2002). The complement system consists of three activation pathways: (1) the classical pathway, (2) the lectin pathway, and (3) the alternative pathway (Figure 1.2). All three pathways eventually converge to a single terminal pathway involving regulatory proteins and complement receptors (Kinoshita 1991).

The classical pathway is commonly activated by antibodies binding to an antigen, but it also can be activated by aggregated amyloid proteins, petraxins, C-reactive

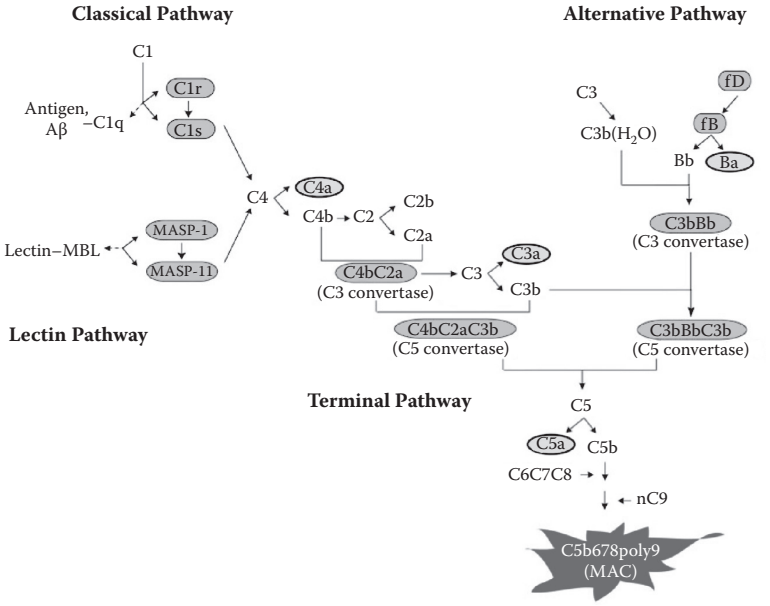


FIGURE 1.2 Schematic diagram of complement activation. According to the initial stimuli, complement activation can be induced by the classical, alternative, and lectin pathways. All three pathways can converge into the terminal pathway inducing target cell disruption if the membrane attack complex (MAC) is formed once the complement system is fully activated. Abbreviations: Aβ, amyloid β peptide; C1q, complement component 1 subcomponent q; MBL, mannose-binding lectin, MASP, mannose-activating surface protein; fB, factor B; fD, factor D. Gray tone circles indicate proteases with enzymatic activities. Oval shapes with dark outlines indicate generated anaphylatoxins during a series of events of complement activation.

protein, and necrotic or apoptotic cells without antigen–antibody interactions (Rogers et al. 1992; McGeer and McGeer 2004). The classical pathway is initiated by the attachment of C1q to a target that causes autoactivation of serine proteases C1r and C1s. Active C1s cleaves C4 into C4a and C4b, and then forms C3 convertase (C4bC2b) following C4b binding to C2 and release of C2a. The C3 convertase cleaves C3 into C3a and C3b, which in turn incorporates into C5 convertase (C4bC2bC3b) to cleave C5 into C5a and C5b. The attached complement fragments then become ligands for complement receptors on phagocytes, such as the microglia of the brain (Pinckard et al. 1975). The small fragments, such as C3a and C5a anaphylatoxins, have multiple pro-inflammatory effects (Hugli 1990).

The lectin pathway is initiated by binding of serum mannose-binding lectin (MBL) to simple carbohydrates (such as mannose and fucose) expressed on bacteria and viruses in a pathogen-specific manner (Matsushita 1996). The mannose-binding lectin-associated serine proteases MASP1 and 2 are structurally and functionally similar to the C1r and C1s in the classical pathway by their ability to cleave C4 and then C2 to generate C3 and C5 convertases (Thiel et al. 1997).

The alternative pathway is initiated by spontaneous activation of C3 by water molecules, without engaging early components of the classical pathway. The

spontaneously hydrolyzed C3 molecule, called C3 (H_2O), is able to form complexes with factor B. In the context of this complex, factor B is subject to proteolytic attack by factor D, resulting in a smaller fragment Ba (that gets released into the fluid phase), while the larger Bb fragment remains bound to the hydrolyzed C3 molecule. Under normal circumstances factors H and I mediate dissociation of the above complex that is capable of proteolytic cleavage of C3 into C3a and C3b. Amplification of the alternative pathway is initiated when cellular surfaces of pathogenic microorganisms (yeast cell wall component zymosan or LPS in the bacterial cell wall) provide a site where spontaneously activated C3b molecules are protected from the regulatory functions of factors H and I. Following stabilization of C3bBb molecule by properdin (that reduces the rate of decay of the complex), the amplification C3 convertase is formed. This convertase creates more C3bBb via an amplification loop that involves cleavage of more native C3 in the fluid phase. After several rounds of amplification, multimers of C3bBb molecules then reach a critical mass, sufficient to form the alternative pathway C5 convertase, and the pathway then proceeds through the common membrane attack complex (MAC) formation stage (McGeer and McGeer 2002).

Although each of the three complement pathways is initiated differently, all three have a common terminal portion of the pathway that begins with cleavage of C5 into C5a and C5b. The formation of C5b initiates a sequence of conformational and hydrophobic changes of complement components C6 through C9, resulting in the formation of the lytic C5b678poly9 complex or membrane attack complex (D'Ambrosio et al. 2001). Pores 9–12 nm in diameter form within the target membrane, depending upon the number of C9 proteins assembling. The MAC is usually inserted into foreign bacteria and viruses and can also damage host cells if cells are inadequately protected.

To counteract deleterious complement activation, cells are equipped with a series of endogenous membrane-bound complement inhibitors, such as binding proteins, receptors, and cofactor proteins. This is to ensure that the small complement fragments that stimulate inflammation do not harm host cells from uncontrolled complement-mediated damage (McGeer and McGeer 2002). Moreover, depending on the type of stimulus, although the complement system is meant to confer protection in the short term, it can also cause damage to the brain due to its strong cytotoxic capabilities by amplifying neuroinflammation unless tightly regulated (Rogers et al. 1992; McGeer et al. 1993). Many of the adverse effects of complement proteins can be counteracted by immunoglobulins, and data suggest that intravenous immunoglobulin can reduce injury and improve functional outcome in experimental models of stroke (Arumugam et al. 2007).

1.3 CELLULAR AND MOLECULAR CHANGES THAT OCCUR IN THE BRAIN DURING AGING

Several major alterations that occur in the brain during the aging process set the stage for hyperactivation of innate and humoral immune pathways. Similar to other tissues, these changes include oxidative stress, increased production of pro-inflammatory

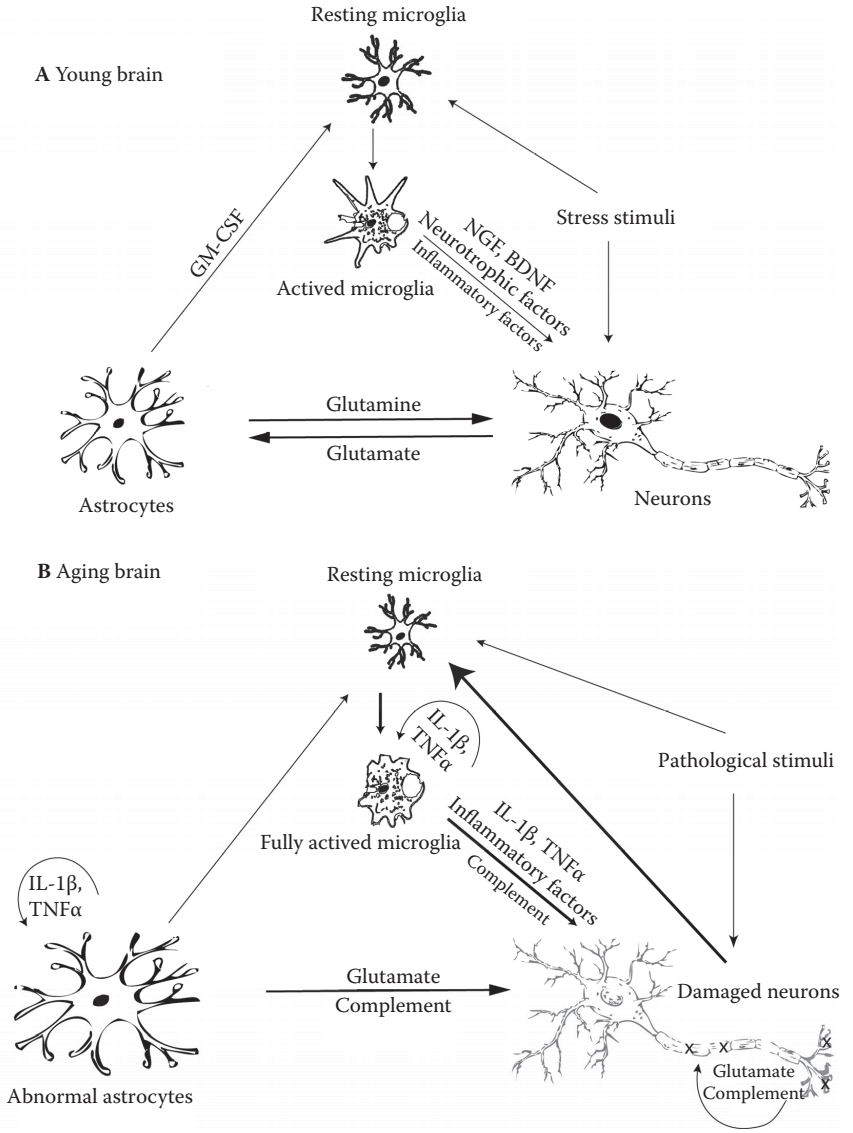


FIGURE 1.3 A simplified schematic representation of interactions of key cells in the young and aged brain.

cytokines, cellular damage and death, and the recruitment and activation of innate immune effector cells (microglia/macrophages) and circulating lymphocytes. These inflammatory processes may be relatively subtle in some individuals or more pronounced and closely associated with the disease process in those who suffer from AD, PD, and other age-related neurodegenerative conditions. A simplified illustration of the key cells that are affected in the brain during aging is shown in Figure 1.3. In this section we describe immunity-related changes that occur during normal aging

in the major cell types in the brain (astrocytes, microglia, and neurons). As this topic has been reviewed in considerable detail previously (Bishop et al. 2010; Mrak et al. 1997; Lucin and Wyss-Coray 2009), we will pay particular attention to the impact of age-related immune alterations on neural stem cells (NSCs) because of their potential to replace neurons and glial cells damaged in aging, injury, and disease.

1.3.1 Changes in Glial Cells during Aging

The numbers of activated astrocytes and microglia are increased during normal aging (Morgan et al. 2007). Data suggest that total numbers of astrocytes and pericytes may increase by ~20% in the aged cortex and other brain regions, whereas the number of oligodendrocytes and microglia does not change (Pilegaard and Ladefoged 1996; Peinado et al. 1998; Rozovsky et al. 1998). Astrocytes with an activated phenotype increase during aging in multiple brain regions (Cotrina and Nedergaard 2002). The increase in activated glial cells with age is sex-dependent and region-specific (Mouton et al. 2002), and in some brain regions such as the hippocampus, the numbers of astrocytes and microglia may not increase with age (Long et al. 1998).

1.3.1.1 Inflammation and Gliosis

Upon activation by tissue damage, severe cellular stress, or infection, microglia proliferate and undergo a morphological transformation from a ramified to an amoeboid appearance. Depending upon the nature of the activation stimulus, microglia respond so as to perform a specific task that is usually beneficial (e.g., removal of apoptotic cells or abnormal proteins). If the disturbance is relatively minor, microglia may secrete anti-inflammatory cytokines and supportive growth factors. This type of activation is also regarded as alternative activation, or M2 (Colton and Wilcock 2010). If the disturbance poses a more serious threat, such as a pathogen invasion, microglia can release toxic factors to kill the pathogen and recruit help by releasing pro-inflammatory cytokines. This type of activation is also referred to as classical activation, or M1 (Mantovani et al. 2004). M2 microglia are typically considered less inflammatory than M1 cells and are characterized by reduced nitric oxide production and increased anti-inflammatory cytokine production. Accordingly, there is heterogeneity of microglial activation states depending upon the nature, intensity, and duration of a pathological condition (Colton et al. 2006; Maier et al. 2008). The microglial phenotype could mean the difference between a beneficial outcome and a detrimental outcome if the response is either too aggressive or too passive (Lucin and Wyss-Coray 2009). This is especially important in the CNS, in which superfluous inflammation could result in excessive collateral damage to neurons, with dire functional and cognitive implications.

In response to injury and acute infection, microglia may release a combination of factors that function to limit the extent of the injury or infection. These include factors known to promote the survival and plasticity of neurons, including TNF-α and neurotrophic factors like brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) (Morgan et al. 2004; Cullheim and Thams 2007; Trapp et al. 2007), and protect neurons against glutamate-mediated excitotoxicity by, for example, increasing the expression of the glutamate transport

protein GLT-1 (Persson et al. 2005; Shaked et al. 2005) and Ca^{2+}-binding proteins (Cheng et al. 1994). On the other hand, parenchymal microglia derived from old mice exhibit an altered inflammatory profile (Streit 2006; Sierra et al. 2007), which may play a role in the previously characterized decrease in neurogenesis with aging (Kuhn et al. 1996). A lower turnover rate of parenchymal microglia during aging may result in a preponderance of microglia with the M1 phenotype that appears to dominate the scene in age-related neurodegenerative disorders.

1.3.2 Changes in Neurons during Aging

During normal aging, in the absence of disease, neuron numbers are believed to remain relatively stable in some brain regions, with modest neuronal loss occurring in other brain regions. For example, a stereological analysis of the cerebellum from human subjects aged 19 to 94 years of age revealed no evidence for loss of either granule neurons or Purkinje cells; however, the volumes of white matter and Purkinje cell somata were reduced, suggesting a reduction in the size of individual neurons (Andersen et al. 2003). Even dopaminergic neurons in the substantia nigra, which are considered to be particularly prone to degeneration and exhibit extensive loss in PD, are maintained at constant levels during aging in some individuals (Alladi et al. 2009). As described in Section 1.3.2.1, however, there is considerable evidence that the functionality of neuronal circuits declines during aging in many regions of the nervous system. While most neurons in the adult mammalian brain are believed not to be replaced, at least two populations of neurons are replaced: granule neurons in the dentate gyrus of the hippocampus and interneurons in the olfactory bulb (Conover and Notti 2008). The impact of aging on this process of neurogenesis is described in Section 1.3.2.2.

1.3.2.1 Neuronal Dysfunction and Degeneration during Aging

The efficiency and accuracy of most behaviors declines during aging. Decrements in vision, hearing, smell, and taste contribute to worsening functions in activities of daily living (Corso 1971). However, there are also deficits in the central processing of information, as reflected in poorer performance in tests of learning and memory (Lister and Barnes 2009) and motor coordination (Seidler et al. 2010), for example. Because neuronal loss is minimal during normal aging, it is likely that age-related deficits in neuronal circuit function are the result of more subtle structural and functional alterations. In this regard, changes in numbers of synapses or functional plasticity of synapses occur in at least some brain regions. Perhaps the most thorough analysis of normative brain aging has been performed by Peters et al., who examined area 46 in the prefrontal cortex of rhesus monkeys (Luebke et al. 2010). Their findings reveal loss of white matter, regression of dendritic arbors, and loss of dendritic spines and synapses. In a study of CBA mice of five ages (4, 8, 12, 18, and 24 months) there was an impairment of cerebellum-dependent delay eyeblink conditioning in the 24-month-old mice (Woodruff-Pak et al. 2010). Stereological analysis of their cerebella indicated significant loss of Purkinje neurons in the 18- and 24-month-old mice, and electrophysiological analysis demonstrated a significant deficit in long-term depression, whereas hippocampal long-term depression was normal in the old mice.

Neurons are maintained well beyond the age of 65 in both lamina III and V of the frontal cortex in human subjects (Scheff et al. 2001). The lack of synaptic decline in the frontal cortex in neurologically normal individuals older than 65 years lends support to the idea that many stereotypic views of age-related changes in the CNS do not apply to all brain regions. Therefore, neuron loss may contribute to age-related dysfunction in some brain regions, whereas in other regions the dysfunction results from loss or dysfunction of synapses.

Changes in neurons that occur during normal aging may be antecedents of common age-related neurodegenerative disorders, including AD and PD. Synapse loss occurs in the frontal cortex of AD patients, as demonstrated by electron microscope-based analysis of biopsy tissue samples (DeKosky and Scheff 1990). Analysis of the dentate gyrus of the hippocampus demonstrated a reduction in synapse number and an associated decline in the width of the molecular layer; interestingly, there was an increase in the size of remaining synapses (Scheff et al. 1996). Individuals with mild AD had fewer synapses in region CA1 of the hippocampus than age-matched individuals who were neurologically normal or were experiencing mild cognitive impairment (MCI); those with MCI had significantly fewer synapses than controls (Scheff et al. 2007). There was a positive correlation between performance on cognitive tests and total CA1 synapses; however, the total number of synapses showed no relationship to numbers of plaques and neurofibrillary tangles. Interestingly, despite the abundance of neurofibrillary pathology, there was no change in synaptic density in the entorhinal cortex between control and Alzheimer subjects (Scheff et al. 1993). Thus, the entorhinal cortex differs from other cortical areas that show a significant decline in synaptic numbers with AD.

1.3.2.2 Neurogenesis in the Aging Brain

Adult neurogenesis is a complex process involving pools of self-renewing progenitor cells that, upon receiving certain signals in their immediate environmental niche, can stop dividing and differentiate into neurons (Lathia et al. 2007). The newly generated neurons can grow axons and dendrites, and form functional synapses with preexisting neurons (Klempin and Kempermann 2007). While neurogenesis dramatically decreases in late embryonic and early postnatal periods, it does take place during adulthood and is thought to play a physiological role in learning and memory (Garthe et al. 2009).

In the adult mammalian brain, neural progenitor cells (NPCs) are located in the hippocampal dentate gyrus subgranular zone (SGZ) and the subventricular zone (SVZ) lining the lateral ventricles (Suh et al. 2009). The production of new neurons from NPCs continues throughout life in rodents, nonhuman primates, and humans. In the olfactory bulb two types of interneurons are generated from a dividing precursor cell population in the SVZ (Altman 1969; Corotto et al. 1993; Luskin 1993; Winner et al. 2002). The continuous addition of interneurons, which modulate spatial and temporal coding of olfactory information, might provide a substrate for adapting to environmental changes (Cecchi et al. 2001; Doetsch and Hen 2005). In the dentate gyrus of the hippocampus, new granule cells are continuously generated from precursor cells in the subgranular zone (Altman and Das 1965; Kaplan and Hinds 1977; Cameron et al. 1993; Kuhn et al. 1996). The formation of new granule cells in the

SGZ is modulated by a large number of physiological stimuli, including exercise (van Praag 2009), dietary energy restriction (Lee et al. 2002b), and environmental enrichment (Rossi et al. 2006). Increasing evidence suggests a role for hippocampal neurogenesis in learning and memory and mood regulation, and a role for olfactory bulb neurogenesis in olfactory discrimination and memory (Abrous et al. 2005).

Across the life span, a progressive reduction of adult hippocampal neurogenesis occurs. With advancing age, there is a decline in precursor cell proliferation and net neurogenesis (Kuhn et al. 1996). This reduction takes place in the context of other structural changes (Rosenzweig and Barnes 2003; Driscoll and Sutherland 2005). Recent findings have shown there are at least two subpopulations of NPCs in the hippocampus, only one of which is adversely affected by aging (Lugert et al. 2010). Regular exercise can counteract the adverse effect of aging on hippocampal neurogenesis in mice (van Praag et al. 2005). Similar to the hippocampus, neurogenesis is impaired in the olfactory bulb of old, compared to young, animals (Brown et al. 2003; Enwere et al. 2004; Luo et al. 2006). Aged mice show olfactory discrimination deficits, attributed to a decline in olfactory neurogenesis (Enwere et al. 2004).

Aging has sometimes been designated as a strong negative regulator of adult hippocampal neurogenesis. Although neurogenesis decreases with advancing age and in old age lingers at a few percent of the value in early adulthood, whether age regulates neurogenesis is problematic. The decline need not be "regulated," but instead may be secondary to general age-related changes, including oxidative stress and elevated glucocorticoid levels. Indeed, experimental clamping of corticosterone at its level in young animals resulted in maintenance of neurogenesis as the animals aged (Cameron and McKay 1999). While most studies have demonstrated a reduction of neurogenesis during aging, counting numbers of proliferating cells alone might be deceiving. For example, even if the baseline level of adult neurogenesis is very low in old age, the relative regulation that is possible from this baseline might be much larger than early in life (Kempermann et al. 1998).

1.3.2.3 The Effects of Inflammation on Neurogenesis: A Role for Microglia

An important component of the disease process in many neurological disorders is inflammation; microglia are major players in such states (Kerschensteiner et al. 2009). With their diversity of cell types and activation states, microglial effects on adult neurogenesis may range from detrimental to supportive (Simard and Rivest 2004). The turnover rate of microglia in the healthy adult brain is probably low (Lawson et al. 1992). However, during pathological conditions, both intrinsic proliferation of parenchymal microglia and recruitment of monocytes could be substantial (Flugel et al. 2001; Ladeby et al. 2005; Djukic et al. 2006; Ajami et al. 2007). Microglial processes and arborizations are highly mobile and malleable, which may enable microglia to scan the environment without disturbing neuronal networks (Davalos et al. 2005; Nimmerjahn et al. 2005). Sudden appearance of factors that are not usually detected, damage to neuronal membranes, and loss of inputs can all result in focal and transient changes in the microglial activation profile (van Rossum and Hanisch 2004; Hanisch and Kettenmann 2007; Pocock and Kettenmann 2007).

While microglial activation and the resulting inflammation could be detrimental to adult neurogenesis, this may not always be the case. Evidence indicates that microglia

under certain circumstances can be beneficial and support the different steps in adult neurogenesis. Most studies so far have primarily focused on the microglial reaction after an acute injury, or have used exogenous administration of the bacterial endotoxin lipopolysaccharide (LPS; a TLR4 ligand) (Ekdahl et al. 2003; Monje et al. 2003). LPS mimics the infection by Gram-negative bacteria, which results in a massive TLR4-mediated antimicrobial defense reaction with an acute excessive activation of microglia that can trigger the death of newly formed neurons (Hanisch and Kettenmann 2007). However, as mentioned above, microglial functional phenotype is context-dependent and probably adapts as the microenvironment changes in order to cope with altered homeostasis. Therefore, LPS-stimulated microglia do not reflect all microglial functions, but can only provide proof of principle that this particular functional phenotype (probably the M1 phenotype) is detrimental for survival and differentiation of newly formed neurons in the adult brain (Ekdahl et al. 2003; Monje et al. 2003). Further support for a detrimental effect of the M1 microglial activation state can be inferred from observations in transgenic mice, which exhibit chronic expression of interleukin-6 (IL-6) by LPS-activated microglia and an associated decrease in the production of new neurons (Vallieres et al. 2002). In addition, cell culture studies showed that NSC survival is compromised when NSC are exposed to IL-6 (Monje et al. 2003). Together with other inflammation-induced cytokine products like interferon-γ (IFN-γ), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α), IL-6 may suppress neurogenesis in inflammatory states (Ben-Hur et al. 2003; Monje et al. 2003; Cacci et al. 2005; Iosif et al. 2006; Koo and Duman 2008).

Further evidence for an adverse effect of microglia on neurogenesis comes from a study in which minocycline, a microglia inhibitor, administered for 5 weeks resulted in an increase in the number of newly formed neurons, while the microglia population decreased (Monje et al. 2003). However, other studies have shown that the natural killer cell- and T cell-derived protein IFN-γ can be both neurotoxic and supportive of neurogenesis. The deleterious effect of IFN-γ is well-characterized, but recent observations have indicated that on the contrary, microglia stimulated with low levels of IFN-γ support neurogenesis (Butovsky et al. 2006), and that IFN-γ enhances neuronal differentiation directly when administered to NSCs or neuronal cell lines (Wong et al. 2004; Song et al. 2005). In addition, IFN-γ transgenic mice exhibit increased NSC proliferation and differentiation in the adult dentate gyrus associated with neuroprotection and improved spatial cognitive performance (Baron et al. 2008). The previously reported neurotoxic effect by IFN-γ in this area could be due to its occurrence in high concentrations or to the concomitant presence of inflammatory mediators such as LPS or TNF-α during severe infection (Baron et al. 2008).

When acutely activated microglia change into an anti-inflammatory M2 phenotype following an injury, the cells either maintain their phenotype or divert into a more deleterious activation state. This transition can be demonstrated by changes in their cytokine production profile when cultured microglia are exposed to LPS for a prolonged time period. Newly formed neurons that did not die during the first month of deleterious microglial activation continued to survive for at least 6 months following the insult (Bonde et al. 2006). Interestingly, this long-term survival occurred despite the concomitant chronic microglia response, suggesting an ability of NSC to adapt to an inflammatory environment (Bonde et al. 2006). An instructive,

beneficial role of microglia in adult neurogenesis is supported by studies of NSCs cocultured with microglia or grown in conditioned media from microglia (Aarum et al. 2003; Morgan et al. 2004; Walton et al. 2006; Nakanishi et al. 2007).

Studies exploring the interaction between brain inflammation and neurogenesis have so far mainly focused on the short- and long-term influence of microglia on progenitor proliferation and survival of new neurons. However, it is conceivable that microglia could also influence the functional properties and synaptic connectivity of the new neurons. For example, activated microglia secrete cytokines and growth factors, such as TNF-α and BDNF, which can modulate excitatory (Pickering et al. 2005) and inhibitory (Henneberger et al. 2005) synaptic transmission and alter dendritic spine morphology (Schratt et al. 2006; von Bohlen und Halbach et al. 2006). In fact, recent findings support roles for inflammatory mediators in the development of the functional synaptic connectivity of the new neurons. Thus, new hippocampal neurons born after induction of epileptic seizures received decreased excitatory and increased inhibitory synaptic drive (Jakubs et al. 2006). The latter response may involve TNFα, a cytokine that modulates synaptic plasticity and vulnerability of neurons to seizure activity (Bruce et al. 1996; Albensi and Mattson 2000). Moreover, LPS-induced inflammation without seizure activity and neuronal death, leading to chronic elevation of the numbers of activated microglia, resulted in enhanced inhibitory input to the new hippocampal neurons (Ekdahl et al. 2009).

The risk for brain tumors increases with advancing age (Flowers 2000). The cellular environment surrounding a brain tumor differs considerably from the pro-inflammatory environment in affected brain regions of patients with neurodegenerative disorders. Soluble factors released by the tumor cells change the phenotype of surrounding microglia; TNFα and IL-6 are downregulated, and expression of metalloproteases is upregulated, which stimulates the growth and invasiveness of the tumor cells (Markovic et al. 2005; Sliwa et al. 2007). Conversely, the intrinsic properties of the microglia population seem to be an important factor. A reason that glioblastomas are almost always fatal is that they harbor a small population of cancer stem cells that are resistant to chemotherapy and radiation. Similar to NPCs, self-renewal of glioblastoma stem cells depends upon the tonic repression of neuron-specific genes by a transcriptional repressor called REST and an associated protein called TRF2 that stabilizes REST (Zhang et al. 2009). Both NSCs and cancer stem cells can be induced to stop dividing and establish a neuronal phenotype by experimental treatments that target REST or TRF2. Though not yet established, it may also be possible to suppress neural tumor growth by activating innate immune pathways that induce differentiation of cancer stem cells or reduce their survival. Consistent with the latter possibility, it was recently shown that both TLR3 (Lathia et al. 2008) and TLR2 (Okun et al. 2010) suppress neurogenesis.

1.3.2.4 Microglia and Acute Brain Injuries

Neurogenesis may be increased in response to an acute brain injury such as severe epileptic seizures and stroke; such injury-induced neurogenesis can occur in the SVZ and the SGZ in the hippocampus (Bengzon et al. 1997; Parent et al. 1997; Arvidsson et al. 2001, 2002). Interestingly, ischemia-induced neurogenesis gives rise to new neurons not only in the SGZ and SVZ, but also in areas that are nonneurogenic in

the intact brain, the striatum, and to a minor extent, the cerebral cortex (Lindvall and Kokaia 2010). Similarly, following hippocampal damage caused by epilepsy, aberrant migration of new neurons is seen toward the necrotic area in the dentate hilus (Parent et al. 1997; Scharfman et al. 2000, 2002). These findings raised the possibility that stimulation of neuronal replacement by neurons produced by endogenous neurogenesis could become of value for restoring function after stroke and other conditions leading to neuronal loss. However, although there are animal studies reporting that increased neurogenesis may be associated with improved recovery after stroke, definite proof for a causal relationship is lacking (Lindvall and Kokaia 2010).

Also after stroke, the microglial population changes over time with respect to morphology, phenotype, and cytokine expression. Consistent with a cytotoxic action of microglia early after the insult, administration of the anti-inflammatory drug indomethacin improved the survival of the stroke-generated neuroblasts in the striatum (Hoehn et al. 2005). Similarly, delivery of minocycline, which reduces microglia activation, during 1 month after MCAO increased the number of new neuroblasts and mature neurons in the dentate gyrus (Liu et al. 2007). Importantly, data suggest that neurogenesis continues for at least 1 year after a stroke (Kokaia et al. 2006; Thored et al. 2006), suggesting the possibility of the formation of new neuronal circuits and restoration of function even long after the stroke occurred.

The inflammatory system may also be involved in the migration of the new neurons toward ischemic areas, acting through the chemokine stromal cell-derived factor-1α (SDF-1α) and its receptor CXCR4 (Thored et al. 2006), the latter being highly expressed by neural progenitors (Ni et al. 2004). SDF-1 is upregulated by the glial population after a stroke and is implicated as an inflammatory stimulus that could enhance both progenitor proliferation and chain migration (Imitola et al. 2004). Moreover, SDF-1 has been reported to promote the differentiation of newly generated neurons into inhibitory GABAergic neurons (Luo et al. 2008), which may provide a mechanism to suppress unrestrained neuronal activity that can occur in brain injury.

1.4 INFLAMMATORY RESPONSES ARE EXAGGERATED IN THE BRAIN DURING LATE LIFE

As in many other organ systems (Libby 2007), inflammatory processes increase during aging as the result of oxidative stress, and cell damage and death. Hypersensitivity to innate immune activation in the brain is evident in several models of aging. Mixed glial and coronal sections from the brains of aged rodents are hyperresponsive to LPS stimulation and produce more inflammatory cytokines (e.g., IL-1β and IL-6) than those of cultures from younger animals (Ye and Johnson 2001; Xie et al. 2003). Further, older mice are more sensitive to septic shock induced by intracerebroventricular (ICV) administration of LPS. Old mice had elevated TNF-α production in the brain and plasma after LPS challenge compared with young adult controls (Kalehua et al. 2000). In another murine model of aging, microarray analysis revealed that peripheral injection of LPS induced a higher expression of IL-1β and TNF-α in the hippocampus of aged mice compared to young mice (Terao et al. 2002). Another microarray analysis

showed increased markers of glial reactivity, including major histocompatibility complex (MHC) class II, CD68, and glial fibrillary acidic protein (GFAP), in the brains of aged mice (Godbout et al. 2005). In this model peripheral stimulation of the innate immune system with LPS caused an exaggerated inflammatory cytokine response in the aged brain with increased production of IL-6 and IL-1β.

Aged mice that experienced an amplified and prolonged neuroinflammatory response to LPS showed a delayed recovery from sickness behavior (Godbout et al. 2005). In a rat model of aging, in which increased reactive glia with MHC class II expression were detected, peripheral injection of *Escherichia coli* promoted higher levels of IL-1β in the hippocampus of old compared to young animals (Barrientos et al. 2006). This increased IL-1β production in the hippocampus of aged rats after *E. coli* challenge was associated with impaired long-term hippocampus-dependent memory (Barrientos et al. 2006). Neither of these studies (Godbout et al. 2005; Barrientos et al. 2006) found peripheral inflammatory cytokines to be a reliable indicator of the exaggerated inflammatory responses in the CNS. We recently measured levels of a panel of cytokines, neurotrophic factors, and stress response proteins in brain tissue samples (cerebral cortex and striatum) of young, middle-age, and old mice that had been subjected (or not) to a stroke. Old mice exhibited higher levels of pro-inflammatory cytokines (TNFα, IL-1β, and IL-6) and lower levels of neuroprotective proteins (BDNF, bFGF, HSP70, GRP78, and HO-1) than young mice (Arumugam et al. 2010). Interestingly, even the side of the brain not directly affected by the stroke exhibited elevated levels of pro-inflammatory cytokines in old compared to young and middle-age mice, suggesting aging reduces the ability of the brain to contain inflammatory processes within the brain region directly affected by the injury. Taken together, these results suggest that the presence of reactive glia in the aged or diseased brain is permissive to an amplified, spreading, and prolonged neuroinflammatory response, which may lead to subsequent behavioral and cognitive complications.

1.5 HORMONAL CHANGES IN THE BRAIN DURING AGING THAT AFFECT IMMUNITY

One well-known aspect of aging is progressive changes in the status of multiple neuroendocrine systems. In women, estrogen levels decline precipitously at menopause, and in men testosterone levels decline steadily with advancing age (Chahal and Drake 2007). There is considerable evidence from experimental cell culture and animal models that estrogen and testosterone can suppress inflammatory processes (e.g., microglial activation and pro-inflammatory cytokine production) in the brain, and can protect neurons against dysfunction and death (Bruce-Keller et al. 2000; Pike et al. 2009). Insulin resistance and consequent diabetes are increasingly common with advancing age and may promote inflammatory processes in the brain as a result of increased oxidative stress caused by this metabolic state (Craft 2007). Mice that are insulin resistant as the result of either overeating a normal diet or consuming a diet high in saturated fats and sugar exhibit deficits in learning and memory and impaired hippocampal synaptic plasticity and neurogenesis (Stranahan et al. 2008a, 2008b). The relative contribution of innate and humoral immune systems to

insulin resistance/diabetes-induced brain dysfunction and degeneration remains to be determined.

Another endocrine system that is altered during aging is the hypothalamic–pituitary–adrenal (HPA) axis, which controls the production of the glucocorticoid cortisol. Basal levels of corticosteroids are generally elevated during aging, probably due to impairment of negative feedback mechanisms that normally suppress the HPA axis after a surge of corticosteroids. Aging is also associated with a blunted activation of the HPA axis by stress or inflammation (Nicolson et al. 1997; Terrazzino et al. 1997; Kudielka et al. 2004). Moreover, the diurnal fluctuations of corticosteroids are moderated or lost during aging. Persistent elevation of corticosteroid levels might be responsible for the documented age-related downregulation of glucocorticoid receptors in the brain, most obviously in hippocampus (Sapolsky et al. 1983). Their diminution—along with that of the sex steroids—might become permissive for exaggerated and prolonged activation of microglia. Excessively high levels of glucocorticoids may play a particularly important role in cognitive decline during aging because data suggest that chronic stress during mid- and late life can increase the risks for depression, cognitive impairment, and AD (Rothman and Mattson 2010). Moreover, recent findings suggest a role for adrenal glucocorticoids in the impaired neuroplasticity and cognitive deficits caused by diabetes and insulin resistance (Stranahan and Mattson 2008; Stranahan et al. 2008b).

1.6 INNATE IMMUNE EFFECTORS IN THE BRAIN DURING AGING

In the elderly, systemic infection is associated with an increased frequency of behavioral and cognitive complications (Penninx et al. 2003; Evans et al. 2005). Stimulation of the peripheral immune system in aged mice causes exaggerated neuroinflammation (Henry et al. 2008) that is paralleled by prolonged sickness behavior (Godbout et al. 2005), impaired working memory (Chen et al. 2008), and protracted depressive-like behavior (Godbout et al. 2008). In the following paragraphs we will describe the roles of TLRs and complements in mediating such adverse effects of aging on the brain.

1.6.1 TLRs

Aging of the brain is associated with changes in the expression of innate immune receptors. The expression of TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, and CD14 is upregulated during aging, with TLR4 showing the strongest response. In contrast, TLR9 expression in the brain decreases during aging, whereas levels of TLR8 are unchanged (Letiembre et al. 2007). A recent study assessed the response of microglial cells from aged mice to systemic LPS challenge (Henry et al. 2009). Peripheral LPS injection causes exaggerated microglial mRNA and protein induction of both inflammatory IL-1β and anti-inflammatory IL-10 in aged mice compared to young adult mice. Moreover, a large fraction of microglial cells in aged mice coexpress MHC-II and high levels of IL-1β, implying that these cells are in a primed or reactive state.

The reason why the expression of these innate immune receptors is altered in brain aging despite the absence of any overt pathology is unclear. During normal

brain aging, altered protein turnover is believed to contribute to the aggregation of multiple proteins (e.g., β, τ, and α-synuclein), and oxidized lipofuscin-ceroid accumulation (Kato et al. 1998; Terman and Brunk 1998, 2006). The accumulation of these proteins and lipids is, in part, the consequence of oxidative stress and believed to contribute to the dysfunction and damage to neurons that occurs in normal aging and, more dramatically, in AD, PD, and other age-related neurodegenerative disorders (Keller et al. 2004). Recently, several studies demonstrated the involvement of TLR2 and TLR4 in oxidative stress (Frantz et al. 2001; Miller et al. 2003; Walton et al. 2003a, 2003b; Holvoet et al. 2006), and that endogenous heat shock proteins (HSPs) that are elevated in brain aging (Calabrese et al. 2005) can interact with CD14, TLR2, and TLR4 (Asea et al. 2000, 2002; Dybdahl et al. 2002; Vabulas et al. 2001, 2002a, 2002b, 2002c). Thus, aging-induced oxidative stress generates HSPs that could potentially activate different TLRs, including TLR2 and TLR4. In addition, an exercise program resulted in a reduction in TLR4 signaling in circulating lymphocytes of human subjects (Stewart et al. 2005), which may contribute to the beneficial effects of exercise on the aging brain (van Praag 2009). Similarly, alternate-day fasting suppressed age-related and stroke-induced increases in pro-inflammatory cytokines in the brains of mice (Arumugam et al. 2010).

Downregulation of TLR9 in the brain during aging might be a mechanism that dampens inflammatory responses previously reported in brain aging (Blalock et al. 2003; Godbout and Johnson 2004; Lu et al. 2004). This would be consistent with a microglial dystrophy characterized by deramification, spheroid formation, and fragmentation of processes in brains of the elderly (Streit et al. 2004). An altered profile of innate immune receptors on these cells might be a correlate of such age-related microglial dystrophy. Interestingly, a TLR4 polymorphism was also associated with successful aging (Candore et al. 2006), which further indicates a role of innate immune receptors in aging.

1.6.2 The Complement System

The role of the complement system during brain aging is not clear. A DNA microarray study in mice demonstrated that cellular immunity and inflammation are elevated during brain aging (Lee et al. 2000). Analysis of gene transcription from the neocortex and cerebellum of aged mice showed increased transcription of complement C4, C1qa, C1qb, and C1qc. The mRNA increase of those selected markers during aging was confirmed by quantitative real-time PCR, which indicates that concurrent production of complement proteins in the brain might lead to the generation of pro-inflammatory peptide fragments contributing to functional alterations in the brain during aging or in age-related disease. Expression of several specific immune response genes was also elevated in the cortex, cerebellum, and hippocampus with aging (Lee et al. 2000). The complement C1q B and C chains also showed a steady increase in the aged hippocampus (Terao et al. 2002). In addition, expression of both the classical and alternative complement pathway components, such as C1q, C3, C4, C5, and factor B mRNA, shows an age-dependent increase in control mice. The protein level of C1q in the

brain was elevated in 15-month-old mice compared to young mice, which was correlated with a similar elevation of C1q mRNA levels (Reichwald et al. 2009).

It was reported that levels of C3a and MAC are higher in the CSF of elderly subjects than in younger subjects (Loeffler et al. 1997). The C3a concentration in normal aged subjects was threefold higher than in normal younger subjects. There was also a trend toward increased MAC levels in the brain during normal aging (Loeffler et al. 1997). In another study, levels of C4d and C3b fragments were elevated in hippocampal tissue samples from aged compared to young individuals, suggesting that early components of the complement cascade increase in the brain during normal aging (Loeffler et al. 2004). A finding from the comparison of brain complement activation between young and aged rhesus monkeys shows significantly higher activation of the early component of complement cascade, but with no detectable terminal component activation. Since the activation of terminal complement pathway is tightly controlled by regulatory proteins, as described above, lack of activation of a terminal component suggests that the complement cascade is restrained in brain cells during normal aging in the absence of pathology. In the case of pathology, such as intracerebral hemorrhage (ICH), levels of complement factor C9 and clusterin in ipsilateral basal ganglia were elevated more in aged rats than in young rats (Gong et al. 2008). More C9- and clusterin-positive cells were found around the hematoma in aged rats. However, myeloperoxidase (a marker for the detection of neutrophil infiltration)-positive cells in ipsilateral basal ganglia were fewer in aged rats after ICH. This suggests that ICH causes more severe complement activation and less neutrophil infiltration in aged rats (Gong et al. 2008).

1.7 OXIDATIVE STRESS AND INFLAMMATION IN THE BRAIN DURING AGING

Reciprocal, cross-amplifying interactions between cellular oxidative stress and inflammation occur in many tissues during usual aging. Many of the same oxidative and inflammatory cascades are clearly activated excessively in many different neurological disorders, and these pathological processes are often associated closely (in space and time) with hallmark histopathological lesions, including Aβ aggregates in AD and Lewy bodies in PD (Mattson 2002; Nunomura et al. 2007). In normal aging there is an accumulation of oxidized proteins, DNA, and lipids in brain cells (Cutler et al. 2004; Haripriya et al. 2005; Markesbery et al. 2005; Poon et al. 2005). DNA bases within the promoter regions of several important neuronal genes, including those that encode proteins involved in synaptic plasticity and mitochondrial function, are prone to oxidative modification during brain aging in humans (Lu et al. 2004). Several genes critical for inhibitory GABAergic transmission are markedly downregulated in brain cells during normal aging (Loerch et al. 2008). Reduced inhibition with aging may result in excessive activity in some neuronal circuits, which would be expected to promote cellular Ca^{2+} overload (Bezprozvanny and Mattson 2008). Moreover, genes encoding proteins involved in DNA protection and repair are suppressed in multiple brain regions during aging (Xu et al. 2007).

Oxidative stress in brain cells activates innate and humoral immune systems in the following ways. Oxidative modification of cell surface and secreted proteins by lipid peroxidation products and glycation can be recognized by receptors on microglia and lymphocytes (Wang et al. 2008; Yun et al. 2008). Oxidative stress activates several cellular signaling pathways in glial cells that result in the induction of genes encoding pro-inflammatory proteins. One such pathway involves the transcription factor NF-κB, which induces the expression of TNF-α, IL-1β, and IL-6 by microglia, cytokines that may damage neurons, particularly under conditions (metabolic, oxidative, and proteotoxic stress) that occur in aging (Fine et al. 1999; Kaushal and Schlichter 2008). However, it should be appreciated that activation of NF-κB in neurons upregulates the expression of several cytoprotective proteins, including Mn superoxide dismutase and Bcl-2, as well as proteins involved in synaptic plasticity (Mattson and Meffert 2006). In addition, oxidative stress upregulates the expression of several TLRs, including TLR2 and TLR4, in neurons (Tang et al. 2007).

Activation of innate and humoral immune systems promotes oxidative stress in brain cells in the following ways. As described above, TLRs 2 and 4 are coupled to NF-κB and the production of pro-inflammatory cytokines, which can promote oxidative stress in neurons and glial cells (Scirocco et al. 2010; Qin et al. 2005). Activation of the complement cascade induces oxidative stress by elevating intracellular Ca^{2+} levels (Xiong and McNamara 2002; Luo et al. 2003), resulting in generation of superoxide by the activity of oxidases and the mitochondrial electron transport chain (Hongpaisan et al. 2004; Ibi et al. 2008). In addition, infiltrating macrophages and lymphocytes produce pro-inflammatory cytokines and also reactive oxygen species (ROS) and excitotoxins (Guo et al. 2004). Figure 1.4 illustrates the involvement of TLRs and complement cascades in the inflammatory process during aging.

1.8 THE CONTRIBUTION OF INFLAMMATION TO AGING-ASSOCIATED COGNITIVE DECLINE AND ALZHEIMER'S DISEASE

This section reviews the evidence that innate and humoral immune signaling pathways are aberrantly activated in brain regions involved in learning and memory processes in Alzheimer's disease (AD), and to a lesser extent in age-related mild cognitive impairment. Similar alterations in these inflammatory pathways are also believed to occur in several other neurodegenerative disorders, including PD, HD, stroke, and amyotrophic lateral sclerosis. For information on the latter disorders, the reader is referred to previous articles (Singhrao et al. 1999a, 1999b; McGeer and McGeer 2005a, 2005b; Moisse and Strong 2006; Tang et al. 2007; Wang et al. 2007; Holmoy 2008; Stone et al. 2009; Tansey and Goldberg 2010). AD is a progressive neurodegenerative disease characterized by gradual onset and advancement of memory loss and other cognitive deficits. Definitive diagnosis of AD is based on the presence of extracellular amyloid plaques comprised of neurotoxic amyloid β-peptide (Aβ), which is generated by proteolysis of the β-amyloid precursor protein (APP), and intracellular neurofibrillary tangles composed of hyperphosphorylated insoluble forms of τ protein (Mattson 2004). Genetic factors that either cause or predispose

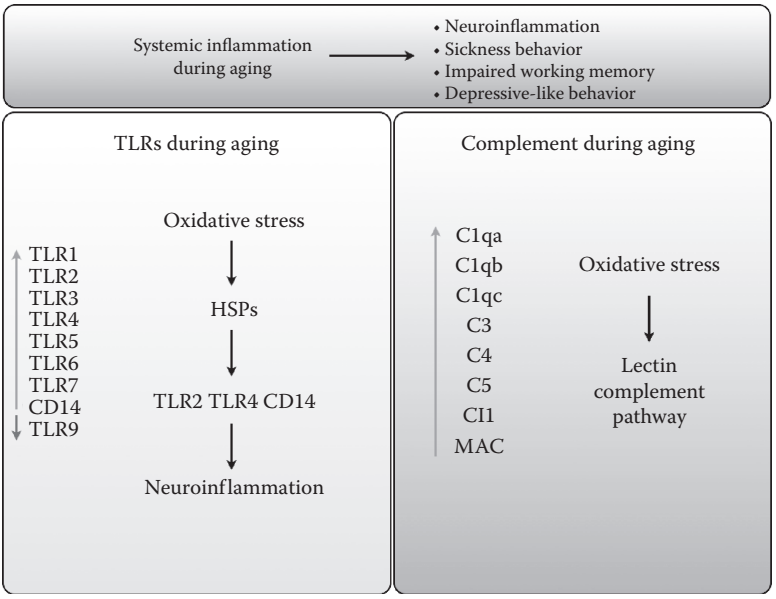


FIGURE 1.4 Aging-induced oxidative stress activates TLRs and complement cascades. Aging-related oxidative stress and inflammation result in impaired working memory and depressive-like behavior. Multiple mechanisms are responsible for these alterations, including increased expression of TLRs 1–7 and CD14, and decreased expression of TLR9. Oxidative stress activates heat shock proteins (HSPs), which in turn activate TLR2, TLR4, or CD14 to induce neuroinflammation. In addition, increased expression of the complement components C1qa, C1qb, C1qc, C3, C4, C5, and C11 occurs in the brain during aging, along with increased levels of the membrane attack complex (MAC). Oxidative stress associated with aging activates the lectin complement pathway contributing to neuroinflammation.

to AD include mutation in APP and presenilins 1 and 2 (which cause early-onset autosomal-dominant inherited AD) and polymorphisms in apolipoprotein E (ApoE4 increases the risk of AD). Activation of the innate immune response by reactive glia in association with Aβ and neurofibrillary tangles is a consistent pathological feature of AD. Neuroinflammation in the AD brain is concentrated at sites of Aβ plaques, which exhibit increased levels of pro-inflammatory cytokines, complement components, and proteases (Akiyama et al. 2000; McGeer et al. 2006). Aβ plaques are surrounded and infiltrated by activated astrocytes and microglia, which are believed to be the major source of local inflammatory components. Neuroinflammation is proposed to play a major role in AD pathogenesis, because long-term treatment with nonsteroidal anti-inflammatory drugs reduces AD risk and may delay disease progression (Stewart et al. 1997; in t'Veld et al. 2001).

1.8.1 TLRs

The expression of several TLRs is elevated in the AD brain. TLR2 and TLR4 expression is increased in the brain of AD patients (Walter et al. 2007). Further,