Nihar Jana · Anirban Basu
Prakash Narain Tandon *Editors*

Inflammation: the Common Link in Brain Pathologies

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Preface

The role of neuroinflammation, as a common denominator of diverse neurological disorders including ageing, infections, trauma, stroke, demyelinating and degenerative diseases, was overlooked till recently since it lacked the classical markers of inflammation elsewhere in the body. It was only after the tools and techniques of molecular biology were utilized to investigate the pathophysiology of these conditions that the tell-tale evidence of inflammation in all these pathologies came to light. Not only inflammation was found to accompany these lesions but also it soon became evident that neuroinflammation plays a critical role in the pathogenesis of these conditions.

Over the years voluminous literature has accumulated on the subject but the knowledge is dispersed and not available as a comprehensive overview. It was realized that a number of neuroscientists in different parts of the country were studying various aspects of neuroinflammation in specific disease entities. This prompted us to bring together at one place the current knowledge on the subject (the proverbial nine blind men and the elephant!).

This monograph has 13 chapters contributed by investigators from institutions in different parts of the country. The first chapter is an overview providing a definition of neuroinflammation, its biomarkers and its cellular and molecular components. An attempt is made to answer a series of questions regarding its significance in different pathologies and a brief mention is made on the role played by ageing, obesity, metabolic disorders and systemic infection/inflammation. It outlines its clinical implications. Patro and his colleagues (Chap. 2) elaborate the role of microglia as the dominant player in initiating and promoting the inflammatory cascade, while in the Chap. 3 Tiwari and Seth discuss the role of astrocytes in the process. They specially highlight their role in pathogenesis of HIV-associated neurodegenerative disorders. Dutta, Ghosh and Basu, in Chap. 4, elaborate the dangerous liaison between infections and inflammation. They provide an account of the immune responses (which form the basis of inflammation), to different types of infections affecting the central nervous system. Chapter 5 by Singh and Das Sharma deals with role of neuroinflammation in demyelinating disease. Tripathi and Jana, in Chap. 6, present an overview of neuroinflammation related to neurodegenerative disorders, taking Huntington's disease as an example. This is followed by a chapter on neuroinflammation during Parkinson's disease by Sinha et al.,

amyotrophic lateral sclerosis (ALS) by Upadhyay et al., and by Alam et al., neuroinflammation in ischemic stroke. Irshad, Madan and Chosdol (Chap. 10) have dealt with role of inflammation in augmenting tumour progression, angiogenesis, promoting tumour cell proliferation and survival. Nivedita Chatterjee (Chap. 11) discusses the dysfunction of glia as a cause of many retinal disorders. Kaur et al. deals with, till recently unexpected, systemic disorder, obesity and its complementary role in augmenting neuroinflammation triggered by any aetiology. The possible therapeutic implications of the new knowledge have been referred to by all authors. The last chapter by Ghosh and Ghosh discusses the role of microglia in adult neurogenesis.

The editors take this opportunity to thank all authors and their collaborators to accede to their request to contribute to this book, which will hopefully be of great utility to students and researchers interested in neurosciences.

Special thanks are due to our publisher "Springer" and persons associated with production specially Madhurima Kahali and Muthu Rajan for their help and support in bringing out this attractive publication.

Gurgaon, India

Nihar Jana
Anirban Basu
Prakash Narain Tandon

Contents

About the Editors

Nihar Jana is presently working as Professor at National Brain Research Centre (NBRC), Manesar. Dr. Jana obtained his Ph.D. from Visva-Bharati University in 1996. After completing his post doctoral training at RIKEN Brain Science Institute, Japan, he joined NBRC in 2001. His laboratory is primarily interested in exploring the role of ubiquitin ligases in cellular protein quality control and how loss of function of quality control ubiquitin ligases leads to neuronal dysfunction or neurodegeneration observed in various neurological disorders. Dr. Jana is elected fellow of National Academy of Sciences, India (2008) and West Bengal Academy of Science and Technology (2012). He is a recipient of National Bioscience Award (2008, DBT) and TATA Innovation Fellowship (2014, DBT), VASVIK award (2012) and KT Shetty memorial award (2013 from Indian Academy of Neurosciences). Currently, he is serving as an editorial board member of PLoS ONE, Frontier of Molecular Neurosciences and Annals of Neurosciences.

Anirban Basu is a Senior Scientist and Additional Professor at National Brain Research Centre, Manesar, Haryana. He received his Ph.D. degree in Immunology from the Indian Institute of Chemical Biology, Kolkata. He then obtained post-doctoral training in neuro-immunology at Neural and Behavioral Science Department in Pennsylvania State University College of Medicine, US. So far he has trained 4 Masters students, 9 PhD students and 9 postdocs, and numerous short-term and long-term research trainees in his lab. Dr. Basu has long been interested in curing diseases of the nervous system. His current research is focused on identifying the role of microglia and neural stem/progenitor cells in the healthy and diseased central nervous system, with specific reference to CNS infections, and neurodegenerative diseases. The students currently working with him are testing strategies to develop disease modifying therapy by abrogating inflammation in CNS disorders. Dr Basu is the recipient of National Bioscience Award for Career Development (2010), Vasvik Industrial Research Award (2011), Dr. J.B. Srivastav Oration Award (2011), Rajib Goyal Prize (2012) and NASI- Reliance Industries Platinum Jubilee Award (2013), Tata Innovation Fellowship (2015) from the Department of Biotechnology, and Senior Scientist Oration Award (2015) from the Indian Immunology Society. He is also an elected Fellow of the National Academy of Sciences, India and West Bengal Academy of Science and Technology. Dr Basu sits on the editorial boards of the Journal of Neurochemistry, Scientific Reports,

Journal of Neuroinflammation, PLoS One, Frontiers in Molecular Neuroscience, and Metabolic Brain Diseases. He is also a faculty member in the Faculty of 1000 in the section Neurological Disorders.

Prakash Narain Tandon is a National Research Professor and President of National Brain Research Centre Society, Manesar. He has graduated with an MBBS and an MS from the University of Lucknow in 1950 and 1952, respectively. He was trained at the University of London and obtained his FRCS in 1956. He further obtained his specialist training in neurosurgery at Oslo, Norway and Montreal, Canada. After a brief tenure as Professor at the K.G. Medical College, Lucknow (1963–1965), he moved to the prestigious All India Institute of Medical Sciences, New Delhi where he founded the neurosurgery department and has been a Professor of Neurosurgery. He received Bhatnagar award (CSIR). He is an elected fellow of the National Academy of Medical Sciences and Indian National Science Academy. He also served as President of Indian National Science Academy in 1991–1992. He has been awarded Padma Shri (1973) and Padma Bhushan (1991) by the Government of India.

Biology of Neuroinflammation: A Common Denominator in Brain Pathologies

1

Prakash Narain Tandon

Abstract

Neuroinflammation is a common denominator of diverse neurological diseases. While acute inflammatory response is considered to be neuroprotective, chronic inflammation induces cascades of inflammatory reactions that leads to neurodegeneration. The harmful effect of chronic inflammation in modulating the course of disease has been well documented in a wide range of neurodegenerative disorders like Alzheimer's disease, Huntington's disease, Amyotrophic lateral sclerosis, etc., Overall goal of this review is to provide a broad description of the current state of knowledge of neuroinflammation associated with various acute and chronic neurological disorders.

1.1 Introduction

Oskar Fisher (1910) recognising the fact that the neuritic and glial changes associated with the extra cellular fibrils in Alzheimer brain as a tissue reaction to a foreign substance was surprised not to find the characteristic morphological signs of inflammation, as described by Celsus in other tissues. He posed the question: "Aber! wo bleibl dann die entzundliche Reaktion?" (However! where is then the inflammatory reaction) {Quoted by Eikelenboom et al. 2002}. It is now common knowledge that in the nervous system inflammation is a common denominator of diverse neurological diseases not only of infective origin but others, like—trauma, ischaemia, tumours, degeneration—but lacks its classical signs, "dolor, tumor, calor and rubor" described by Celsus. It is the molecular evidence of response to an

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external or internal insult to the brain that constitutes neuroinflammation (Mc Geer and Mc Geer 2001a, b). It is not just a response to the pathology but in many instances it is responsible for augmenting and perpetuating the disease. While in the acute phase of the disease inflammatory response may be neuroprotective, chronic inflammation destroys the neurons which in turn perpetuates the inflammation. Thus, the deleterious role of chronic inflammation in adversely affecting the course of disease has been well documented in a wide range of conditions other than infective disorders. Although probably triggered by many different initiating events in the early stages, many neurodegenerative diseases share chronic immune activation as a common feature. Thus its role in accentuating the original insult as in case of head injury, or stroke, or being responsible for neurodegeneration in Alzheimer's disease, Huntington's disease, Amyotrophic Lateral Sclerosis, Autism, Multiple Sclerosis, even ageing, metabolic disorders has attracted a large number of publications (Streit 2004, 2005; Chen et al. 2003; Eikelenboom et al. 2002; Akiyama et al. 2000; Mc Geer and Mc Geer 2004). This review, while attempting to provide a comprehensive account of the current state of knowledge is also aimed at answering some important questions

- What constitutes the minimum markers of neuroinflammation?
- What is the critical role of different cells—microglia, astrocytes, neurons, macrophages—and different cytokines and chemokines and complements in the inflammatory response?
- What are the differences between neuroinflammation secondary to infections—viral, bacterial, mycobacterial, fungal and neurodegenerative disorders?
- Are there differences between neuroinflammatory cascades in different neurodegenerating disorders—ageing, Alzheimer's disease (AD), Parkinson's disease (PD), Amyotrophic lateral sclerosis (ALS), Multiple sclerosis (MS), Autism disorders, Metabolic Syndrome?
- Is the temporal pattern and pathogenic mechanism identical in different conditions?
- What are the therapeutic implications of this knowledge?

1.2 Markers of Neuroinflammation

Mc Geer and Mc Geer (2001a, b) remarked, "Even though inflammation is a well recognised and well researched area the precise definition of inflammation remains obscure". This is so, in case of neuroinflammation, which is recognised not on basis of the classical signs of inflammation elsewhere in the body, e.g., infiltration of leucocytes, but on the basis of cytological and molecular evidence of innate immune response as manifested by activated microglia and astrocytes and presence of pro-inflammatory cytokines and chemokines. This may or may not be augmented by involvement of the adaptive immune system with infiltration of circulating leucocytes or macrophages depending upon the nature of pathological insult

(Mc Geer and Mc Geer 2001a, b; Eikelenboom et al. 2002; Amor et al. 2010). It is now generally accepted that despite the immune-privileged environment both innate and adaptive immune responses occur in the central nervous system (CNS). While invading microorganisms initiate adaptive immune response initially, endogenous signals switch on the innate responses to start with. As disease progresses both innate and adaptive responses come into play to varying extent in the inflammatory process. Amor et al. (2010) in their Table 1 summarise immune responses in neurodegenerative disorders.

1.3 Critical Role of Different Cells

1.3.1 Microglia

They are conventionally recognised as the resident macrophages and are responsible for the innate immune mechanism in the brain. They kill invading microorganism in the brain, remove debris, facilitate tissue repair after injury (Tandon 2007). They become readily activated as a result of endogenous stimuli associated with ischaemic, demyelinating or degenerative disorders. They serve an immune surveillance function. Microglia can sense subtle changes in micro-environment through a variety of surface receptors (Nimmerjahn et al. 2005; Kreutzberg 1996; Barron 1995). While the role of microglia in acute insults to the brain was well known, it is only in last couple of decades that their involvement in neuroinflammation associated with neurodegeneration has been brought into light. Block and Hong (2005) extensively reviewed, "Microglia and Inflammation—mediated neurodegeneration". Extensive reviews are also available on the role of microglial involvement in neuroinflammation associated with diverse CNS insults and diseases such as head injury, infective disorders, stroke, neurodegeneration, autism, ALS, multiple sclerosis, brain ageing. The trigger for activation of microglia is different in acute neurological diseases and trauma and chronic neurodegenerative conditions. In the former it is the ischaemic or necrotic lesions which trigger the activation while in majority of the latter it is the accumulated abnormal proteins (Sherman and Goldberg 2001; Walker and LeVine 2000). In order to detect potential insults microglia possess a vast array of highly conserved pattern recognition receptors. While toll-like receptors (TLRs) have received most attention, for their ability to recognise both pathogen-associated molecular patterns (PAMPs) and endogenous danger-associated molecular patterns (DAMPs), many other receptors that recognise specific molecular patterns have been described (Lucin and Wyss-Conray 2009). Activated microglia are capable of releasing a variety of soluble factors which are pro-inflammatory in nature and potentially cytotoxic (Streit et al. 1999; Stoll and Jander 1999; Lucin and Wyss-Conray 2009; Walter et al. 2007). Block and Hong (2005) enumerated more than 30 such factors including NO, H_2O_2, OH, NOO, TGFβ, PGE_2 and a variety of interleukins which influence cell survival. They summarised, "Microglia are critical actors of self-propelling mechanisms of neurotoxicity contributing mechanism to degenerative disorders. And further, "thus, multiple triggers of microglia derived

oxidative stress fuelling the progressive nature of several independent neurodegenerative diseases". Since neuronal death itself can be a trigger for activating microglia neuronal damage may continue even in absence of the original trigger as has been observed in most degenerative disorders (Eikelenboom et al. 2002). There is a common thread of microglial activation across numerous neurodegenerative diseases though there can be diverse ways of activating microglia. The resulting neuronal damage is itself responsible for a self-propelling cycle of neuronal death (Block and Hong 2005).

While a great deal has been written about the neurotoxic effect of activated microglia, enough investigations have not been done on their neuroprotective role. Inflammation is an evolutionary—conserved defense strategy of the immune system that can be mounted in response to injury or infection. Acute inflammation is traditionally considered a beneficial mechanism to limit damage and invoke tissue repair and resolution of injury (Cuartero et al. 2013). There is enough evidence that microglia being the first line of defense following acute neuronal injury not only act as a scavenger of the debris but also secrete a number of growth factors like BDNF, EGF, NGF, etc., (Streit 2005; Lucin and Wyss-Conray 2009; Murray et al. 2015). The molecular basis for neuroprotection has been detailed by Streit (2005). However, during chronic inflammation the activated microglia produce cytotoxic molecules which leads to destruction of neurons. Similarly features of microglial activation can result in diverse localization, pathology and clinical symptoms of each unique disease (Block and Hong 2005). The intriguing question remains as to how the generalised phenomena of microglial activation can result in diverse and localised neurodegeneration. According to Lucin and Wyss-Conray (2009) microglia heterogeneity exists during neurodegeneration and may influence disease outcome. Chapter 2 in this book have discussed this subject further.

1.3.2 Astrocytes

It has been known for a long time that following damage, degeneration and loss of neuronal tissue there is proliferation of the glial elements, particularly astrocytes, to replace it. However, their role in neuroinflammation has been brought to light only recently. Like microglia neuronal insult or damage also activates astrocytes which then secrete a variety of cytokines which contribute to neuroinflammation (Miller 2005; Tandon 2007). Although neuroinflammatory function of microglia is not as prominent as that of microglia (Streit et al. 1999) they become activated in response to immunologic challenge or brain injury (Aloisi 1999). The relative role of microglial and astroglial reactions to inflammatory lesions of experimental autoimmune encephalomyelitis has been studied in details by Matsumoto et al. (1992). They observed that microglia reacted to inflammatory foci at the very early stage while astrocytes encased the lesions at the peak stage of EAE. However, total neural tissue destruction produced by cold injury induces much faster and stronger astrological responses than the autoimmune inflammation. This suggested that the magnitude of astrological reaction is variable depending on the nature and severity

of damage. In general in most degenerative disorders the role of astrocytes is much less and later than microglia (Teismann et al. 2003; Vehmas et al. 2003). They postulated that more than one chemotactic factor is produced for regulation of different stages of inflammation. There is evidence for cross-talk between these cells. Ultimately the glial responses results in deleterious—effects on the neurons through production of pro-oxidant reactive species and pro-inflammatory cytokines and prostaglandin (Teismann et al. 2003). Tewari and Seth have discussed the role of astrocytes in neuroinflammation in this book.

1.3.3 Neurons

Contrary to the idea that neurons play only a passive role, recent findings indicate that neurons themselves appear to be active players in neuroinflammation. Many of their products, like increased expression of complement factors and the inducible cyclooxinase2 (COX-2), as well as neuronal membrane proteins CD22, CD47, CD200, CX3CLI (fractalkine), ICAM5, NCAM, Semaphorin all regulate inflammation (Eikelenboom et al. 2002; Amor et al. 2010; Tian et al. 2009; Oka and Takashima 1997; Hoozemans et al. 2001; Doll et al. 2014). In addition neurons are capable of expressing COX-2 which has been found to be upregulated in early stages of AD but down regulated in advanced stages. IL-1β induces COX-2 expression in neurons (Hoozemans and O'Banion 2005).

1.4 Infiltration of Cells from the Periphery (Role of Adaptive Immune System)

Chronic activation of the microglial cells leads to recruitment of cells of adaptive immune system into the CNS. Despite the otherwise immuno-suppressive environment T-cells do enter and survive in the CNS as observed in substantia nigra in PD patients, MS and traumatic brain injury (Mantovani et al. 2009; Ankeny and Popovich 2009; Neumann et al. 2002). Most neurodegenerative disorders are characterised by both local inflammation from resident cell types in the brain and by the infiltration of leucocytes from the periphery (Mc Geer et al. 1989). Post-mortem brain tissue from patients with AD shows an atypical inflammatory response dominated by cells of the macrophages lineage, with activation of the resident microglial cells and possible recruitment of monocytes from the blood (Perry et al. 2007). Table 1 of the review by Amor et al. (2010) summarises the literature on the innate and adaptive responses in different neurodegenerative diseases. For reasons not well understood the response is not identical. Several factors including CCL2 serve as a chemoattractant for the peripheral monocytes (El Khoury et al. 2007). Many classical immune regulatory factors are produced by glial cells or neurons in the CNS which are increased following injury. On the other hand neurodegenerative changes in the brain appear to be associated with changes in the peripheral immune system as observed in patients with AD, HD and PD. Elevated levels of

IL-6 were observed in preclinical HD mutation carriers up to 16 years before the onset of motor abnormalities and changes in the levels of 18 cellular communication factors in plasma predicted AD progression several years prior to clinical manifestation (Lucin and Wyss-Conray 2009; Ray et al. 2007). Details of cerebral infiltration of immune cells in neurodegenerative diseases are somewhat different in different disorders. The precise role of resident microglia versus infiltrating monocytes CD4, CD8, T-cells, etc., need further studies (Lucin and Wyss-Conray 2009).

1.5 Cytokines and Other Pro-inflammatory Molecules

Following a CNS insult, multiple cytokines are generated to cause, exacerbate, mediate and/or inhibit cellular injury and repair (Allan and Rothwell 2003; Allan et al. 2005). Primarily, produced by activated microglia these cytokines include IL-1, TNFα, NO, PGE2, Superoxide among others. These are responsible for chronic inflammation and self-perpetuating cycle of neural death (Block and Hong 2005; Swardfager et al. 2010; Murray et al. 2015). These have been extensively studied in Alzheimer's disease (Misiak et al. 2012; Swardfager et al. 2010; Vehmas et al. 2003; Hoozemans and O'Banion 2005; Lue et al. 1996; Akiyama et al. 2000; Mc Geer and Mc Geer 2001a, b), in Parkinson's disease (Teismann et al. 2003; Allan et al. 2010, Chap. 7 in this book), multiple sclerosis (Block and Hong 2005), amyotrophic lateral sclerosis (Henkel et al. 2004), brain ischaemia and stroke (Doll et al. 2014; Murray et al. 2015), physiological ageing (Lucin and Wyss-Conray 2009; Villeda et al. 2011), gliomas (Dikshit et al. 2013; Sen 2011), head injury (Harish et al. 2015; Johnson et al. 2013; Gentleman et al. 2004). Pathological studies have been shown that pro-inflammatory cytokine interleukin (IL)-1β is over-expressed six folds in the brains of AD patients compared with control subjects (Griffin et al. 1995).

A meta-analysis of cytokines (in blood and CSF) of patients with Alzheimer's disease concluded that, "these results strengthen the clinical evidence that AD is accompanied by an inflammatory response, particularly higher peripheral concentration of IL-6, TNF, IL-1β, IL-12 and IL-18 and higher CSF concentration of TGF-β (Swardfager et al. 2010)".

Release of these cytokines generates an inflammatory cascade, resulting in the synthesis of various downstream mediators (Murray et al. 2015). IL-1 has been reported to be the key pro-inflammatory mediator. It has two main ligands IL-1∝ and IL-1β. However, the relative role of other cytokines mentioned above and their role in perpetuating the chronic inflammation has not been clearly defined.

1.6 Complement System

Most complement components, nearly 30 of them, and their receptors are expressed by microglia, astrocytes and neurons. This is particularly prominent in neurodegenerative disorders (Bonifati and Kishore 2007; Lucin and Wyss-Conray 2009). The increase of these proteins in CNS in AD, ALS, Huntington's disease, MS, PD indicates a broad role for complement in neuronal degeneration (Amor et al. 2010). An increase in complement proteins can initiate neuroinflammatory processes by activating inflammatory cells, promoting their migration, and up-regulating phagocytosis (Song et al. 2000).

During AD, PD, HD and prion disease the levels of various complement components have been reported to be increased (Akiyama et al. 2000; Singh-rao 1999; Dandoy-Dron et al. 1998).

1.7 Factors Predisposing/Influencing Neuroinflammation

Role of neuroinflammation in the pathogenesis of neuronal damage due to injury, ischaemia or infection, endogenous toxins like accumulation of mis-folded proteins as seen in various neurodegenerative disorders—AD, PD, HD, Prion diseases or autoimmune diseases—is well established. It is not as well recognised that the involvement of the CNS can be influenced by comorbidities. The important among these are ageing, obesity, metabolic syndrome and diabetes.

1.7.1 Ageing

Microarrays of aged human and mouse brains show that genes related to cellular stress and inflammation increase with age, while genes related to synaptic functions/transport, growth factors and trophic support decrease (Lee et al. 2000; Lu et al. 2004). However, it is unclear why inflammation increases with age. It has been pointed out that microglia existing in ageing environment may be "a different beast altogether" (Lucin and Wyss-Conray 2009). Age related neuron degeneration itself activates microglia and promotes neuroinflammation. Ageing is the most common risk factor for neurodegenerative diseases, it results in a significant increase in glial activation, complement factors and inflammatory mediators (Lu et al. 2004; Streit et al. 2008). To make matters worse neurogenesis also decreases with age-possibly as a result of factors secreted by activated microglia (Carpentier and Palmer 2009). On the other hand Streit et al. (2004) and Streit (2005) suggested that microglial cells becoming increasingly dysfunctional with advancing age may result in a loss of their neuroprotective properties that could contribute to the development of age-related degeneration. Similarly advancing age is the single most important risk factors for stroke. Increase in serum levels of inflammatory cytokines increases the vulnerability of the aged brain to stroke (Jenny et al. 2002).

1.7.2 Obesity, Diabetes, Metabolic Syndrome

Although a considerable number of pro-inflammatory cytokines come from microglia and astrocytes, their peripheral source located in adipose tissue cannot be excluded (Misiak et al. 2012). The contribution of comorbidities to inflammation has been studied in details especially in relation to ischaemic stroke as also in AD. Accumulating evidence from clinical and experimental studies suggest that pre-existing inflammation and elevated levels of IL-1 can affect patient susceptibility and severity of CNS injury (Murray et al. 2015; McColl et al. 2007, 2009; Lee et al. 2008). A raised systemic inflammatory profile is a characteristic feature of obesity, evidenced by raised serum levels of C-reactive protein (CRP) and IL-6 (Visser et al. 1999). Obesity alone is an independent risk factor for ischaemic stroke (Suk et al. 2003; Yatsuya et al. 2010; Kurth et al. 2002; Terao et al. 2008). Adipose tissue is considered to be a highly active endocrine organ that liberates several cytokines and chemokines (collectively referred to as adipokines) that can produce an inflammatory response in distant tissues (Trujillo and Scherer 2006), playing a major role in pathogenesis of ischaemic stroke, by increasing its risk, contributing to increased size of infarct and worst outcome. IL-1 plays the key role as a mediator of acute neuronal injury. These adipokines may interact with central sub-clinical inflammation and contribute to the initiation of the pathology underlying AD. In the Framingham Heart Study, a high level of adiponectin was found to be a risk factor for all types of dementia including AD (van Himbergen et al. 2012). Chapter 12 have contributed to this subject in this book.

Metabolic syndrome has been associated with AD, vascular dementia and with cognitive decline (Panza et al. 2010). Insulin resistance, together with hyper insulinemia has been reported to promote neurodegeneration and facilitate the onset of AD. Excessive insulin production results in an increase in the level of Abeta and inflammatory agents, effects that are exacerbated by age and obesity (Fishel et al. 2005).

1.7.3 Pre-existing Systemic Inflammation

Pre-existing inflammation can present either chronically (as in obese individuals or in patients with rheumatoid arthritis) or as an acute event such as a viral infection. A systemic inflammatory challenge in an animal with a chronic neurogenerative disease leads to exaggerated brain inflammation, exaggerated sickness behaviour and a significant increase in acute neurodegeneration (Perry et al. 2007). A raised systemic inflammatory profile, a characteristic feature of obesity, metabolic syndrome and diabetes, is not only a risk factor for stroke but also increases the size of the infarct and a poor outcome. The risk of first time stroke was found to be higher after diagnosis of a systemic infection. This adverse effect of pre-existing systemic infection has been attributed to elevated levels of IL-1 (Murray et al. 2015). Systemic inflammatory challenges in mouse models of ALS, PD, prion disease lead to exaggerated CNS inflammation and a significant increase in neurodegeneration

(Cunningham et al. 2005; Misiak et al. 2012; Palin et al. 2008). Chronic systemic expression of IL-1β in mouse models of PD was found to enhance CNS inflammation and neurodegeneration (Godoy et al. 2008). Systemic infections and elevated plasma levels of IL-1 expression of IL-1β were found to be associated with increased rate of cognitive dysfunction in patients with AD (Holmes et al. 2003). Evidence that systemic inflammation in general is a risk factor for the future development of AD has been found in a number of studies. Inflammatory proteins in plasma, notably C-reactive protein and IL-6 were found to be increased 5 years before the clinical onset of dementia compared with age-matched individuals who did not develop dementia (Engelhart et al. 2004; Dunn et al. 2005).

1.8 Are There Differences in the Inflammatory Response in Different CNS Pathologies?

Voluminous information is now available regarding the role of inflammation as an important element of most CNS diseases both acute and chronic. However, most such studies deal with a single entity and are limited to only a few markers of inflammation. Furthermore, the temporal sequence of appearance of these markers is seldom described. The timing of cross-talk between the various cellular elements involved or a complete picture of the various cytokines, chemokines or complements participating in the inflammatory process is generally not available. Hence to say whether the inflammatory process involved in various CNS pathologies is identical, similar or at variance is not obvious. If it is same process, involving identical elements, following similar cascades of events, in same temporal sequence, and hence amenable to a common therapeutic strategy defies a clear answer.

That there would be differences is obvious from the standard neuropathological studies. For example the inflammatory lesions caused by viral, bacterial, mycobacterial, parasitic and fungal infections of the brain are quite distinct from each other. These are, of course, totally different from the inflammation associated with degenerative diseases. Similarly secondary inflammation following acute brain insults, e.g. traumatic brain injury and ischaemic stroke have their own pathophysiology. Whether at molecular level the pathogenetic mechanism of inflammation in these conditions is identical or similar is not known.

Some information is available on the time of onset of inflammation, the temporal sequence of involvement of various participants in the inflammatory process, e.g. the microglia, astrocytes, blood derived cells in various diseases, but a predictable generally applicable picture does not emerge. It is obvious that the triggers for activation of microglia are different for acute lesions like infective conditions, traumatic brain injury and stroke compared to most neurodegenerative disorders. The former activate the adaptive immune responses earlier, while endogenous signals in case of neurodegeneration switch on the innate responses to start with. As the disease progresses both innate and adaptive immune systems come into play. There appears to be a common thread of microglial activation across different

neurodegenerative disease though there are diverse ways of activating microglial cells which are known to possess a large number of surface receptors. Activated microglia release a number of pro-inflammatory cytotoxic soluble factors and a variety of interleukins. Do these play a specific role in determining the pathogenesis or pathology in a particular degenerative disorder with its characteristic involvement of well defined neuronal groups? Answer to such questions would require a systems approach for future investigations.

There is yet another feature of neuroinflammation induced neurodegeneration as observed in long-term effects of a single insult as seen in some cases of acute traumatic brain injury which requires further investigations. As reported by Gentleman et al. (2004) and Johnson et al. (2013), a single traumatic injury to the brain is associated with increased risk of dementia and, in a proportion of patients surviving a year or more from injury, there is development of hallmark Alzheimer's disease like pathologies (Guo et al. 2000; Plassman et al. 2000). Evidence of persistent inflammation and ongoing white matter degeneration for many years after a single traumatic brain injury has been well documented in humans as well as animal models (Johnson et al. 2013; Gentleman et al. 2004; Pierce et al. 1998; Holmin and Mathiesen 1999). The precise mechanism for development of this condition and its temporal dynamics is ill-understood.

1.9 Clinical Consideration

The role of neuroinflammation in pathogenesis of diverse CNS pathologies has been established beyond doubt during the last 2–3 decades. Various cellular and molecular participants in this process have been carefully identified and dissected. It prompted exploration as to how this knowledge can be utilised for therapeutic purposes. The working hypothesis was that combating the inflammation should result in prevention, impeding progression and amelioration of the primary disease itself. While this strategy has been advocated for treatment of several CNS diseases and disorders like autism (Vargas et al. 2005; Lv et al. 2013; Siniscalco et al. 2013), traumatic and ischaemic CNS damage (Hailer 2008), multiple sceloris (Pluchino et al. 2005), HD, AD (Chap. 6 in this book). Japanese encephalitis (Chap. 4 in this book), has been subject of many detailed studies—epidemiological, pathological, experimental, animal models and even clinical trials.

The observations that neuroinflammation, a common denominator in a large number of CNS pathologies suggested it to be a possible therapeutic target. This premise found support from a number of epidemiological studies showing slowing down the development of AD among individuals receiving non-steroidal anti-inflammatory drugs (NSAIDS) like those with rheumatoid arthritis (Beard et al. 1991; Mc Geer and Rogers 1992; Breitner et al. 1994, 1995; Mc Geer et al. 1996; Int'Veld et al. 2001). This prompted a number of clinical trials for the treatment of AD using different anti-inflammatory drugs—NSAIDS, Cortiosteroids, Cox-1, COx-2 inhibitors, (Rogers et al. 1993; Rogers and O'Barr 1996; Rother et al. 1998; Sainetti et al. 2000).

Notwithstanding the very promising leads from molecular, pathological, and in vitro studies (Hoozemans et al. 2001; Landreth and Heneka 2001; Mc Geer and Mc Geer 2001a, b) and epidemiological studies mentioned earlier, clinical trials in AD patients with anti-inflammatory drugs failed to slow the progression of dementia in AD (Aisen et al. 2000; Sainetti et al. 2000; Van Gool et al. 2001). Akiyama et al. (2000) and Hoozemans and O'Banion (2005) provide excellent reviews on the subject. The reasons for this failure are not forthcoming.

Inflammation being an immune response attempts were made to try immunotherapy both active and passive for this purpose. Immunisation with synthetic Abeta 1-42 peptide against a key component of the pathological process, amyloid beta-peptide was shown to be effective in a transgenic animal model of AD (Schenk et al. 1999). On the other hand, Bard et al. (2000) and De Mattos et al. (2001) demonstrated that peripherally administered antibodies against amyloid beta-peptide entered the CNS and reduced pathology in a mouse model of AD. The first clinical trial with Abeta vaccine in human beings was considered a success at the end of one year. However, development of meningo-encephalitis in some patients in a phase II trial resulted in its termination (Dodel et al. 2003).

1.10 Concluding Remarks

The role of inflammation in the pathogenesis of CNS damage in diverse pathological conditions has been unequivocally established during the last 2–3 decades. This is especially true for most of the degenerative disorders of the CNS—AD, PD, HD, F-T dementia, MS, ALS-among others. The role of neuroinflammation in the pathogenesis of these diseases was overlooked till recently because of the absence of the classical signs of inflammation observed in inflammatory lesions in the periphery. The established belief in CNS being immunologically privileged added to this misconception. Advances in molecular biology and renewed interest in glial biology finally led to unmasking neuroinflammation as a common denominator in vastly diverse pathological conditions of the CNS.

Over the years the role of various cellular, molecular, biochemical factors involved in neuroinflammation have been dissected. The reductionist approach followed for this purpose has led to the accumulation of vast amount of information. The relative role of both innate and adaptive immune responses has been defined. A large number of cytokines, chemokines, complements and other immunologically important molecules have been identified.

Notwithstanding the vast amount of new knowledge generated, it has not yet resulted in major clinical advances, be it for early diagnosis, prevention, prognosis or therapy. Like in many other fields of medicine the leads obtained from the promising animal model studies have belied expectations in clinical trials. It appears that time is ripe to utilize a systems approach to identify if the cascade of events is identical for different pathologies or not, the significant differences if any and whether more reliable leads can be identified for clinical purposes.

References

Aisen PS, Davis KL, Berg JD et al (2000) A randomised controlled trial of prednisone in Alzheimer's disease co-operative study. Neurology 54:588–593

Akiyama H, Berger S, Barnum S et al (2000) Inflammation and Alzheimer's disease. Neurobiol Aging 21:383–421

Allan SM, Rothwell NJ (2003) Inflammation in central nervous system injury. Philos Trans R Soc Lond B Biol Sci 358:1669–1677

Allan SM, Tyrrell PJ, Rothwell NJ (2005) Interleukin-1 and neuronal injury. Nat Rev Immunol 5:629–640

Allan LE, Geraldine HP, Brundin P (2010) Cell transplantation in Parkinson's disease: problems and perspectives. Curr Opin Neurol 23:1350

Aloisi F (1999) The role of microglia and astrocytes in CNS immune surveillance and immunopathology. Adv Exp Med Biol 468:123–133

Amor S, Puentes F, Baker D, van der Valk P (2010) Inflammation in neurodegenerative diseases. Immunology 129:154–169

Ankeny DP, Popovich PG (2009) Mechanisms and implications of adaptive immune responses after traumatic spinal cord injury. Neuroscience 158:1112–1121

Bard F, Cannon C, Barbour R et al (2000) Peripherally administered anti-bodies against amyloid beta-peptide enter the nervous system and reduce pathology in a mouse model of Alzheimer's disease. Nat Med 6:916–919

Barron KD (1995) The microglial cell: a historical review. J Neurosci 134:57–68

Beard CM, Kokman E, Kurland LT (1991) Rheumatoid arthritis and susceptibility to Alzheimer's disease. Lancet 337:1426

Block ML, Hong JS (2005) Microglia and inflammation—mediated neurodegeneration: multiple triggers with a common mechanism. Prog Neurobiol 76:77–98

Bonifati DM, Kishore U (2007) Role of complement in neurodegeneration and neuroinflammation. Mol Immunol. 44:999–1010

Breitner JC, Gau BA, Welsh KA et al (1994) Inverse association of anti-inflammatory treatments and Alzheimer's disease: initial results of a co-twin control study. Neurology 44:227–232

Breitner JC, Welsh KA, Helms MJ et al (1995) Delayed onset of Alzheimer's disease with non-steroidal anti-inflammatory and histamine H2 blocking drugs. Neurol Aging 16:523–530

Carpentier P, Palmer T (2009) Immune influence on neural stem cell regulation and function. Neuron 64:79–92

Chen Y, Hallenbeck JM, Ruetzlar C, Bol D et al (2003) Overexpression of monocyte chemoattractant protein 1 in the brain exacerbates ischaemic brain injury and is associated with recruitment of inflammatory cell. J Cereb Blood Flow Metab 23:748–755

Cuartero MI, Ballesteros I, Moraga A (2013) N2 neutrophils novel players in brain inflammation after stroke modulation by PPARr against rosiglita zone. Stroke 44:3498–3508

Cunningham C, Wilcockson DC, Campion S, Lunnon K (2005) Central and systemic endotoxin challenges exacerbate the local inflammatory response and increase neuronal death during chronic neurodegeneration. J Neurosci 25:9275–9284

Dandoy-Dron F, Guillo F, Benboudjema L, Deslys JP (1998) Gene expression in scrapie. Cloning of a new scrapie-responsive gene and the identification of increased levels of seven other mRNA transcripts. J Biol Chem 273:7691–7697

De Mattos RB, Bales KR, Cummins DJ et al (2001) Peripheral anti-A beta antibody alters CNS and plasma A beta burden in a mouse model of Alzheimer's disease. Proc Natl Acad Sci USA 98:8850–8855

Dikshit B, Irshad K, Madan E, Aggarwal N et al (2013) FAT1 acts as an upstream regulator of oncogene and inflammatory pathways, via PDCD4, in gliomas cells. Oncogene 32:3798–3808

Dodel RC, Hampel H, Du Y (2003) Immunotherapy for Alzheimer's disease. Lancet 2:215–220

Doll DN, Barr TL, Simpkins JW (2014) Cytokines: their role in stroke and potential use as biomarkers and therapeutic targets. Aging Dis 5:294–306

Dunn N, Mullee M, Perry VH, Holmes C (2005) Association between dementia and infectious disease: evidence from a case-control study. Alzheimer Dis Assoc Disord 19:91–94
Eikelenboom P, Bate C, Van Gool WA, Hoozemans JJ et al (2002) Neuroinflammation in Alzheimer's disease and prion disease. Glia 40:232–239
El Khoury J, Toft M, Hickman SE, Means TK (2007) Ccr2 deficiency impairs microlgial accumulation and accelerates progression of Alzheimer-like disease. Nat Med 13:432–438
Engelhart MJ et al (2004) Inflammatory proteins in plasma and the risk of dementia: the rotterdam study. Arch Neurol 61:668–672
Fishel MA, Watson GS, Montine TJ, Wang Q (2005) Hyperinsulinemia provokes synchronous increases in central inflammation and beta-amyloid in normal adults. Arch Neurol 62: 1539–1544
Gentleman SM, Leclercq PD, Moyes L, Graham DI et al (2004) Long-term intracerebral inflammatory response after traumatic brain injury. Forensic Sci Int 146:97–104
Godoy MC, Tarelli R, Ferrari CC, Sarchi MI, Pitossi FJ (2008) Central and systemic IL-1 exacerbates neurodegeneration and motor symptoms in a model of Parkinson's disease. Brain 131:1880–1894
Griffin WS, Sheng JG, Roberts GW, Mrak RE (1995) Interleukin-1 expression in different plaque types in Alzheimer's disease: significance in plaque evolution. J Neuropathol Exp Neurol 54:294–297
Guo Z, Cupples A, Kurz A (2000) Head injury and the risk of AD in the MIRAGE study. Neurology 54:1316–1323
Hailer NP (2008) Immunosuppression after traumatic or ischemic CNS damage: it is neuroprotective and illuminates the role of microglial cells. Prog Neurobiol 84:211–233
Harish G, Mahadevan A, Pruthi N, Sreelakshmi K et al (2015) Characterization of traumatic brain injury in human brains reveals distinct cellular and molecular changes in contusion and preicontusion. J Neurochem 134:156–172
Henkel JS, Engelhard JI, Siklo's L, Simpson EP (2004) Presence of dendritic cells, MCP-1 and activated microglia/macrophages in amyotrophic lateral sclerosis spinal cord tissue. Ann Neurol 55:221–235
Holmes C, El-Okl M, Williams AL, Cunningham C (2003) Systemic infection, interleukin 1beta, and cognitive decline in Alzheimer's disease. J Neurol Neurosurg Psychiatry 74:788–789
Holmin S, Mathiesen T (1999) Long-term intracerebral inflammatory response after experimental focal brain injury in rat. NeuroReport 10:1889–1891
Hoozemans JJ, O'Banion MK (2005) The role of COX-1 and COX-2 in Alzheimer's disease pathology and the therapeutic potentials on non-steroidal anti-inflammatory drugs. Curr Drug Targets CNS Neurol 4:307–315
Hoozemans JJ, Rozemuller AJM, Janssen I et al (2001) Cyclo-oxygenase expression in microglia and neurons in Alzheimer's disease and control brains. Acta Neuropathol (Berl) 101:208
Int'Veld BA, Ruitemberg A, Holfman A et al (2001) Nonsteroidal anti-inflammatory drugs and the risk of Alzheimer's disease. N Engl Med 345:1515–1521
Jenny NS, Tracy RP, Ogg MS, le Luong A et al (2002) In the elderly, interleukin-6 plasma levels and the -174G> C polymorphism are associated with the development of cardiovascular disease. Arterioscler Throm Vas Biol 22:2066–2071
Johnson VE, Stewart JE, Begbie FD, Trojanowiski JQ et al (2013) Inflammation and white matter degeneration persist for years after a single traumatic brain injury. Brain 136:28–42
Kreutzberg GW (1996) Microglia: a sensor for pathological events in the CNS. Trends Neurosci 19:318–321
Kurth T, Gaziano JM, Berger K, Kase CS (2002) Body mass index and the risk of stroke in men. Arch Int Med 162:2557–2562
Landreth GE, Heneka MT (2001) Anti-inflamatory actions of peroxisome proliferator-activated receptor gamma agonists in Alzheimer's disease. Neurobiol Aging 22:937–944
Lee CK, Weindruch R, Prolla TA (2000) Gene expression profile of ageing brain in mice. Nat Genet 25:294–297

Lee YH, Tharp WG, Maple RL, Nair S et al (2008) Amyloid precursor protein expression is upregulated in adipocytes in obesity. Obesity 16:1493–1500
Lu T, Pan Y, Kao SY, Li C et al (2004) Gene regulation and DNA damage in the ageing human brain. Nature 429:883–891
Lucin KM, Wyss-Conray T (2009) Immune activation in brain aging and neurodegeneration: too much or too little? Neuron 64:110–122
Lue LF, Brachova L, Civin H, Rogers J (1996) Inflammation Aβ deposition, and neurofibrillary tangle formation as correlates of Alzheimer's disease neurodegeneration. J Neuropath Exp Neurol 55:1083–1088
Lv Y-T, Zhang Y, Liu M et al (2013) Transplantation of human cord blood mononuclear cells and umbilical cord-derived mesenchymal stem cells in autism. J Transl Med 11:196–205
Mantovani S, Garbelli S, Pasin A, Alimonti D et al (2009) Immune system alterations in sporadic amyotrophic lateral sclerosis patients suggest an ongoing inflammatory process. J Neuroimmunol 210:73–79
Matsumoto Y, Ohmori K, Fujiwara M (1992) Microglial and astroglial reactions to inflammatory lesions of experimental autoimmune encephalomyelitis in the rat central nervous system. J Neuroimmunl 37:23–33
Mc Geer PL, Mc Geer EG (2001a) Inflammation, autotoxicity and Alzheimer's disease. Neurobiol Aging 22:799–809
Mc Geer PL, Mc Geer EG (2001b) Inflammation, autotoxicity and Alzheimer's disease. Neurobiol Aging 22:799–809
Mc Geer PL, Mc Geer EG (2004) Inflammation and the degenerative diseases of aging. Ann N Y Acad Sci 1035:104–116
Mc Geer PL, Rogers J (1992) Anti-inflammatory agents as a therapeutic approach to Alzheimer's disease. Neurology 42:447–449
Mc Geer PL, Akiyama H, Itagaki S, Mc Geer EG (1989) Immune system response in Alzheimer's disease. Can J Neurol Sci 16:561–567
Mc Geer PL, Schulzer M, Mc Geer EG (1996) Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease: a review of 17-epidemiology studies. Neurology 47:425–432
McColl BW, Rothwell NJ, Allan SM (2007) Systemic inflammatory stimulus potentiates the acute phase and CXC chemokine responses to experimental stroke and exacerbates brain damage via interleukin-1- and neutrophil-dependent mechanisms. J Neurosci 27:4403–4412
McColl BW, Allan SM, Rothwell NJ (2009) Systemic infection, inflammation and acute ischemic stroke. Neuroscience 158:1049–1061
Miller G (2005) Neuroscience: the dark side of glia. Science 308:778–781
Misiak B, Leszek J, Kiejna A (2012) Metabolic syndrome, mild cognitive impairment and Alzheimer's disease—the emerging role of systematic low-grade inflammation and adiposity. Brain Res Biol 89:144–149
Murray KN, Parry-Jones AR, Allan SM (2015) Interleukin-1 and acute brain injury. Front Cell Neurosci 9:1–17
Neumann H, Medana IM, Bauer J, Lassmann H (2002) Cytotoxic T lymphocytes in autoimmune and degenerative CNS diseases. Trends Neurosci. 25:313–319
Nimmerjahn A, Kirchhoff F, Helmnchen F (2005) Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308:1314–1318
Oka A, Takashima S (1997) Induction of cyclo-oxygeanse 2 in brains of patients with Down's syndrome and dementia of Alzheimer's type: specific localization in affected neurons and axons. Neuro Rep 8:161–164
Palin K, Cunningham C, Forse P, Perry VH, Platt N (2008) Systemic inflammation switches the inflammatory cytokine profile in CNS Wallerian degeneration. Neurobiol Dis 30:19–29
Panza F, Frisarde V, Cupurso C, Imbimbo BP et al (2010) Metabolic syndrome and cognitive impairment: current epidemiology and possible underlying mechanisms. J Alzheimer's Dis 21:691–724

Perry VH, Cunningham C, Holmes C (2007) Systematic infection and inflammation affect chronic neurodegeneration. Nat Rev/Immunol 7:161–167
Pierce JE, Smith DH, Trojanowski JQ, McIntosh TK (1998) Enduring cognitive, neurobehavioral and histopathological changes persist for up to one year following severe experimental brain injury in rats. Neuroscience 87:359–369
Plassman BL, Havlik RJ, Steffens DC, Helms MJ (2000) Documented head injury in early adulthood and risk of Alzheimer's disease and other dementias. Neurology 55:1158–1166
Pluchino S, Zanoti L, Rossl B et al (2005) Neurosphere-derived multipotent precursors promote neuroprotective by immune-modulatory mechanisms. Nature 436:266–271
Ray S, Britschgi M, Herbert C, Takeda-Uchimura Y et al (2007) Classification and prediction of clinical Alzheimer's diagnosis based on plasma signalling proteins. Nat Med 13:1359–1362
Rogers J, O'Barr S (1996) Inflammatory mediators in Alzheimer's disease 1996 (Quoted by Akiyama et al 2000)
Rogers J, Kirby SR, Hempelman D et al (1993) Clinical trial of indo-methacin in Alzheimer's disease. Neurology 43:1609–1611
Rother M, Erkinjuntti T, Roessner M et al (1998) Propentofylline in the treatment of Alzheimer's disease and vascular dementia: a review of phase III trials. Dement Geriatr Cog Dis Ord 9:36–43
Sainetti SM, Ingrim DM, Talwalkar S, Geis GS (2000) Results of a double-blind, randomised, placebo-controlled study of celecoxib in treatment of progression of Alzheimer's disease (Quoted by Eikelenboom et al 2002)
Schenk D, Barbour R, Dunn W et al (1999) Immunization with amyloid-beta attenuates Alzheimer's disease-like pathology in the PDA PP mouse. Nature 400:173–177
Sen E (2011) Targeting inflammation—induced transcription factor activation: an open frontier for glioma therapy. Drug Discov Today 16:1044–1051
Sherman MY, Goldberg AL (2001) Cellular defences against unfolded proteins: a cell biologist thinks about neurodegenerative disease. Neuron 29:15–32
Singh-rao SK (1999) Increased complement biosynthesis by microglia and complement activation on neurons in Huntington's disease. Exp Neurol 159:362–376
Siniscalco D, Bradstreet JJ, Antonucci N (2013) Therapeutic role of hematopoietic stem cells in autism spectrum disorder-related inflammation. Front Immunol 4:1–6
Song WC, Sarrias MR, Lambris JD (2000) Complement and innate immunity. Immunopharmacology 49:187–198
Stoll G, Jander S (1999) The role of microglia and macrophages in the pathology of the CNS. Prog Neurobiol 58:233–247
Streit WJ (2004) Microglia and Alzheimer's disease pathogenesis. J Neurosci Res 77:1–8
Streit WJ (2005) Microglia and neuroprotection: implications for Alzheimer's disease. Brain Res Rev 48:234–239
Streit WJ (2009) Microglia and neuroprotection: implication for Alzheimer's disease. Brain Res Rev 48:234–239
Streit WJ, Walter SA, Pennel NA (1999) Reactive microgliosis. Prog Neurobiol 57:563–581
Streit WJ, Sammons NW, Kuhns AJ, Sparks DL (2004) Dystrophic microglia in the aging human brain. Glia 45:208–212
Streit WJ, Miller KR, Lopes KO, Njie E (2008) Microglial degeneration in the aging brain–bad news for neurons? Front Biosci 13:3423–3438
Suk SH, Sacco RL, Boden-Albala B, Cheun JF (2003) Abdominal obesity and risk of ischemic stroke: the Northern Manhattan Stroke Study. Stroke 34:1586–1592
Swardfager W, Lanctot K, Rothenberg L, Wong A et al (2010) A meta-analysis of cytokines in Alzheimer's disease. Biol Psychiat 68:930–941
Tandon PN (2007) Brain cells-recently unveiled secrets: their clinical significance. Neurol India 55:322–327
Teismann P, Tieu K, Cohen O, Chot D-K et al (2003) Pathogenic role of glial cells in Parkinson's disease. Mov Disord 18:121–129

Terao S, Yilmaz G, Stokes KY, Ishikawa M et al (2008) Inflammatory and injury responses to ischemic stroke in obese mice. Stroke 39:943–950

Tian L, Rauvala H, Gahmberg CG (2009) Neuronal regulation of immune responses in central nervous system. Trends Immunol 30:91–99

Trujillo ME, Scherer PE (2006) Adipose tissue-derived factors: impact on health and disease. Endocr Rev 27:762–778

Van Gool WA, Weinstein HC, Scheltens PK, Walstra GJ (2001) Effect of hydrochloroquine on progression of dementia in early Alzheimer's disease: an 18-month randomised double-blind, placebo-controlled study. Lancet 358:455–460

Van Himbergen TM, Beiser AS, Ai M, Seshadri S (2012) Biomarkers for insulin resistance and inflammation and the risk for all-cause dementia and alzheimer disease: results from the Framingham Heart Study. Arch Neurol 69:594–600

Vargas DI, NAsclmbene C, Krishnan C (2005) Neuroglial activation and neuroinflammation in the brain of patients with autism. Ann Neurol 57:67–81

Vehmas AK, Kawas CH, Stewart WF, Troncoso JC (2003) Immune reactive cells in senile plaques and cognitive decline in Alzheimer's disease. Neurobiol Aging 24:321–331

Villeda SA, Luo J, Mosher KI, Zhou B et al (2011) The ageing systematic milieu negatively regulates neurogenesis and cognitive function. Nature 477:90–94

Visser M, Bouter LM, McQuillan GM et al (1999) Elevated C-reactive protein levels in overweight and obese adults. JAMA 282:2131–2135

Walker LC, LeVine H (2000) The cerebral proteo-pathies—neurodegenerative disorders of protein conformation and assembly. Mol Neurobiol 21:83–95

Walter S, Letiembre M, Liu Y, Heine H (2007) Role of the toll-like receptor 4 in neuroinflammation in Alzheimer's disease. Cell Physiol Biochem 20:947–956

Yatsuya H, Folsom AR, Yamagishi K, North KE et al (2010) Race and sex-specific associations of obesity measures with ischemic stroke incidence in the Atherosclerosis Risk in Communities (ARIC) study. Stroke 41:417–425

General Physiology and Pathophysiology of Microglia During Neuroinflammation

2

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Abstract

Microglia are the immunocompetent resident macrophages of the central nervous system and constitutes 15–20 % of the glial population. They provides the first line of defence against any disease or insult and display enormous structural and functional plasticity. Microglial cells are also well establised to play a very important role in the pathogenesis of various neurological disorders. Microglial activation not only protect and repair the damaged tissue by eliminating the dying cell and assisting the restorative process but are also implicated in inducing neurodegeneration. This review provides a comprehensive account of development and various physiological states of microglia and their role in healthy and disease brain.

2.1 Introduction

Our understanding of microglia has moved from being a 'silent' cell in healthy brain to an actively involved component in brain physiology, neurogenesis, cognition and behavioural functions. They are the surveyors of the healthy brain with actively retracting and extending their processes and thus maintaining the pre- and post-synaptic elements and fine tuning of the neuronal circuits. Thus, a disruption of this homeostatic act of microglia becomes the prime cause of neuronal disorders. Microglia are nomadic cells of the brain that continuously survey the central

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nervous system (CNS) with their highly motile extensions for any kind of brain insult (Gehrmann et al. 1995) and constitute 15–20 % of the total glial population in the central nervous system (Carson et al. 2006). They are the resident macrophages of CNS and are immunocompetent phagocytic cells and constitute the first line of defence against any disease or insult and exhibit structural and functional plasticity. Microglia are considered responsible to maintain the homeostasis within the brain and undergo appropriate structural transformations to perform various immunological functions. First recognized by Nissl in 1880, later Pio-del Rio Hortega, a Spanish neuroanatomist, described microglia as resting ramified cells using silver staining methods (Del Rio-Hortega 1932).

Microglial cells are now well recognized as an elementary contributor in the pathogenesis of various neurological diseases and disorders (Heneka et al. 2010; Parpura et al. 2012; Verkhratsky et al. 2014). As affiliate of brain defence system, on any immune breaching or insult, microglia become activated (Saxena et al. 2007; Patro et al. 2010a, b, 2013; Nagayach et al. 2014a, b, 2015; Sharma et al. 2015). On activation, these immune cells get rapidly transformed into the reactive phenotype and slack their highly ramified morphology not only to protect but also to repair the damaged tissue by removing the dying cell debris and facilitating the healing process (Hanisch and Kettenmann 2007; Kettenmann et al. 2011). On the contrary, microglial activation is also responsible in aggravating the neurodegeneration (Block and Hong 2005; Venero et al. 2011). Understanding of the imperative and multitasking attribute of microglial cells, like its stature and response following neuroinflammation deserves pertinent investigation.

2.2 Physiological States of Microglia

Morphologically, microglia have three major transitional stages that can be distinguished as: amoeboid, ramified or resting and reactive or activated (Fig. 2.1) and these states perform varied functions in the brain.

Amoeboid microglia are round or irregular in shape. They are more prevalent during development, originate from the yolk sac and populate the developing brain early. Association of developmental neuronal cell death and microglia has been reported in most parts of the CNS (Pont-Lezica et al. 2011). Because of phagocytosis as well as their ability to induce apoptosis in unwanted neurons in developing brain, microglia are important participant in the process of CNS development. They also interact with the synapses and modulate synaptic plasticity via pruning of excessive unwanted synapses and this is mediated by the complement pathway (Schafer et al. 2012; Ginhoux et al. 2013; Neiva et al. 2014). Morphologically, they closely resemble the macrophages. Amoeboid microglia are generated from primitive myeloid/ haematopoietic progenitor cells during the embryonic and perinatal stage and sustain up to the early postnatal stages in rats (Prinz and Mildner 2011; Gomez et al. 2013) and finally transform into ramified

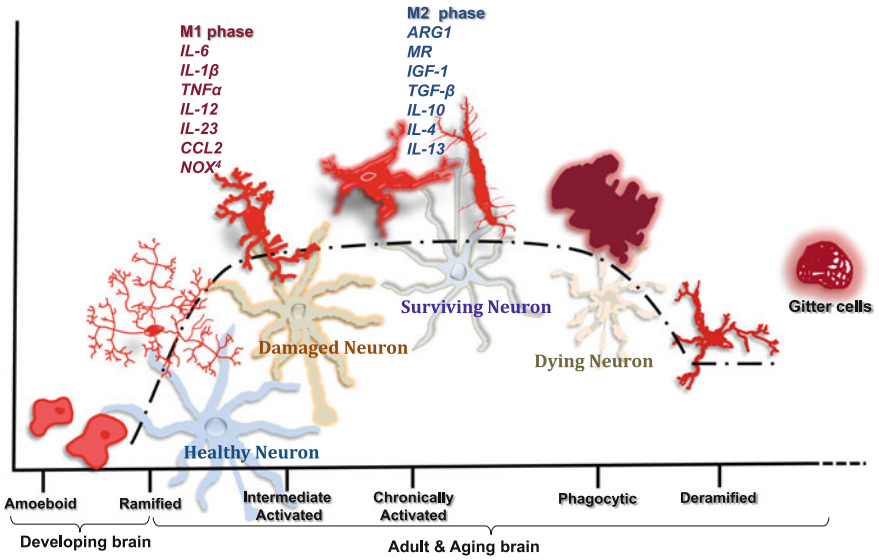

Fig. 2.1 Microglial transformations both in terms of phenotype and secretory molecules with the advancing age: In developing and adult brain, amoeboid and ramified microglia supports the survival of healthy neurons. During state of insult microglia get activated and attain either of the two phases of activation, i.e. M1 and M2 on the basis of severity and generation of secretory molecules. M1 phase exacerbates microglial activation directed neuronal damage via releasing plethora of pro-inflammatory molecules while M2 phase mitigates the neuroinflammation and promotes tissue repair and neuron survival by secreting growth factors and anti-inflammatory molecules. On disease progression and neuronal death, microglia turn deramified and phagocytic. Gitter cells are the microglia crammed with the phagocytic debris

microglia (Kaur et al. 1985). The development of microglia and role of microglia in brain development have been reviewed by Pont-Lezica et al. (2011) and Nayak et al. (2014).

Ramified or '*resting surveillent microglia*' of the adult CNS consist of small cell body with short, wispy and fine processes. These processes extend into the brain microenvironment creating a matrix-like structure that helps to better perceive the CNS milieu. Yamasaki et al. (2014) have reviewed the available information on the differentiation of the resident microglia and the monocytes in neuroinflammatory states. Microglia are considered to be the critical effectors and regulators of changes in CNS homeostasis in health and disease (Prinz and Priller 2014) as well as during CNS development. Microglia even in healthy brain continuously survey the CNS for any damage or insult as shown in in vivo time-lapse video microscopy and hence they are never in a state of rest (Nayak et al. 2014). The studies of Hellwig et al. (2013) have established the active role of such cells as depletion of ramified microglia prior to experimental stroke exacerbated the damage, establishing the active and protective role of the so called 'resting microglia'.

Reactive microglia: Following any unfavourable stimuli ramified microglia get transformed into a reactive or activated state. Such cells have thick and retracted processes with a large and irregular shaped cell body. Reactive microglia even start proliferating to ascertain better screening and support as a hallmark of microglial activation (Niquet et al. 1994). Depending upon the stimulus and progression of the diseased state, microglial activation acts in two ways; either help in efficacious restoration of the injured brain cells or generate a threatening environment that results in exaggerated brain damage. Recent studies with mouse models of neurodegenerative disorders have helped us in better understanding to an extent the role of microglia in health and disease (Hellwig et al. 2013). To overcome the confusion, the activity-dependent microglial activation spectrum (Tang and Le 2015) was developed on the basis of cytoactive factors released by the reactive microglia (Fig. 2.1). The 'classical activation (popularly known as M1 phase)' represents the initial innate immune response induced by Toll-like receptor (TLR) ligands and interferon-γ (IFN-γ) followed by the generation of pro-inflammatory cytokines. The reactive release of a plethora of pro-inflammatory molecules like tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-1β (IL-1β), interleukin-12 (IL-12), superoxide anions, nitric oxide synthase, redox molecules like nitrogen dioxide 2 (NOX2), nitrogen dioxide1 (NOX1), a member of Rho family of GTPases (RAC1), inducible nitric oxide synthase (iNOS), nitric oxide synthase 2 (NOS2) and excitotoxic molecules like group II metabotropic glutamate receptor (MGluR2), glutamate transporter-1 (GLT-1), purinergic P2X7 receptor (P2X7-R), etc. (Benoit et al. 2008). The next alternate phase of microglial activation is 'M2 or alternate activation' phase, that dampen inflammation by switching over to the anti-inflammatory state by secreting molecules like interleukin-4 (IL-4), interleukin-3 (IL-13), interleukin-1 receptor antagonist (IL-1RA), scavenging receptors and extracellular matrix molecules (Luo and Chen 2012). The cytoactive molecules thus released mitigate the generation of pro-inflammatory molecules. This accelerates the process of wound healing and damaged tissue repair (Martinez et al. 2008). The third or subtype of M2 phase is 'acquired deactivation' associated with deactivation of glial inflammation and uptake of apoptotic cells or oxidized lipids via release of anti-inflammatory cytokines like transforming growth factor-β (TGF-β) and interleukin-10 (IL-10; Gregory and Devitt 2004; Colton 2009).

2.3 Development of Microglia

The origin of microglia and its cell lineage still remains highly controversial and debatable. Microglia arise early during development from precursor cells in the embryonic yolk sac that seed the brain rudiment and appear to persist throughout the life. Microglia are the only cell population in the CNS that originate outside the brain. The differentiation of yolk sac macrophages into typical microglia is dependent on transcription factors like IFM regulatory factor-8 (IRF-8; Ginhoux et al. 2013; Prinz and Priller 2014). Bone marrow-derived progenitors or monocytes

are also considered to be recruited for supplementing the microglial population (Saijo et al. 2013; Ginhoux et al. 2013; Prinz and Priller 2014).

The neuroectodermal matrix cells and yolk sac cells are the two distinct sources of microglial precursors (Saijo and Glass 2011). Prenatally, these cells invade the brain through meninges, choroid plexus and ventricles (Boya et al. 1991; Ginhoux et al. 2010). This primeval microglial population was reported in human gestation week 5.5 near the di-telencephalic fissure (Monier et al. 2006). First, the neuroectodermal and yolk sac cells populate the brain during first two trimesters in humans and between embryonic days 10/9.5–10.5 in rodents, and grow as amoeboid microglia (Ginhoux et al. 2010). Subsequently in early days of postnatal development, the circulating monocytes developed from blood borne precursors later give rise to amoeboid microglia (Rezaie and Male 2002). The hematopoietic stem cells in developing and adult brain have also been reported to transform into microglia (Alliot et al. 1991). This has been supported by chimeric animal study following irradiation (Hickey et al. 1992) and in experimental model of allergic encephalomyelitis (EAE; Lassmann and Hickey 1993). However, as a contrast, it has also been reported that microglia also existed before brain vascularization and production of monocytes in hematopoietic tissues indicating thereby that all microglia are not hematopoietic in origin (Shepard and Zon 2000; Takahashi 2001). The perivascular microglia are the only cell population that are continuously replaced in the adulthood by bone marrow-derived haematopoietic precursors (Hickey and Kimura 1988). While we continue debating the microglial lineage and origin, interestingly two independent reports claim that microglia can themselves act like pluripotent stem cells and can also transform into astrocytes, neurons and oligodendrocytes (Yokoyama et al. 2004; Matsuda et al. 2008) although the lineage of microglia is different than the astrocytes and neurons. This is being actively investigated and remains to be established and explored.

2.4 Microglia in Healthy Brain

2.4.1 In Developing Brain

Brain development and maturation involves a continuous refinement of synapses involving pruning of inappropriate synapses and strengthening of the established ones. Microglia have been implicated as a major player for the developmental synaptic pruning (Rakic and Zecevic 2000). The activated microglia surround the regions undergoing developmental synapse turnover, and remove the unnecessary synapses (Paolicelli et al. 2011). This happens in a complement-dependent manner. During the embryonic and early postnatal life amoeboid microglia expressing DNAX associated protein 12 (DAP12), complement and fractalkine receptors are directed towards the developing synaptic sites. Such microglia engulf the complement proteins (C1q and C3) and tagged synapses (Paolicelli et al. 2011; Schafer et al. 2013). Thus, any kind of deviation in microglial involvement leads to deficits in synaptic remodelling and maintenance, resulting in developmental disorders.

Microglia are also believed to be involved in regulation of neuronal differentiation (Farinas et al. 2002) and apoptosis (Miller and Kaplan 2001) by producing neurotrophins (Nakajima et al. 2001) and the presence of microglia secreted basic fibroblast growth factors (bFG; Bansal 2002) and cytokine IL-1β have been reported to enhance proliferation and differentiation of oligodendrocytes and astrocytes. Microglia undoubtedly play an authoritatively supportive and directive role in both neurogenesis and gliogenesis in developing brain (Thored et al. 2009).

2.4.2 In Adult Brain

'*Ramified/(resting?) surveillent*' microglia reside at strategic locations throughout the mammalian brain and spinal cord. Such microglia are unremittingly surveying the healthy brain for any disparaging condition at a speed of 1.47 μm s^{-1} with their long thin processes (Nimmerjahn et al. 2005). Recent in vivo studies have recorded the region specific speed of process motility to be between 0.2 and 6.5 μm/min (Tremblay et al. 2010). During such scrutiny microglial processes constantly establish a direct contact with neuronal synapses (Wake et al. 2009). Such microglia release various neurotrophic growth factors to promote the neuronal survival and also to enhance neurogenesis (Ekdahl et al. 2009). In neurodegenerative diseases and following brain insults the resident microglia get stimulated and transform into activated or reactive state. In such circumstances microglia release numerous inflammatory molecules, growth factors, matrix proteins, chemokines, prostanoids and reactive free radicals (Fig. 2.2) either contributing to neuronal dysfunction and cell death or to provide support in the healing process (Gomes-Leal 2012). The detrimental or beneficial role of microglia depends upon the type and intensity of the insult and associated microglia activation stature. This may even call for microgliosis. Microglia in adult brain are not evidenced to have the ability of restoring their normal density, if depleted experimentally from the pool of precursor cells dispersed all over the brain (Parkhurst et al. 2013; Elmore et al. 2014), rather than depending upon the influx from the peripheral bloodstream as reported previously (Hughes and Bergles 2014). This may be one of the mechanisms how the old and/or damaged microglia are replaced with new healthy microglia during progression of a disease and ageing conditions.

It is now clear that microglia are also important in both learning and synaptic remodelling (Parkhurst et al. 2013) and take part in activity-dependent structural remodelling both driven by sensory input and age-related factors (Wake et al. 2009; Tremblay et al. 2012). Microglia in adult brain help in regulation of long-term potentiation (LTP) and tuning of synaptic strength, which is responsible for consistent long-term neural networks (Ben Achour and Pascual 2010; Kettenmann et al. 2011). Microglia also maintain the synaptic plasticity by releasing various soluble molecules responsible for regulating learning and memory and augmentation of *N*-methyl-D-aspartate (NMDA)-mediated LTP responses. It has been

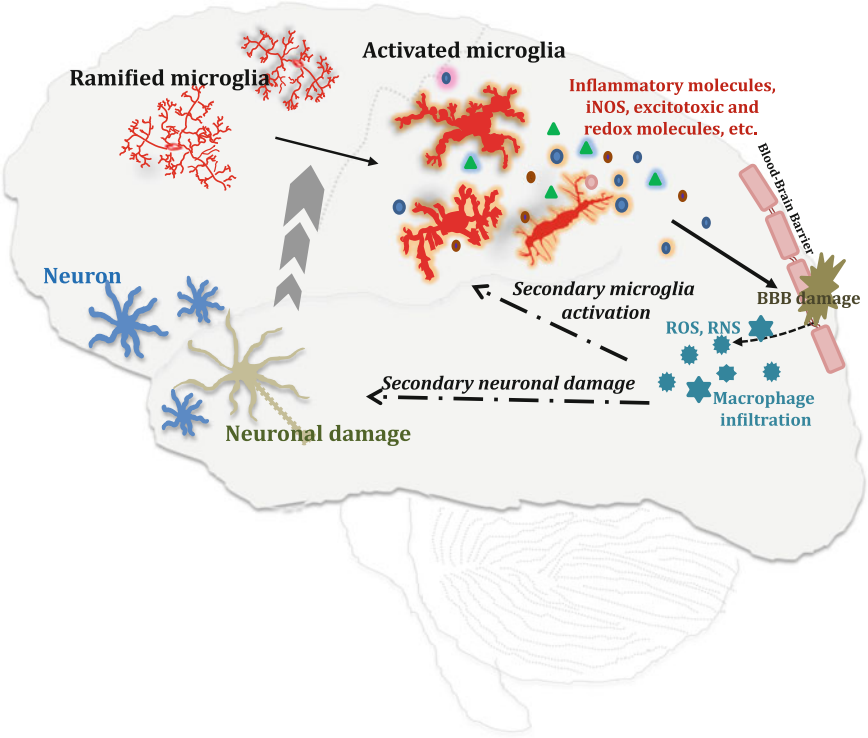

Fig. 2.2 Microglial pathology following neuroinflammation: in response of immune breaching and neuronal damage, a state of neuroinflammation developed inside the brain foremostly activates the microglia. Ramified microglia get transformed into activated microglia and release various neuroinflammatory molecules that leads to blood–brain barrier damage. Such damage promotes the macrophagic infiltration that later on exaggerates the influx of inflammatory cytokines and aggravate the existing neuroinflammatory state in CNS by causing secondary neuronal damage and subsequent microglial activation

predicted that the absence of microglia-mediated fractalkine receptor CX3CR1 signalling and secretion of glycine and L-serine by experimental intervention results in diminished learning and memory (Hayashi et al. 2006; Rogers et al. 2011). Moreover, microglia also mediate the modulation of GABAergic transmission and basal glutamatergic signalling via brain-derived neurotrophic factor (BDNF) and adenosine triphosphate (ATP; Coull et al. 2005; Pascual et al. 2012). BDNF is required for tyrosine kinase B (TrkB) phosphorylation responsible for synaptic plasticity. This has now been established in mice models depleted of microglia that have impaired ability in multiple learning tasks. Such mice also presented a reduction in motor learning-associated synaptic formation.

2.4.3 In Aged Brain

The immune components in the ageing brain are equally affected with age-associated challenges. The immune system in aged brain is also more susceptible towards age-associated damage and dysfunction (Yung and Julius 2008). Microglial dystrophy has been noted as an indication of microglial senescence in brain (Streit et al. 2004; Kushwaha 2009; Patro et al. 2010c). With advancing age, microglia become more reactive (Streit 2006; Godbout and Johnson 2009), exhibit an amoeboid-like morphology and present an upregulation of major histocompatibility complex class II antigens, toll-like receptors 4 (TLR4) and cluster of differentiation 14 (CD14) receptors on their surface. Concomitantly, microglia also express an elevated pro-(TNF-α, IL-1β, IL-6) and anti-(TGF-β, IL-10) inflammatory cytokines in the healthy senile brain of aged mice (Sierra et al. 2007; Godbout and Johnson 2009). However, it still remains to be established either such primed state is associated with the ageing changes of the brain or ageing of the microglial cells themselves.

Ageing, age-associated exposure to stress and neurodegenerative diseases, all induce a 'priming' stimulus to microglia. Microglial priming and impaired microglial response is suggestive of age-related changes in microglial regulation (Wynne et al. 2009). Amplified cytokine response by primed microglia has been related to the behavioural distortions like maladaptive sickness response studied in aged subjects exposed to peripheral stimulation (Dilger and Johnson 2008). Increased cytokine secretion by such 'primed' microglia following altered immune reaction also cause cognitive impairment in aged brain (Chen et al. 2008).

2.5 General Microglial Physiology

2.5.1 Ion Channels

Myriad of microglial patch-clamp studies in tissue slices and in cell culture showed that microglia possess various ion channels, comprising K^+, Ca^{2+} and Na^+ channels. These ion channels undoubtedly play a potential role in both regulation and maintenance of microglial functions (Färber and Kettenmann 2005, 2006a, b; Eder 2005; Schilling and Eder 2007; Black et al. 2009). In general, ion channels in all living cells may influence several cellular processes like, proliferation, migration, apoptosis, secretion and excitability, etc. via movement of cations or anions across the membrane through hydrophilic pores. The functional stature of the microglia evidently states the expression patterns of the ion channels. Expression of various cytokines or immune molecules fluctuate the pH along the gradient and/or activation of the G proteins or protein kinase C, that in turn could modulate the microglial ion channels. The functional coherence and transforming ability of microglial ion channels during various conditions make them a suitable target to study under pathophysiological process like neuroinflammation that further contributes to the onset or progression of neurological disorders.

2.5.2 Sodium Channels

In the healthy CNS (in vivo) the evidential data regarding the functional activity of voltage-operated Na^+ channels in microglial cells is scanty (Black and Waxman 2012). However, in vitro study in rat microglia (Black et al. 2009) depicted the three major isoforms of sodium channels enlisted as, $Na_v1.1$, $Na_v1.6$ (tetrodotoxin-sensitive) and $Na_v1.5$ (tetrodotoxin-insensitive). Reportedly, $Na_v1.6$ is the most abundant isoform that also participate in the modification of microglial functions. In an experiment of primary cultures, mice lacking $Na_v1.6$ express decrement in the LPS-exposed phagocytosis (Craner et al. 2005).

2.5.3 Calcium-Permeable Channels

Expression of classical voltage-operated Ca^{2+} channels is considered to be absent in microglia in the CNS (both in vivo and in vitro). Store-operated channels and channels of TRP family are the two main types of Ca^{2+} permeable channels in microglia. Similar to all other non-excitable cells, microglial cells also possess store-operated Ca^{2+} entry that was mediated by the Ca^{2+} release activated channels, i.e. TRP channels.

2.5.4 Calcium Signalling in Microglia

Calcium signalling is a homeostatic mechanism controlled by an evolutionary conserved cascade of molecules, that directs both intracellular calcium buffering and calcium transportation across the cellular membrane (Petersen et al. 2005). In resting microglial cells, calcium signalling is triggered by the calcium entry through ligand-gated plasmalemma and store-operated calcium permeable channels that further direct the release of intracellular stored calcium (Färber and Kettenmann 2006a). Microglia constitute both type of intracellular calcium channels, i.e. ryanodine receptors (RyRs) and inositol 1,4,5-trisphosphate (InsP3)-gated Ca^{2+} (InsP3Rs). Microglial calcium signalling is mainly initiated by InsP3Rs that further activates the G-protein-coupled metabotropic receptors connected to phospholipase C (PLC; Kettenmann et al. 2011).

2.5.5 Potassium Channels

Potassium channels (Kv) were credited as one of the first ion channels characterized in the microglia (Kettenmann et al. 1990). Precisely, the inward rectifier Kv (K_{IR}), is the first marker channel identified as the marker of activated microglia. Potassium channels in microglia have largely been studied in cultures and/or in tissue slices. The inward rectifier K^+ currents are the main source of membrane permeability in invading amoeboid microglia during perinatal brain development. Such currents

become almost undetectable as the cells get transferred to their ramified surveillance (or so called resting) states (Boucsein et al. 2000).

2.5.6 Anion Channels

Microglial proliferation, phagocytic activity, the control of ramified morphology mainly of cell volume and microglial resting potential are all considered to be regulated by the volume-regulated Cl^- channels. These channels are activated by a hypo-osmotic state. Such channels have been largely studied in microglia cultures and such cells also express the chloride intracellular channel-1 (CLIC-1). CLIC-1 play a major role in release of pro-inflammatory factors from microglia and have been considered to play an important role in progression of brain disorders.

2.5.7 Proton Channels

Functionally H^+ channels are considered to be associated with the regulation of respiratory bursts in phagocytic states of microglia. These are voltage-operated proton channels with single channel conductance having high selectivity to H^+. Extracellular pH is supposed to be regulating these channel expression in microglia in culture. Activated states of microglia decrease the H^+ current in cell culture experiments. In respiratory bursts, activation of NADPH oxidase generates protons and superoxide anions. These ions are effluxed by H^+ channels and protect the cytosol by regulating the intracellular pH.

2.6 Potent Immune Response During Brain Insult: Neuroinflammation

Neuroinflammation is an essential biological progression that stands as the foreground of various acute and chronic neuropathological conditions. Any alteration in brain's cellular and functional integrity grounds the incidence of neuroinflammation. Neuroinflammation as a defending responder aims to refurbish the tissue homeostasis via inducing several repair processes (Goldszmid and Trinchieri 2012). However, if the regulation of this mechanism remains uncontrolled then the initial inflammatory response amplifies exceedingly and the protective mode shifts towards the collateral destruction that would further result in severe disease progression.

Neuroinflammation is a dynamic process in which both microglia and astroglia may migrate, proliferate, release potentially harmful factors (i.e. cytokines and reactive oxygen species), display different surface proteins (i.e. MHC-I/II, etc.) and blend in functions such as antigen presentation and phagocytosis in response to

signals like protein aggregates, neuronal degeneration and glial products (i.e. colony stimulating factor and cytokines, etc.). Cytokine signalling following neuroinflammation is actively involved in the regulation of various brain functions like synaptic signalling modulation, neurotransmission, neuroendocrine functions and neural circuitry of behaviour and cognition (Camacho-Arroyo et al. 2009; del Rey et al. 2013; Aprile-Garcia et al. 2013; Cuartas and Jorge 2014). Therefore, it is relatively apparent to presume an altered behavioural and cognitive outcome as a consequence of a dysregulation in cytokine signalling which might result in depression, anxiety, behavioural deficits and cognitive dysfunction as observed previously (Lynch 2002; Bains and Oliet 2007; Baune et al. 2008; McAfoose and Baune 2009). Additionally in various cross-sectional and prospective population studies, it was shown that any alteration in the level of these cytokines in hippocampus lead to Alzheimer's disease (AD), dementia and cognitive impairment (Dik et al. 2005; Magaki et al. 2007; Holmes et al. 2009; Brosseron et al. 2014).

2.7 Microglia in Immune Regulation

Following injury (Patro et al. 2005, 2010a), inflammation (Patro and Patro 2004; Patro et al. 2010b), blood–brain barrier (BBB) damage (Davies et al. 1998) or metabolic disorder (Nagayach et al. 2014a, b) associated stimuli, microglia get activated. Microglia have also been activated exogenously by various inflammatory stimuli such as lipopolysaccharide (Rivest 2003; Sharma et al. 2015), β-amyloid (Sondag et al. 2009), interferon-γ (Chao et al. 1993), thrombin (Möller et al. 2006), Poly I:C (Patro and Patro 2004), etc. for screening the stature and pathological role of activated microglia. Such activated microglia release an array of immunocompetent molecules comprising of numerous chemokines like KC, MIP-1α (Macrophage-Inflammatory Protein-1α), MIP-1β, MIP-2, MCP-1 (Monocyte chemoattractant protein-1), RANTES (regulated on activation, normal T cell expressed and secreted), IP-10 (IFN-γ-inducible protein-10), and interleukins like IL-1α/β, IL-3, IL-6, IL-10, IL-12, IL-15, IL-18, tumour necrosis factor α (TNF-α), interferon gamma inducing factor (IGIF), inflammatory proteins, TGF-β, etc. Collectively, these molecules not only control the inflammatory processes, but also regulate immune response of the brain and even contribute to neuropathogenesis in CNS inflammation. Activated microglia also promote neuroprotection by releasing anti-inflammatory molecules and growth factors like nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), basic fibroblast growth factor, etc. following immunological stimuli (Hanisch and Kettenmann 2007). In conclusion, microglia as immune regulators of the nervous system can secrete several types of molecules or express various receptors that facilitate the integration of microglial response towards the changing microenvironment (Saxena et al. 2007; Neumann et al. 2008; Patro et al. 2014).

2.7.1 Phagocytic Behaviour of Microglial Cells

Microglia that maintain homeostasis in normal cells that include phagocytic clearance of neuronal damage products or debris. During early development they are innate immune cells and clear the supernumerary synaptic processes and apoptotic neurons. In the adult CNS this phagocytic ability becomes a boon following injury and associated frequent loss of neurons and microglial recruitment at the site of injury. Such microglial presence does more than just debris clearance, which includes axonal and myelin debris in spinal cord injury or multiple sclerosis, amyloid-β deposits in AD, etc. Earlier in this review, we have explained how inefficiency of microglia not only affects clearing up the injury site but also fail reorganization of the neuronal circuits. With age, such inefficiency also enhances the prevalence of neurodegenerative disease and inadequate regeneration. However, the mechanism, action and consequence of microglial phagocytosis have not been deciphered. Thus, there is now a call for considering new therapeutic avenues involving the mechanisms of microglia-mediated tissue repair.

While phagocytosis is beneficial as it cleans up CNS and induces anti-inflammatory response but also produces toxic ROS which we have discussed above. For more details, we would refer you to Neumann and Takahashi (2007) and Sierra et al. (2013). In physiological conditions the highly motile ramified processes respond to the chemotactic 'find me' signals like fractalkine, ATP, UDP, etc. from apoptotic cells. Subsequently 'Eat me' signals, i.e. the ligands for a plethora of microglial receptors are produced by the apoptotic cells that manifest cutting and engulfing of apoptotic debris. Following this the phagocytic microglia with the help of lysosomes and other organelles finally degrade and digest the debris.

2.8 Microglial Pathophysiology Following Neuroinflammation

2.8.1 ATP Signalling

Microglia gets activated through various signalling pathways including chemokine/chemokine receptors, nucleotides/purinergic receptors (P1, P2X and P2Y) and high-mobility group box (HMGB)/toll-like receptors. Consequently, activated microglia may secrete various soluble factors that act in inflammatory, trophic or protective manner (Suzuki et al. 2004; Di Virgilio et al. 2009). Microglia express purinergic (P2) receptors by means of elevated concentrations of extracellular ATP-induced intracellular Ca^{2+} elevation in a receptor-dependent manner (Fig. 2.3; Ferrari et al. 1996). Different concentrations of purine mediate ATP- and ADP-induced microglial chemokinesis and chemotaxis (Honda et al. 2001; Davalos et al. 2005). ATP signalling plays a major role not only in normal CNS function but also during the pathological states. Signals triggering microglia activation following any insult are directed by the release of purine nucleotides, comprising ADP, ATP

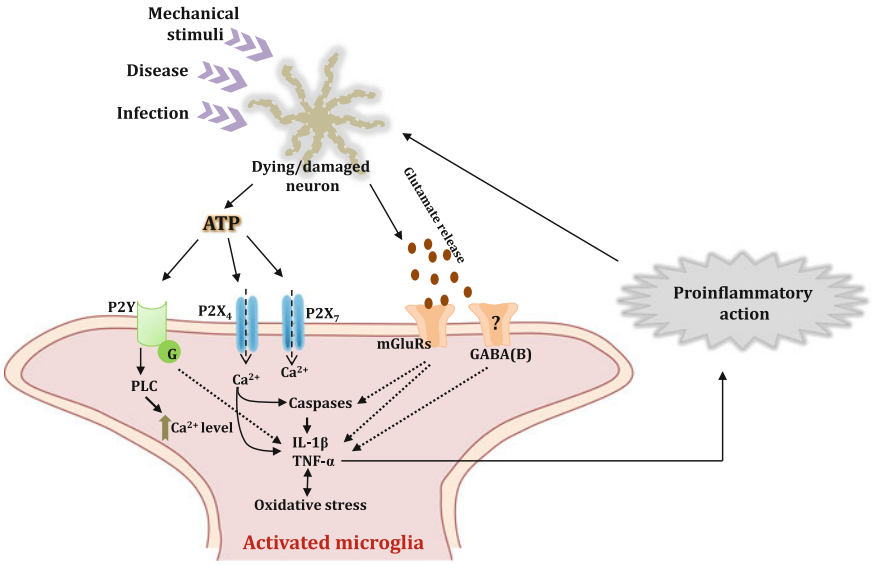

Fig. 2.3 Microglial physiology following neuroinflammation: Neuroinflammation mediated by various brain insults result in neuronal damage. Dying neurons and activated microglia themselves increases the level of extracellular ATP that further triggers the purinergic receptors (P2Y, $P2X_4$ and $P2_7$) present in the microglial cells. Activation of these receptors trigger an inward cationic current and initiate a cascade of second messenger signalling via G protein-phospholipase C (PLC) signal transduction pathway. This further elevates intracellular calcium. Iontropic receptors, P2X are involved in the expression, posttranslational processing and secretion of several inflammatory molecules and reactive oxidative radicals (ROS, RNS). P2X7 receptors eventually mediate apoptosis by caspase activation that also modulates the secretion of inflammatory molecules. Excessive release of glutamate via dying neurons activates glutamate receptors in microglia and exert inflammatory effect and aggravate the pro-inflammatory action

and UTP by damaged neuronal cells (Nimmerjahn et al. 2005; Domercq et al. 2013). These signals are received by the P2Y receptors present in both microglia and astrocytes-mediating chemotactic response of microglia. During neuroinflammatory condition, the extracellular ATP concentration increase is noted that further inhibit activation and overexpression of P2X7 receptors in microglial cells.

2.8.2 Sodium Channel Signalling

Microglial voltage-gated sodium channels (Na_v) are involved in a wide range of regulatory functions such as proliferation, morphological alterations, migration and phagocytosis (Eder 2005; Black et al. 2009) in response to inflammatory stimulus. Activated sodium channels stimulate a transient and rapid depolarization in microglial cells. Such depolarization of microglial cell membrane triggers the signalling cascades that further activates the microglial cell and subsequent immune activation. Furthermore, activated microglial membrane depolarization is a critical

participant in the conformational change of MHC I molecule, which is essentially required for the antigen presentation functions (Bene et al. 1997). Studies on human multiple sclerosis lesions and animal models of experimental autoimmune encephalopathy (EAE), have indicated that expression of Na_v 1.6 isoform of sodium channel in activated microglia gets increased (Craner et al. 2005). Consequently, the blocking of these channels was evidently used in developing the antiepileptic therapies and drugs (Black et al. 2007).

2.8.3 Potassium Channel Signalling

Inward rectifier K^+ currents are generally recorded in the activated states of microglia in various pathological conditions. Insults to the nervous tissue like ischemia, peripheral nerve damage have been recorded to induce a several fold increase in the amplitudes of inward rectifier K^+ channels (Boucsein et al. 2000).

The activated (delayed) receptor K^+ channels in microglia are KV1.2, KV1.3 and KV1.5. The microglia in prenatal brain also express KV1.1 and KV1.2 channels. These channels are responsible for the increased delay rectifier currents in the activated state of microglia. An increased expression of delayed rectifier channels is considered as indicatives of functional responses of hyperactive microglia and during active proliferation. Such increased expression have been experimentally evidenced in microglial cells in cultures with LPS or interferon-γ (Norenberg et al. 1992), in situ following axotomy (Boucsein et al. 2000) and as a reference to microglial activation following exposure to experimental condition like in vitro exposure to LPS, interferon-γ, β-amyloid or HIV-1 regulating protein Tat, etc. (Norenberg et al. 1992; Boucsein et al. 2000). High conductance (BK) and small conductance (KCNNG/KCa3.1/SK4/IK1) type Ca^{2+} dependent potassium (KCa) channels are also considered to be responsible in regulation of activated state of microglia in various pathological conditions (Schlichter et al. 2010). Interestingly, studies have also reported that the stimulation of ATP-sensitive K^+ channels (K_{ATP}) decreases the probability of microglial activation and are neuroprotective in several models of neurodegeneration involving neuroinflammation (Dolga and Culmsee 2012; Ortega et al. 2012).

2.8.4 Calcium Signalling

Calcium receptor activation generates two intracellular second messengers, the InsP3 and the diacylglycerol (DAG) in activated microglia which further in response activates InsP3Rs of the endoplasmic reticulum, and thus directs calcium release that regulates various cellular functions. In cell culture of rodent microglia, Ca^{2+} release activates Ca^{2+} currents (ICRAC). In activated microglia, the amplification of such currents gets decreased. ICRAC occurs after the activation of a complex of ORAI (pore forming) and STIM (Ca^{2+} sensor) protein (Ohana et al. 2009). Microglial cells

also express a range of TRPM, TRPV and TRPC channels considered to produce intracellular Ca^{2+} signals regulating release of cytokines.

2.8.5 Neurotransmitter Receptors

Microglia express several neurotransmitter receptors (Färber and Kettenmann 2006a, b) such as glutamate receptors (AMPA/Kainate, NMDA receptors, metabotropic glutamate receptors, GABA, adrenergic, dopaminergic and cholintergic receptors. Interestingly, the pathological and physiological role of these receptors in microglial cells is still under investigation (Fig. 2.3). Although studies had depicted that these neurotransmitters could exert inflammatory (both pro- and anti-) effects on microglial cells (Hagino et al. 2004; Pocock and Kettenmann 2007). Like, GABA receptors can modulate the interleukin (IL-6 and IL-12) release and glutamate receptors are capable in controlling the TNF-α release. Microglial activation of metabotropic glutamate receptors induces TNF-α and Fas ligand secretion which further trigger the neuronal caspase-3 activation through Fas receptor and TNFR1 (also known as p55), leading to neuronal damage (Taylor et al. 2005). Via receiving the signals from dying neurons microglia NMDA receptor gets activated and triggers the secretion of neurotoxic factors through microglia directing towards the microglia ability of inducing and aggravating the neurological damage (Kaindl et al. 2012). In the neurodegenerative diseases like hypoxia, AD and multiple sclerosis the altered concentration and expression of glutamate receptors in microglia depict the possibility of glutamate mediated toxicity in the progression of these pathological states (Newcombe et al. 2008; Sivakumar et al. 2010). Furthermore, a lipopolysaccharide (LPS) induced activated microglia culture study shows the releases of pro-inflammatory molecules which was attenuated by the simultaneous activation of the GABA(B) receptors directing towards the role of GABA(B) receptors in the modulation of microglia immune response (Kuhn et al. 2004).

The role and relevance of ATP and calcium signalling and neurotransmitters in microglia during neuroinflammatory conditions of several pathological diseases makes them a valuable target for developing therapeutic strategies for neuroprotection. Aspects of neurotransmitter signalling in the pathophysiology of microglia have been aptly reviewed by Domercq et al. (2013).

2.9 Microglia in Neurological Diseases

Neuroinflammatory mechanism is comprised of an organized set of interaction between varied mediators like cytokines, chemokines and prostaglandins, etc. Rather than pathological conditions, the secretion of inflammatory molecules following neuroinflammation is highly influenced by the microglial activation. In response to any injury, insult or disparaged conditions, the apparent activation of microglia triggers the secretion of inflammatory molecules (Fig. 2.2) that circumstantially become superfluous at chronic glial activation (Block et al. 2007).

Microglia through various pattern–recognition receptors (like Toll-like receptors, NOD-like receptors, receptors of cell wall components or DNA/RNA of pathogens), purinergic receptors, advanced glycation endproducts receptors and scavenger receptors receive signals from injured or dying cells and vascular damage (Block et al. 2007; Brown and Neher 2010). Connections of these receptors initiate a microglial activation cascade with the expression of various proteins, comprising CD-45, COX-II, iNOS, MHC-II and various co-stimulatory molecules amongst others. These molecules facilitate the microglial expression of antigens to T cells, entered through the damaged BBB during neuroinflammation (Aloisi 2001; Carson 2005; Gertig and Hanisch 2014). Activity-dependent microglial morphological heterogenity and population segregation has previously been discussed and exemplified via the proliferative ability and/or release spectrum of the constitutive or inducible mRNAs, proteins (e.g. major histocompatibility complex class II, TNF-α, IL-6/12/1β, integrins, IGF-I, CD4/11c/34/40/86/45, FcγRII, iNOS and molecules of the neurotrophin family), superoxide anions, nitric oxide synthase and proteases, etc. redox (NOX2, NOX1, RAC1, iNOS, NOS2, etc.) and excitotoxic molecules (MGluR2, GLT-1, P2X7-R, etc.).

Furthermore, microglia also shares a bidirectional collaborative relationship with neurons that assumed to be imperative in establishing a pertinent physiological, behavioural and immunological response against any injury or disorder. Neurons via a set of unique ligand-receptor pairs (CX3CL1-CX3CR1 and CD200-CD200R), microRNA-124 (mir-124), neurotransmitters (GABA, glutamate, catecholamines), peptides and/or growth factors, CD22, CCL21, fraktalkine (that act on receptors present on microglial membrane) maintain and regulate the microglial activation (Gomes-Leal 2012; Eyo and Wu 2013).

As described above, the secretion of pro-inflammatory cytokines following microglia activation is the classic theory of neuroinflammation recognized and reviewed widely (Carson et al. 2006; Luo and Chen 2012; Boche et al. 2013). Microglia activation is generally accompanied by the proliferation of cells, mobilization towards the damaged or dying cell and the expression and secretion of pro-inflammatory cytokines, like IL-6, TNFα. IL-1β and chemokines, such as cytokine (C-C motif) ligand (CCL)2, CCL3, CCL4, CCL5, CXCL10 and/or CCL12 (Olson and Miller 2004; Semple et al. 2010). Later on, inflammatory molecules stimulate other astroglia and microglia leading to the exacerbation of glial (microglia and astroglia) activation. Furthermost alterations in cytokines expression are a result of stimulation of the transcription factor NF-κB (nuclear factor kappa enhancer of B cells) via phosphorylation-induced activation of IκB kinase (Brown and Neher 2010). Neurotransmitter signalling in the pathophysiology of microglia has been aptly reviewed by Domercq et al. (2013).

During pathological condition, cellular damage following activation of microglial cells further initiates and perpetuates the state of excitoxicity and oxidative stress within the brain. The oxidative stress and cell death caused by the microglial activation are also contributing in the generation and propagation of

pro-inflammatory cytokines as documented widely (Shi et al. 2013; Sandireddy et al. 2014; Muriach et al. 2014). Furthermore, oxidative stress leads to the excessive dicarbonyl glycation which further activates the calpain expression and degrades the brain-derived neurotrophic factor (BDNF). This contributes to retard the process of neurogenesis and synaptic plasticity and stimulates NFkB-dependent inflammation and secretion of inflammatory molecules. Recurrent increment in cytokine levels increases the permeability of BBB to peripheral immune molecules and prolongs the central immune inflammatory response that generates an inordinate environment of oxidative and inflammatory stresses, accelerating CNS damage and elicits adverse structural and functional consequences. Intriguingly, reciprocal relationship between neuroinflammation, cell death and microglial activation is popularly considered as a prerequisite for the onset and pathogenesis of various psychiatric disorders like AD, PD, dementia and bipolar disorder, etc. (Hojo et al. 2004; Enciu and Popescu 2013; Najjar et al. 2013; Watkins et al. 2014).

Interestingly microglia plays a dual role during various neurological insults including neuroinflammation in CNS (Table 2.1). On the basis of secretory molecules released by the activated microglial cells microglial activation is divided into two major phases (Fig. 2.1), i.e. classical activation (M1 phase) and alternate activation (M2 phase; Colton 2009). During classical activation, microglia get triggered by the activated Toll-like receptors (TLRs) through intracellular proteins or pathogen-associated molecular patterns (PAMPs) released from injured neurons and release an array of inflammatory molecules (TNF-α, IL-6, IL-1β, IL-12), redox molecules (NOX2, NOX1, RAC1, iNOS, NOS2) and excitotoxic molecules (MGluR2, GLT-1, P2X7-R), nitric oxide synthase, superoxide anions, etc. (Ransohoff and Brown 2012; Boche et al. 2013) that further resulted in blood–brain barrier (BBB) disruption. The BBB damage promotes the infiltration of macrophages which later on exaggerate the influx of inflammatory cytokines and aggravate the existing neuroinflammatory state in CNS (Fig. 2.2; Zipser et al. 2007; Lassman et al. 2012; Obermeier et al. 2013). While in alternate activation, microglia secrete molecules like trophic growth factors, IL-4, IL-13, IL-1RA, scavenging receptors and extracellular matrix (Luo and Chen 2012; Cherry et al. 2014). Considering the imperious role of microglia in neuroinflammation, a particular attention is warranted on the microglia mediated neuro-immunological aspects of neurodegeneration and neuroregulation.

In conclusion, brain function and dysfunction, without any reservations, has a direct connection with microglial forms and functional states. They contribute to the pathogenesis and progression of various neurological disorders. Microglial activation also acts as a defence mechanism for various insults to the brain including infections. Being the immune cells of the CNS, they protect and repair the damage as also facilitate the healing process. Our understanding even today on microglial functions in normal and diseased brain is limited. Further insights on the physiology and pathophysiology of microglia using in vivo models are likely to contribute to our knowledge on the mechanisms and role of neuroinflammation for prevention or progression of brain disorders.

Table 2.1 Neurodegenerative and neuroprotective role of microglia in various pathological conditions

Neurological insult	Microglial reaction	
	Degenerative	Protective
Alzheimer's disease (AD)	• Release inflammatory cytokines and chemokines, reactive oxygen neurotoxins and exacerbates neuronal degeneration • Contribute in tau phosphorylation	• Secretes several antioxidant and neurotrophic factors • Aβ plaque removal by secretion of proteolytic enzymes and via receptors, viz., Class A scavenger receptors, the receptors for advanced glycation end products and β-integrins
Parkinson's disease (PD)	• Secrete inflammatory molecules, reactive oxygen radicals and nitrogen species and pro-inflammatory prostaglandins and contribute to the degeneration of dopaminergic neurons	• Release anti-inflammatory and neuroprotective cytokines like, TGF-β and GDNF, etc.
Amyotrophic lateral sclerosis (ALS)	• Via interacting with CX3CR1 receptor generate inflammatory signals and free radicals	• Provide trophic support during early stages of the disease
Huntington's disease (HD)	• Exacerbates the pathogenic extrasynaptic NMDA receptor signalling that further decrease the synaptic connectivity and causes loss of BDNF	• Clear the debris and secrete trophic factors
Multiple sclerosis (MS)	• Release inflammatory molecules • Promote demyelination	• Clear the myelin debris and apoptotic cells • Secrete trophic growth factors
Cerebral ischemia	• Produce an array of chemokines, free radicals and various TLR4 mediated pro-inflammatory cytokines	• Secrete neurotrophic growth factors like BDNF, GDNF, and TGF-β • Provide neuronal survival via expressing integrin CD11a to promote cell to cell contact • Phagocytose the invading neutrophils and remove the excitotoxins present in the extracellular space
Transmissible spongiform encephalopathy (prion disease)	• Cause neuronal death • Secrete various neurotoxic factors, proinflammatory cytokines, chemokines and free radicals	• Secrete neurotrophic growth factors when targeted with Amphotericin B
HIV-AIDS	• Via causing inflammation, cell death and astrogliosis contribute in pathogenesis of HIV-AIDS • Involved in the synaptic damage and neuronal death	• Attenuation of HIV-1-induced microglial activation stimulate the generation of neurotrophic factors

(continued)

Table 2.1 (continued)

Neurological insult	Microglial reaction	
	Degenerative	Protective
Brain tumour	• Amplify the tumour invasiveness, proliferation and migration via the release of multiple cytokines, enzymes, reactive oxygen species, growth factors and extracellular matrix proteases	• Therapeutically targeting the glioma associated microglia inhibit the lymphocyte reactivity, proliferation via polarizing towards the M1-phenotype that further contributes to both adaptive and innate anti-tumour immunity and restrict the glioma growth • Prevention of M2-phenotype activation of microglia control the tumour-infiltrating macrophages
Infections	• Release several inflammatory mediators like reactive oxygen radicals, cytokines and chemokines	• Adopt an "amoeboid" activated phenotype and secrete various pro-inflammatory mediators such as cytokines, chemokines, reactive oxygen species and nitric oxide, which contribute to the clearance of pathogenic infections
Chronic pain	• Via expressing activated TLR2 and TLR4 receptors that further stimulate the release of proinflammatory molecules like IL-6, TNF-α and IL-1β • Microglial p38 MAP kinase participates in Ca^{2+}-sensitive intracellular signalling cascades that lead to the exacerbation of chronic pain via producing inflammatory cytokines	• Express the CB2 (cannabinoid receptors) receptors and that further stimulate the production of anti-inflammatory molecules
Diabetes	• Generate pro-inflammatory cytokines and neuroactive molecules	Not known

Abbreviations *NMDA* *N*-methyl-D-aspartate; *BDNF* brain derived neurotropic factor; *TLR2/4* toll-like receptors2/4; *TGF-β* transforming growth factor-β; *GDNF* glial cell line-derived neurotrophic factor

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References

Alliot F, Lecain E, Grima B, Pessac B (1991) Microglial progenitors with a high proliferative potential in the embryonic and adult mouse brain. Proc Natl Acad Sci USA 88(4):1541–1545

Aloisi F (2001) Immune function of microglia. Glia 36(2):165–179

Aprile-Garcia F, Antunica-Noguerol M, Budziñski ML, Liberman AC, Arzt E (2013) Novel insights into the neuroendocrine control of inflammation: the role of GR and PARP1. Endocr Connect 3(1):R1–R12

Bains JS, Oliet SH (2007) Glia: they make your memories stick! Trends Neurosci 30(8):417–424

Bansal R (2002) Fibroblast growth factors and their receptors in oligodendrocyte development: implications for demyelination and remyelination. Dev Neurosci 24(1):35–46

Baune BT, Ponath G, Rothermundt M, Riess O, Funke H, Berger K (2008) Association between genetic variants of IL-1beta, IL-6 and TNF-alpha cytokines and cognitive performance in the elderly general population of the MEMO-study. Psychoneuroendocrinology 33(1):68–76

Ben Achour S, Pascual O (2010) Glia: the many ways to modulate synaptic plasticity. Neurochem Int 57(4):440–445

Bene L, Szollosi J, Balazs M, Matyus L, Gaspar R, Ameloot M, Dale RE, Damjanovich S (1997) Major histocompatibility complex class I protein conformation altered by transmembrane potential changes. Cytometry 27:353–357

Benoit M, Benoit D, Jean-Louis M (2008) Macrophage polarizaition in bacterial infections. J Immunol 181:3733–3739

Black JA, Waxman SG (2012) Sodium channels and microglial function. Exp Neurol 234(2):302–315

Black JA, Liu S, Carrithers M, Carrithers LM, Waxman SG (2007) Exacerbation of experimental autoimmune encephalomyelitis after withdrawal of phenytoin and carbamazepine. Ann Neurol 62:21–33

Black JA, Liu S, Waxman SG (2009) Sodium channel activity modulates multiple functions in microglia. Glia 57(10):1072–1081

Block ML, Hong JS (2005) Microglia and inflammation-mediated neurodegeneration: multiple triggers with a common mechanism. Prog Neurobiol 76(2):77–98

Block ML, Zecca L, Hong JS (2007) Microglia–mediated neurotoxicity: uncovering the molecular mechanisms. Nat Rev Neurosci 8(1):57–69

Boche D, Perry VH, Nicoll JA (2013) Review: activation patterns of microglia and their identification in the human brain. Neuropathol Appl Neurobiol 39:3–18

Boucsein C, Kettenmann H, Nolte C (2000) Electrophysiological properties of microglial cells in normal and pathologic rat brain slices. Eur J Neurosci 12(6):2049–2058

Boya J, Calvo JL, Carbonell AL, Borregon A (1991) A lectin histochemistry study on the development of rat microglial cells. J Anat 175:229–236

Brosseron F, Krauthausen M, Kummer M, Heneka MT (2014) Body fluid cytokine levels in mild cognitive impairment and Alzheimer's disease: a comparative overview. Mol Neurobiol 50 (2):534–544

Brown GC, Neher JJ (2010) Inflammatory neurodegeneration and mechanisms of microglial killing of neurons. Mol Neurobiol 41(2–3):242–247

Camacho-Arroyo I, López-Griego L, Morales-Montor J (2009) The role of cytokines in the regulation of neurotransmission. NeuroImmunomodulation 16(1):1–12

Carson MJ (2005) Microglia-the professional antigen-presenting cells of the CNS? In: Kropshofer H, Vogt AB (eds) Antigen presenting cells: from mechanisms to drug development. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, FRG. doi:10.1002/3527607021.ch12

Carson MJ, Doose JM, Melchior B, Schmid CD, Ploix CC (2006) CNS immune privilege: hiding in plain sight. Immunol Rev 213:48–65
Chao CC, Hu S, Gekker G, Novick WJ Jr, Remington JS, Peterson PK (1993) Effects of cytokines on multiplication of Toxoplasma gondii in microglial cells. J Immunol 150(8 Pt 1):3404–3410
Chen J, Buchanan JB, Sparkman NL, Godbout JP, Freund GG, Johnson RW (2008) Neuroinflammation and disruption in working memory in aged mice after acute stimulation of the peripheral innate immune system. Brain Behav Immun 22(3):301–311
Cherry JD, Olschowka JA, O'Banion MK (2014) Are "resting microglia more M2"? Front Immunol. doi:10.3389/fimmu.2014.00594
Colton CA (2009) Heterogeneity of microglial activation in the innate immune response in the brain. J Neuroimmune Pharmacol 4:399–418
Coull JA, Beggs S, Boudreau D, Boivin D, Tsuda M, Inoue K et al (2005) BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. Nature 438 (7070):1017–1021
Craner MJ, Damarjian TG, Liu S et al (2005) Sodium channels contribute to microglia/macrophage activation and function in EAE and MS. Glia 49:220–229
Cuartas A, Jorge M (2014) Cognition and inflammation: the role of cytokines in cognitive performance. Int J Psychol Res 7(2):8–10
Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S et al (2005) ATP mediates rapid microglial response to local brain injury in vivo. Nat Neurosci 8:752–758
Davies CA, Loddick SA, Stroemer RP, Hunt J, Rothwell NJ (1998) An integrated analysis of the progression of cell responses induced by permanent focal middle cerebral artery occlusion in the rat. Exp Neurol 154(1):199–212
del Rey A, Balschun D, Wetzel W, Randolf A, Besedovsky HO (2013) A cytokine network involving brain-borne IL-1β, IL-1ra, IL-18, IL-6, and TNFα operates during long-term potentiation and learning. Brain Behav Immun 33:15–23
Del Rio-Hortega P (1932) Microglia. In: Penfield W (ed) Cytology and cellular pathology of the nervous system. Paul B Hoeber, New York, pp 482–534
Di Virgilio F, Ceruti S, Bramanti P, Abbracchio MP (2009) Purinergic signalling in inflammation of the central nervous system. Trends Neurosci 32:79–87
Dik MG, Jonker C, Hack CE, Smit JH, Comijs HC, Eikelenboom P (2005) Serum inflammatory proteins and cognitive decline in older persons. Neurology 64(8):1371–1377
Dilger RN, Johnson RW (2008) Aging, microglial cell priming, and the discordant central inflammatory response to signals from the peripheral immune system. J Leukoc Biol 84 (4):932–939
Dolga AM, Culmsee C (2012) Protective roles for potassium SK/KCa2 channels in microglia and neurons. Front Pharmacol 3:196
Domercq M, Vázquez-Villoldo N, Matute (2013) Neurotransmitter signaling in the pathophysiology of microglia. Front Cell Neurosci 7:49
Eder C (2005) Regulation of microglial behavior by ion channel activity. J Neurosci Res 81:314–321
Ekdahl CT, Kokaia Z, Lindvall O (2009) Brain inflammation and adult neurogenesis: the dual role of microglia. Neuroscience 158(3):1021–1029
Elmore MR, Najafi AR, Koike MA, Dagher NN, Spangenberg EE, Rice RA et al (2014) Colony-stimulating factor 1 receptor signaling is necessary for microglia viability, unmasking a microglia progenitor cell in the adult brain. Neuron 82(2):380–397
Enciu AM, Popescu BO (2013) Is there a causal link between inflammation and dementia? Biomed Res Int 2013:316495
Eyo UB, Wu LJ (2013) Bidirectional microglia-neuron communication in the healthy brain. Neural Plast. doi:10.1155/2013/456857
Färber K, Kettenmann H (2005) Physiology of microglial cells. Brain Res Rev 48(2):133–143
Färber K, Kettenmann H (2006a) Functional role of calcium signals for microglial function. Glia 54(7):656–665

Färber K, Kettenmann H (2006b) Purinergic signaling and microglia. Pflugers Arch 452(5):615–621
Farinas I, Cano-Jaimez M, Bellmunt E, Soriano M (2002) Regulation of neurogenesis by neurotrophins in developing spinal sensory ganglia. Brain Res Bull 57(6):809–816
Ferrari D, Villalba M, Chiozzi P, Falzoni S, Ricciardi-Castagnoli P, Di Virgilio F (1996) Mouse microglial cells express a plasma membrane pore gated by extracellular ATP. J Immunol 156:1531–1539
Gehrmann J, Matsumoto Y, Kreutzberg JW (1995) Microglia: intrinsic immuneffector cell of the brain. Brain Res Brain Res Rev 20(3):269–287
Gertig U, Hanisch UK (2014) Microglial diversity by responses and responders. Front Cell Neurosci 8:101
Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S et al (2010) Fate mapping analysis reveals that adult microglia derive from primitive macrophages. Science 330(6005):841–845
Ginhoux F, Lim S, Hoeffel G, Low D, Huber T (2013) Origin and differentiation of microglia. Front Cell Neurosci 7:45
Godbout JP, Johnson RW (2009) Age and neuroinflammation: a lifetime of psychoneuroimmune consequences. Immunol Allergy Clin North Am 29(2):3213–3237
Goldszmid RS, Trinchieri G (2012) The price of immunity. Nat Immunol 13(10):932–938
Gomes-Leal W (2012) Microglial physiopathology: how to explain the dual role of microglia after acute neural disorders? Brain Behav 2(3):345–356
Gomez PE, Schulz C, Geissmann F (2013) Development and homeostasis of "resident" myeloid cells: the case of the microglia. Glia 61(1):112–120
Gregory CD, Devitt A (2004) The macrophage and the apoptotic cell: an innate immune interaction viewed simplistically? Immunology 113:1–14
Hagino Y, Kariura Y, Manago Y, Amano T, Wang B et al (2004) Heterogeneity and potentiation of AMPA type of glutamate receptors in rat cultured microglia. Glia 47:68–77
Hanisch UK, Kettenmann H (2007) Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat Neurosci 10(11):1387–1394
Hayashi Y, Ishibashi H, Hashimoto K, Nakanishi H (2006) Potentiation of the NMDA receptor-mediated responses through the activation of the glycine site by microglia secreting soluble factors. Glia 53(6):660–668
Hellwig S, Heinrich A, Biber K (2013) The brain's best friend: microglial neurotoxicity revisited. Front Cell Neurosci 7:71
Heneka MT, Rodriguez JJ, Verkhratsky A (2010) Neuroglia in neurodegeneration. Brain Res Rev 63(1–2):189–211
Hickey WF, Kimura H (1988) Perivascular microglial cells of the CNS are bone marrow-derived and present antigen in vivo. Science 239:290–292
Hickey WF, Vass K, Lassmann H (1992) Bone marrow-derived elements in the central nervous system: an immunohistochemical and ultrastructural survey of rat chimeras. J Neuropathol Exp Neurol 51(3):246–256
Hojo K, Kawamata T, Tanaka C, Maeda K (2004) Inflammatory glial activation in the brain of a patient with hereditary sensory neuropathy type 1 with deafness and dementia. Neurosci Lett 367(3):340–343
Holmes C, Cunningham C, Zotova E, Woolford J, Dean C, Kerr S, Culliford D, Perry VH (2009) Systemic inflammation and disease progression in Alzheimer disease. Neurology 73(10):768–774
Honda S, Sasaki Y, Ohsawa K, Imai Y, Nakamura Y, Inoue K et al (2001) Extracellular ATP or ADP induce chemotaxis of cultured microglia through Gi/o-coupled P2Y receptors. J Neurosci 21:1975–1982
Hughes EG, Bergles DE (2014) Hidden progenitors replace microglia in the adult brain. Neuron 82 (2):253–255

Kaindl AM, Degos V, Peineau S, Gouadon E, Chhor V et al (2012) Activation of microglial N-methyl-D-aspartate receptors triggers inflammation and neuronal cell death in the developing and mature brain. Ann Neurol 72:536–549

Kaur C, Ling EA, Wong WC (1985) Transformation of amoeboid microglial cells into microglia in the corpus callosum of the postnatal rat brain. An electron microscopical study. Arch Histol Jpn 48(1):17–25

Kettenmann H, Hoppe D, Gottmann K, Banati R, Kreutzberg G (1990) Cultured microglial cells have a distinct pattern of membrane channels different from peritoneal macrophages. J Neurosci Res 26(3):278–287

Kettenmann H, Hanisch UK, Noda M, Verkhratsky A (2011) Physiology of microglia. Physiol Rev 91(2):461–553

Kuhn SA, van Landeghem FK, Zacharias R, Färber K, Rappert A, Pavlovic S, Hoffmann A, Nolte C, Kettenmann H (2004) Microglia express GABA(B) receptors to modulate interleukin release. Mol Cell Neurosci 25(2):312–322

Kushwaha S (2009) Age related changes in astrocytes and microglia in hippocampus and striate cortex. PhD thesis, Jiwaji University, Gwalior, India, p 93

Lassman ME, McLaughlin TM, Somers EP, Stefanni AC, Chen Z, Murphy BA et al (2012) A rapid method for cross-species quantitation of apolipoproteins A1, B48 and B100 in plasma by ultra-performance liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 26(2):101–108

Lassmann H, Hickey WF (1993) Radiation bone marrow chimeras as a tool to study microglia turnover in normal brain and inflammation. Clin Neuropathol 12(5):284–285

Luo XG, Chen SD (2012) The changing phenotype of microglia from homeostasis to disease. Transl Neurodegeneration 1:9

Lynch MA (2002) Interleukin-1 beta exerts a myriad of effects in the brain and in particular in the hippocampus: analysis of some of these actions. Vitam Horm 64:185–219

Magaki S, Mueller C, Dickson C, Kirsch W (2007) Increased production of inflammatory cytokines in mild cognitive impairment. Exp Geron 42(3):233–240

Martinez FO, Sica A, Mantovani A, Locati M (2008) Macrophage activation and polarization. Front Biosci 13:453–461

Matsuda S, Niidome T, Nonaka H, Goto Y, Fujimura K, Kato M et al (2008) Microtubule–associated protein 2–positive cells derived from microglia possess properties of functional neurons. Biochem Biophys Res Commun 368(4):971–976

McAfoose J, Baune BT (2009) Evidence for a cytokine model of cognitive function. Neurosci Biobehav Rev 33(3):355–366

Miller FD, Kaplan DR (2001) Neurotrophin signalling pathways regulating neuronal apoptosis. Cell Mol Life Sci 58(8):1045–1053

Möller T, Weinstein JR, Hanisch UK (2006) Activation of microglial cells by thrombin: past, present and future. Semin Thromb Hemost 32(Suppl 1):69–76

Monier A, Evrard P, Gressens P et al (2006) Distribution and differentiation of microglia in the human encephalon during the first two trimesters of gestation. J Comp Neurol 499(4):565–582

Muriach M, Flores-Bellver M, Romero FJ, Barcia JM (2014) Diabetes and the brain: oxidative stress, inflammation, and autophagy. Oxid Med Cell Longev 2014:102158. doi:10.1155/2014/102158

Nagayach A, Patro N, Patro I (2014a) Astrocytic and microglial response in experimentally induced diabetic rat brain. Metab Brain Dis 29(3):747–761

Nagayach A, Patro N, Patro I (2014b) Experimentally induced diabetes causes glial activation, glutamate toxicity and cellular damage leading to changes in motor function. Front Cell Neurosci 8:355. doi:10.3389/fncel.2014.00355

Nagayach A, Patro N, Patro I (2015) Microglia in physiology and pathology of brain. Proc Natl Acad Sci, India, Sect-B. doi:10.1007/s40011-

Najjar S, Pearlman DM, Alper K, Najjar A, Devinsky O (2013) Neuroinflammation and psychiatric illness. J Neuroinflammation 10:43

Nakajima K, Honda S, Tohyama Y, Imai Y, Kohsaka S, Kurihara T (2001) Neurotrophin secretion from cultured microglia. J Neurosci Res 65(4):322–331

Nayak D, Roth TL, McGavern DB (2014) Microglia development and function. Annu Rev Immunol 32:367–402

Neiva I, Malva JO, Valero J (2014) Can we talk about microglia without neurons? A discussion of microglial cell autonomous properties in culture. Front Cell Neurosci 8:202

Neumann H, Takahashi K (2007) Essential role of microglial triggering receptor expressed on myeloid cells-2 (TREM-2) for central nervopus tissue immune homeostasis. J Neuroimmunol 184:92–99

Neumann H, Kotter MR, Franklin JM (2008) Debris clearance by microglia: an essential link between degenereation and regeneration. Brain 132(2):288–295

Newcombe J, Uddin A, Dove R, Patel B, Turski L et al (2008) Glutamate receptor expression in multiple sclerosis lesions. Brain Pathol 18:52–61

Nimmerjahn A, Kirchhoff F, Helmchen F (2005) Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308:1314–1318

Niquet J, Ben-Ari Y, Repressa A (1994) Glial reaction after seizure induced hippocampal lesion: immunohistochemical characterization of proliferating glial cells. J Neurocytol 23 (10):641–656

Norenberg W, Gebicke-Haerter PJ, Illes P (1992) Inflammatory stimuli induce a new K^+ outward current in cultured rat microglia. Neurosci Lett 147:171–174

Obermeier B, Daneman R, Ransohoff RM (2013) Development, maintenance and disruption of the blood-brain barrier. Nat Med 19(12):1584–1596

Ohana L, Newell EW, Stanley EF, Schlichter LC (2009) The Ca^{2+} release-activated Ca^{2+} current (I (CRAC)) mediates store-operated Ca^{2+} entry in rat microglia. Channels (Austin) 3(2):129–139

Olson JK, Miller SD (2004) Microglia initiate central nervous system innate and adaptive immune responses through multiple TLRs. J Immunol 173:3916–3924

Ortega FJ, Gimeno-Bayon J, Espinosa-Parrilla JF et al (2012) ATP-dependent potassium channel blockade strengthens microglial neuroprotection after hypoxia-ischemia in rats. Exp Neurol 235:282–296

Paolicelli RC, Bolasco G, Pagani F, Maggi L, Scianni M, Panzanelli P et al (2011) Synaptic pruning by microglia is necessary for normal brain development. Science 333(6048):1456–1458

Parkhurst CN, Yang G, Ninan I, Savas JN, Yates JR 3rd, Lafaille JJ, Hempstead BL, Littman DR, Gan WB (2013) Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. Cell 155(7):1596–1609

Parpura V, Heneka MT, Montana V, Oliet SH, Schousboe A, Haydon PG et al (2012) Glial cells in (patho)physiology. J Neurochem 121(1):4–27

Pascual O, Ben Achour S, Rostaing P, Triller A, Bessis A (2012) Microglia activation triggers astrocyte-mediated modulation of excitatory neurotransmission. Proc Natl Acad Sci USA 109 (4):E197–E205

Patro N, Patro IK (2004) Effect of immunactivator (poly I:C) on the rat cerebral cortex. J Tissue Res 4(1):71–74

Patro IK, Pathak S, Patro N (2005) Central responses to peripheral nerve injury: role of non–neuronal cells. In: Thakur MK, Prasad S (eds) Molecular and cellular neurobiology. Narosa Publishing House, New Delhi, p 217

Patro IK, Amit Shrivastava M, Bhumika S, Patro N (2010a) Poly I: C induced microglial activation impairs motor activity in adult rats. Indian J Exp Biol 48(2):104–109

Patro N, Kushwah S, Patro IK (2010b) Aging of microglia: does it influence neuroprotection? Proc Natl Acad Sci India Sect B 80(1):14–23

Patro N, Nagayach A, Patro IK (2010c) Iba1 expressing microglia in the dorsal root ganglia become activated following peripheral nerve injury in rats. Indian J Exp Biol 48(2):110–116

Patro N, Singh K, Patro IK (2013) Differential microglial and astrocytic response to bacterial and viral infection in the developing hippocampus of neonatal rats. Indian J Exp Biol 51 (8):606–614

Patro IK, Saxena K, Nagayach A, Patro N (2014) Involvement of glilal cells in pathophysiology of peripheral nerve injury. In: Mathur PP (ed) Contemporary topics in life sciences. Narendra Publishing House, New Delhi, pp 153–173

Petersen OH, Michalak M, Verkhratsky A (2005) Calcium signalling: past, present and future. Cell Calcium 38:161–169

Pocock JM, Kettenmann H (2007) Neurotransmitter receptors on microglia. Trends Neurosci 30 (10):527–535

Pont-Lezica L, Béchade C, Belarif-Cantaut Y, Pascual O, Bessis A (2011) Physiological roles of microglia during development. J Neurochem 119(5):901–908

Prinz M, Mildner A (2011) Microglia in the CNS: immigrants from another world. Glia 59 (2):177–187

Prinz M, Priller J (2014) Microglia and brain macrophages in the molecular age: from origin to neuropsychiatric disease. Nat Rev Neurosci 15(5):300–312

Rakic S, Zecevic N (2000) Programmed cell death in the developing human telencephalon. Eur J Neurosci 12(8):2721–2734

Ransohoff RM, Brown MA (2012) Innate immunity in the central nervous system. J Clin Invest 122:1164–1171

Rezaie P, Male D (2002) Mesoglia and microglia—A historical review of the concept of mononuclear phagocytes within the central nervous system. J Hist Neurosci 11(4):325–374

Rivest S (2003) Molecular insights on the cerebral innate immune system. Brain Behav Immun 17 (1):13–19

Rogers JT, Morganti JM, Bachstetter AD, Hudson CE, Peters MM, Grimmig BA et al (2011) CX3CR1 deficiency leads to impairment of hippocampal cognitive function and synaptic plasticity. J Neurosci 31(45):16241–16250

Saijo K, Glass CK (2011) Microglial cell origin and phenotypes in health and disease. Nat Rev 11 (11):775–787

Saijo K, Crotti A, Glass CK (2013) Regulation of microglia activation and deactivation by nuclear receptors. Glia. 61:104–111

Sandireddy R, Yerra VG, Areti A, Komirishetty P, Kumar A (2014) Neuroinflammation and oxidative stress in diabetic neuropathy: futuristic strategies based on these targets. Int J Endocrinol 2014:674987

Saxena K, Patro N, Patro I (2007) FK506 protects neurons following peripheral nerve injury via immunosuppression. Cell Mol Neurobiol 27:1049–1057

Schafer DP, Lehrman EK, Kautzman AG, Koyama R, Mardinly AR, Yamasaki R, Ransohoff RM, Greenberg ME, Barres BA, Stevens B (2012) Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. Neuron 74(4):691–705

Schafer DP, Lehrman EK, Stevens B (2013) The "Quad-Partite" synapse: microglia-synapse interactions in the developing and mature CNS. Glia 61(1):24–36

Schilling T, Eder C (2007) Ion channel expression in resting and activated microglia of hippocampal slices from juvenile mice. Brain Res 1186(1):21–28

Schlichter LC, Kaushal V, MoxonEmre I, Sivagnanam V, Vincent C (2010) The Ca^{2+} activated SK3 channel is expressed in microglia in the rat striatum and contributes to microglia-mediated neurotoxicity in vitro. J Neuroinflammation 7:4

Semple BD, Kossmann T, Morganti-Kossmann MC (2010) Role of chemokines in CNS health and pathology: a focus on the CCL2/CCR2 and CXCL8/CXCR2 networks. J Cereb Blood Flow Metab 30:459–473

Sharma A, Patro N, Patro IK (2015) Lipopolysaccharide-Induced apoptosis of astrocytes: therapeutic intervention by minocycline. Cell Mol Neurobiol. 07/2015. doi:10.1007/s10571-015-0238-y

Shepard JL, Zon LI (2000) Developmental derivation of embryonic and adult macrophages. Curr Opin Hematol 7(1):3–8

Shi X, Chen Y, Nadeem L, Xu G (2013) Beneficial effect of TNF-α inhibition on diabetic peripheral neuropathy. J Neuroinflammation 10:69

Sierra A, Gottfried-Blackmore AC, McEwen BS, Bulloch K (2007) Microglia derived from aging mice exhibit an altered inflammatory profile. Glia 55:412–424

Sierra A, Abiega O, Shahraz A, Neumann H (2013) Janus-faced microglis: beneficial and detrimental consequences of microglial phagocytosis. Front Cell Neurosci 7:355. doi:10.3389/fncel.2013.00006

Sivakumar V, Ling EA, Lu J, Kaur C (2010) Role of glutamate and its receptors and insulin-like growth factors in hypoxia induced periventricular white matter injury. Glia 58:507–523

Sondag CM, Dhawan G, Combs CK (2009) Beta amyloid oligomers and fibrils stimulate differential activation of primary microglia. J Neuroinflammation 6:1

Streit WJ (2006) Microglial senescence: does the brain's immune system have an expiration date? Trends Neurosci 29(9):506–510

Streit WJ, Sammons NW, Kuhns AJ, Sparks DL (2004) Dystrophic microglia in the aging human brain. Glia 45(2):208–212

Suzuki T, Hide I, Ido K, Kohsaka S, Inoue K, Nakata Y (2004) Production and release of neuroprotective tumor necrosis factor by P2X7 receptor-activated microglia. J Neurosci 24:1–7

Takahashi K (2001) Development and differentiation of macrophages and related cells: historical review and current concepts. J Clin Exp Hematopathol 41:1–33

Tang Yu, Le W (2015) Differential roles of M1 and M2 microglia in neurodegenerative diseases. Mol Neurobiol. doi:10.1007/s12035-014-9070-5

Taylor DL, Jones F, Kubota ES, Pocock JM (2005) Stimulation of microglial metabotropic glutamate receptor mGlu2 triggers tumor necrosis factor-alpha induced neurotoxicity in concert with microglial-derived Fas ligand. J Neurosci 25:2952–2964

Thored P, Heldmann U, Gomes-Leal W, Gisler R, Darsalia V, Taneera J et al (2009) Long-term accumulation of microglia with proneurogenic phenotype concomitant with persistent neurogenesis in adult subventricular zone after stroke. Glia 57(8):835–849

Tremblay MÈ, Lowery RL, Majewska AK (2010) Microglial interactions with synapses are modulated by visual experience. PLoS Biol 8:e1000527

Tremblay MÈ, Zettel ML, Ison JR, Allen PD, Majewska AK (2012) Effects of aging and sensory loss on glial cells in mouse visual and auditory cortices. Glia 60(4):541–558

Venero JL, Burguillos MA, Brundin P, Joseph B (2011) The executioners sing a new song: killer caspases activate microglia. Cell Death Differ 18(11):1679–1691

Verkhratsky A, Parpura V, Pekna M, Pekny M, Sofroniew M (2014) Glia in the pathogenesis of neurodegenerative diseases. Biochem Soc Trans 42(5):1291–1301

Wake H, Moorhouse AJ, Jinno S, Kohsaka S, Nabekura J (2009) Resting microglia directly monitor the functional state of synapses in vivo and determine the fate of ischemic terminals. J Neurosci 29(13):3974–3980

Watkins CC, Sawa A, Pomper MG (2014) Glia and immune cell signaling in bipolar disorder: insights from neuropharmacology and molecular imaging to clinical application. Transl Psychiatry 4:e350

Wynne AM, Henry CJ, Godbout JP (2009) Immune and behavioral consequences of microglial reactivity in the aged brain. Integr Comp Biol 49(3):254–266

Yamasaki R, Lu H, Butovsky O, Ohno N, Rietsch AM, Cialic R (2014) Differential roles of microglia and monocytes in the inflamed central nervous system. J Exp Med 211(8):1533–1549

Yokoyama A, Yang L, Itoh S, Mori K, Tanaka J (2004) Microglia, a potential source of neurons, astrocytes and oligodendrocytes. Glia 45(1):96–104

Yung RL, Julius A (2008) Epigenetics, aging and autoimmunity. Autoimmunity 41(4):329–335

Zipser BD, Johanson CE, Gonzalez L, Berzin TM, Tavares R, Hulette CM, Vitek MP, Hovanesian V, Stopa EG (2007) Microvascular injury and blood-brain barrier leakage in Alzheimer's disease. Neurobiol Aging 28(7):977–986

Astrocytes in Neuroinflammation and Neuronal Disorders: *Shifting the Focus from Neurons*

3

Manju Tewari and Pankaj Seth

Abstract

Glial cells have emerged as essential participants in most aspects of brain development, behaviour and disease. Renewed interest into astrocyte biology has transpired due to the fact that astrocytes are active participants with neurons in activities ranging from neural circuit formation, brain information processing, metabolic support to synaptogenesis and synaptic activity. Any perturbations in astrocyte function especially during pathological conditions may thus have profound effects on the optimal functioning or even survival of neurons. In this chapter, we provide a brief history about the discovery of astrocyte, discuss basic questions such as; what do astrocytes do? How astrocytes respond to inflammation? and their role in neuroinflammatory disorders? More specifically, the chapter details the role of astrocytes in the pathogenesis of Human Immuno Deficiency Virus (HIV) associated neurocognitive disorders. The chapter also discusses how changes in astrocyte morphology and function results in dysregulation of astrocytic responses during inflammatory injury and its repercussions that ultimately leads to neuronal dysfunction or death. Finally, we discuss recent advances in how the knowledge about astrocytes has fostered newer ideas about brain functions and disease, which offer therapeutic leads to treat neuroinflammatory disorders. In summary, recent studies have provided novel insights into the role of astrocytes in a wide variety of neuroinflammatory and neurocognitive diseases, and future research on astrocyte pathophysiology is expected to provide new perspectives on importance of astrocytes in healthy and diseased brain.

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Abbreviations

HIV	Human immunodeficiency virus
GFAP	Glial fibrillary acidic protein
GLT-1	Glutamate transporter-1
EAAT1	Excitatory aminoacid transporter1
AQP4	Aquaporin4
PDGF	Platelet derived growth factor
ATP	Adenosine triphosphate
LIF	Leukaemia inhibitory factor
NT	Neurotrophin 3
CNTF	Ciliary neurotrophic factor
IGF	Insulin-like growth factor
CNS	Central nervous system
BDNF	Brain-derived neurotrophic factor
GDNF	Glial cell line-derived neurotrophic factor
GABA	Gamma amino butyric acid
bFGF	Basic fibroblast growth factor
BBB	Blood brain barrier
ADP	Adenosine diphosphate
SOD-1	Superoxide dismutase-1
TNF-α	Tumour necrosis factor alpha
IL	Interleukin
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
NMDAR	*N*-methyl-D-aspartate receptor
LTR	Long terminal region
HDAC	Histone deacetylases
DDX1	DEAD (Asp-Glu-Ala-Asp) Box Helicase 1
TCF 4	Transcription factor 4
CCL5	Chemokine (C-C Motif) Ligand 5

3.1 Astrocytes: An Introduction

3.1.1 Historical Aspects of Astrocytes

On the basis of histological techniques developed by Camillo Golgi, Ramón y Cajal proposed the neuronal doctorine that the neuron is the basic structural and functional unit of the nervous system. For this Cajal and Golgi were awarded Nobel Prize in 1906; however, they both disagreed about the basic structure of the nervous system. Cajal's discovery is said to be one of the most important one for the neuroscience.

Another breakthrough in the field of neuroscience occurred, with the discovery of an important brain cell in 1824 which was termed as nervenkitt or neuroglia in 1856 by famous German neuropathologist Rudolf Virchow. Thirty years later in 1885, using black chrome silver reaction, Golgi confirmed the presence of glial cells in the brain which were undoubtedly different from neurons and were later termed as radial glia and multipolar glia. In 1893, two other type of glial cells; protoplasmic glia in the grey matter and fibrous glia in the white matter were distinguished by Andriezen and speculated to originate from mesoderm and ectoderm, respectively. It was Ramon y Cajal who in 1913 visualized these cells by using specific gold sublimate stain and termed these cells as astrocytes and considered both of them to be of ectodermal origin. In 1920 Pio del Rio Hortega, a student of Cajal classified glia into four types (i) ectodermal protoplasmic neuroglia of grey matter (ii) ectodermal fibrous neuroglia of white matter (iii) mesodermal microglia (iv) interfascicular glia now known as oligodendrocytes (Andriezen 1893; Somjen 1988).

Astrocytes play an important role in human brain evolution and it has now been proved by anatomical, genetic and functional studies on human and other mammalian brains that astrocytes are critical for improved cognitive abilities in humans (Robertson 2014; Zhang and Barres 2013). A very interesting fact about glial cells came to light in 1980 when famous scientist Albert Einstein's brain was studied by Marian Diamond, an anatomist in University of California, in an attempt to understand if there was anything specific in Einstein's brain which made him so intelligent. It was found that there was nothing different in Einstein's brain, neither the brain was large nor it had more number of neurons than others. Rather it contained more number of glia in association cortex, an area of brain which is involved in complex thinking and imagination (Diamond et al. 1985). This was a great surprise to scientists who earlier believed that glia are only passive and supportive cells in the brain.

3.1.2 Origin and Markers of Astrocytes

During brain development astrocytes develop from three different sources: from radial glia, from stem cell itself and from A2B5+/GFAP—precursor cells (Barry and McDermott 2005; Doetsch 2003; Fok-Seang and Miller 1992; Kessaris et al. 2008). Astrocytes are identified by their characteristic star shaped morphology and the presence of their prototypic marker glial fibrillary acidic protein (GFAP) which is found in almost all reactive astrocytes during central nervous system (CNS) injury. However, some mature astrocytes have undetectable label of GFAP in healthy brain which is attributed to the heterogeneity of astrocytes within the same area or different areas of the brain (Oberheim et al. 2012). GFAP is present more in white matter astrocytes as compared to the grey matter astrocytes. Other putative markers for astrocytes are S100B and glutamine synthetase (GS) (Anlauf and Derouiche 2013; Walz and Lang 1998), however these are also not exclusive for astrocytes. A list of other markers used for identification of astrocytes includes glutamate

transporter (GLT-1) and excitatory aminoacid transporters (EAAT1) which are glutamate transporters, inward rectifying potassium channel (Kir4.1), Aquaporin-4 water channel (AQP4) and glycogen granules. One more promising marker discovered from transcriptome database for astrocyte identification is Aldehyde dehydrogenase-1 family member Aldh1L1 which has a broaden pattern for astrocyte expression than GFAP (Cahoy et al. 2008).

3.1.3 Functions of Astrocytes

Astrocytes were once believed to be mere passive, non-excitable, non-neuronal cells that provide structural and trophic support to neuron. Over last decade the list has been expanded several folds and astrocytes are now believed to be indispensible cells for neuronal function. Recently, the importance of astrocytes in normal CNS physiology and pathophysiology has been redefined (http://annalsofneurosciences.org/journal/index.php/annal/article/viewArticle/155/381). Recent discoveries into glial cell biology, particularly those of astrocytes, suggest dynamic crosstalk between astrocytes and neurons to be pivotal to normal brain physiology and function. Currently, the new field of neuron-glia crosstalk has gathered the interest of both basic and clinical neuroscientists. Many critically important functions of the brain physiology as well as pathophysiology are executed by astrocytes, making astrocyte one of the most important cell types in brain.

Astrocytes are most abundant cell type found to be present in all regions of the brain in a non-overlapping manner. They interact with all other cell types present in the brain and regulate their functions in several ways. They play an important role in maintaining the integrity of blood brain barrier by interacting with endothelial cells and acting as a blood–nervous system interface, maintain pH, ionic and water homeostasis and participate in neurotransmitter uptake and release during neuronal activity, provide metabolic support to neurons by making available lactate and help in neuronal transmission and brain information processing, synaptic function and plasticity. Astrocytic processes envelope the neuronal synapse (made by presynaptic and post synaptic neuron) and give rise to a structure called tripartite synapse. The concept of tripartite synapse has helped in improved understanding of neuron-glia crosstalk and has further nurtured our appreciation for role of astrocytes in neuronal activity. Astrocytes send their signals to neighbouring astrocytes in the form of calcium waves; however, signal to neuron is passed via calcium dependent glutamate release, thus regulating the electrical impulses in the brain (Perea et al. 2009). Several lines of evidence suggest the role of astrocytes in modulating neuronal activity; one such example came from the fact that the synaptic activity increased by 10–100-fold in presence of astrocytes or astrocyte conditioned media (Pfrieger and Barres 1997). Various soluble factors released by astrocytes have been shown to promote the formation and maturation of excitatory and inhibitory synapses (Bolton and Eroglu 2009). Astrocytes also interact with oligodendrocytes. ATP released from neurons acts on nearby astrocytes and releases cytokine leukaemia inhibiting factor (LIF) which promotes myelination activity of

oligodendrocyte (Cohen and Fields 2008). Various factors released by astrocytes, e.g. PDGF, LIF, NT-3, NT-4, CNTF and IGF-1 promote differentiation, proliferation and survival of oligodendrocyte precursor cells. These also help in myelin formation and remyelination following injury (Gard et al. 1995). The interaction of astrocytes with other brain cells is shown in Fig. 3.1.

Astrocytes maintain a dynamic crosstalk with neurons to serve important brain function at the "tripartite synapse" hence it is obvious that astrocytes dysfunction

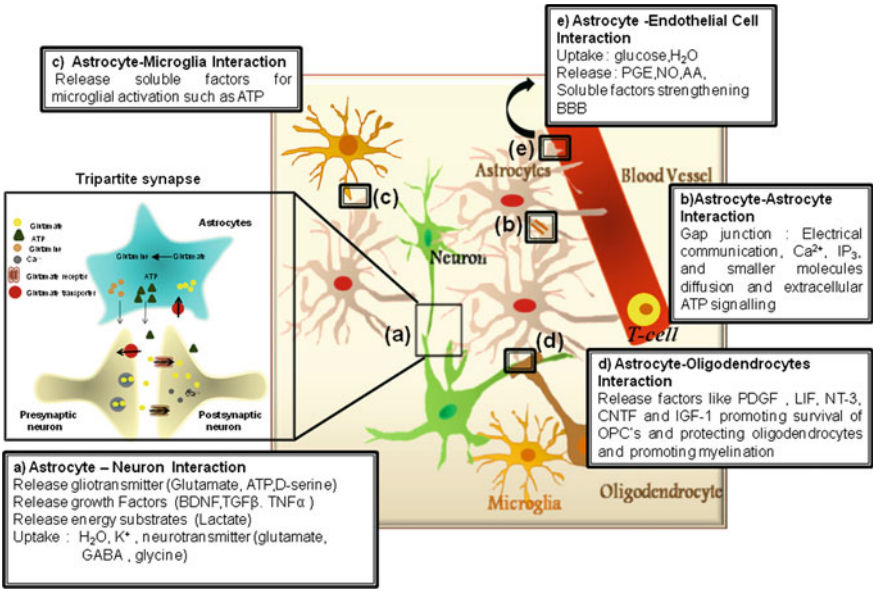

Fig. 3.1 Astrocytes interaction with other brain cells: *a* Astrocyte–neuron interaction: Astrocytes in close proximity with the presynaptic and postsynaptic neuron form the tripartite synapse. At the tripartite synapse a bidirectional communication occurs between astrocytes and neurons. During neuronal activity, the astrocyte detects the neurotransmitter released from neurons and in turn releases gliotransmitters (glutamate, ATP, D-serine) that modulates synaptic activity. Astrocytes release growth factors (BDNF, TGF-β) and energy substrates (lactate) to provide trophic and metabolic support to neurons. Astrocytes maintain the ionic and water homeostasis by removing H_2O and K^+ ion from the extracellular space. *b* Astrocyte–astrocyte interaction: Astrocytes send their signals to nearby astrocytes in the form of calcium waves. Short range calcium signalling occurs as a gap junction mediated metabolic coupling in the form of IP_3. However, the long range calcium signalling occurs through the release of ATP from astrocytes. *c* Astrocyte: microglia interaction: Microglia express purinergic receptors that are stimulated by ATP, released from astrocytes. ATP mediated calcium signalling act as a mode of communication between astrocytes and microglia. Astrocytes also play a regulatory role for differentiation and deactivation of microglial cells. *d* Astrocyte–oligodendrocyte interaction: Astrocytes release leukaemia inhibitory factors (LIF) which promotes myelination activity of oligodendrocytes. Various factors released by astrocytes like PDGF, CNTF, IGF promote survival and differentiation of oligodendrocyte precursor cells. *e* Astrocyte-endothelial cells interaction: Astrocytes send their end feet to enwrap the endothelial cells at the blood brain barrier. Various soluble factors released from astrocytes help in development and strengthening of tight junction between endothelial cells and regulate the entry and exit of various factors of the brain

may lead to neuronal impairment. Various functions of astrocytes have been explored, however, the precise cellular and molecular mechanisms that contribute to astrocyte functions are still not well understood. A better understanding of the cellular and molecular mechanisms of astrocyte functions will help to approach the astrocytes for pharmacological targeting. Identifying the role of astrocytes in CNS pathologies is currently an area of active research and preventing astrocyte damage might prove an important strategy to save the dying neuron in neurological disorders. Some of the important functions of astrocytes are listed in Table 3.1.

Table 3.1 Functions of astrocytes in brain

Functions	Functional components
pH maintenance	Na+/H+ exchanger, bicarbonate transporter
Water homeostasis	AQP4
Potassium (ion homeostasis)	Kir 4.1
Neurotransmitter uptake and release	Uptake via Glutamate, GABA and glycine transporters, calcium dependent vesicular release of ATP and Glutamate
Blood flow control	Secrete prostaglandins, arachidonic acid and nitric oxide to increase and decrease blood vessel diameter
Trophic support	Release BDNF, GDNF, bFGF and neurosteroids
Metabolic support	Convert glutamate to glutamine, convert glycogen to lactate to provide energy to neuron, synthesize cholesterol, produce ATP
Detoxification	Prevent neurons by scavenging free radicals released by neurons
Development	Synapse formation and maturation by thrombospondin and pruning by releasing C1q, axonal guidance
Pathologic	Astrocyte dysfunction is associated with neurological disease like Stroke, Glioma, Alzheimer's, Pain, Amyotrophic Lateral Sclerosis, epilepsy, Huntington disease, Autism Spectrum Disorder, Schizophrenia and Parkinson's disease
Maintenance of blood brain barrier (BBB)	Release soluble factors affecting endothelial cells to influence BBB integrity. Release sonic hedgehog to promote BBB formation and integrity, induce immune quiescence
Structural	Astrocytes are in close contact with neurons and form tripartite synapse, regulate transmission of electrical impulse in the brain
Modulate synaptic transmission	Release ATP which gets converted to ADP and suppress neuronal activity, release glutamate to enhance neuronal activity
Neuronal protection and nervous system repair	Form glial scar at the site of injury to protect healthy tissue, contain antioxidant system like—GSSG-GSH system and antioxidant enzyme like SOD-1 and catalase
Phagocytosis	ced-1/Draper—ced-7—ced-6 phagocytosis pathway for synapse pruning. Also had ced-2—ced-5—ced-12 pathway of phagocytosis
Regulation of neural stem cells	Activate neural stem cells to allow neurogenesis by dampening ephrin A-2 and ephrin A-3, which otherwise keep stem cells in a dormant state

3.2 Neuroinflammation, Response or Cause of Pathology

Central Nervous System (CNS) is immunologically privileged where the brain immune cells-like microglia or astrocytes are maintained in a quiescent state. The entry of peripheral immune cells into the brain is restricted by the blood brain barrier. However, during a pathological insult that may include viral or bacterial infection or neuronal injury, the microglia and astrocytes get activated and release various proinflammatory cytokines or chemokines as an acute neuroinflammatory response to fight against the infection and to restore the normal functioning of brain. Thus, acute neuroinflammation acts as a protective mechanism to neutralize toxic signals and minimize further injury to the brain. However, when inflammation fails to combat the neurological insult and persists for longer period, the chronic neuroinflammation leads to uncontrolled release of proinflammatory cytokines and chemokines release from these activated glial cells. Unprecedented cytokine/chemokine release from activated glial cells creates detrimental environment for neighbouring neurons affecting their normal activity and contributing to severe neurological disorders. Hence, special attention is required on the role of chronic neuroinflammation in brain pathology. Many neurodegenerative disorders including Alzheimer's disease, Parkinson's disease, Huntington's disease and HIV-associated neurocognitive disorders are associated with inflammation. The role of astrocytes in the CNS disorders with special emphasis on their role in neuroinflammation and their pathogenesis is discussed in detail.

3.2.1 Role of Astrocytes in CNS Inflammation

Astrocytes ensheath the brain microvascular endothelial cells and help in maintaining the integrity of blood brain barrier (Abbott 2002). Their position at BBB makes them an ideal candidate to influence the entry of peripheral immune cells into the CNS during disease process and in modulating their activity once these cells enter the CNS parenchyma. During inflammation, astrocytes play a protective function in the brain through release of several trophic factors. Thus, impairment of astrocytic function may have immense potential to contribute to neurological disease. Neuroinflammation can damage astrocytes or influence astrogliosis and scar formation in the CNS. Astrocytes respond to CNS injury by a process known as reactive astrogliosis characterized by increase in the number and size of GFAP expressing cells which act as a major pathological hallmark of astrocytes dysfunction during CNS pathology. Reactive astrogliosis is assumed to be a consequence of increased proliferation of astrocytes or migration of GFAP positive astrocytes from nearby region to the site of pathology (Sofroniew 2009). However, both these phenomenon do not contribute significantly to the total increase in GFAP positive cells, in fact it was found to be a result of phenotypic changes in local astrocytes which otherwise have undetectable level of GFAP (Serrano-Pozo et al. 2013).

As a response to neuroinflammation, astrocytes secrete different cytokines and chemokines. A recent report has analysed the cytokine secretome profile of human astrocytes and demonstrated that astrocytes express distinct set of cytokine and chemokines under normal and inflammatory conditions and are direct target of nuclear transcription factor NF-κB (Choi et al. 2014). The report demonstrated that while some cytokines/chemokines were upregulated in active astrocytes other new cytokines also get expressed.

Centrally or peripherally induced CNS inflammation motivates astrocytes to migrate towards the site of injury and form glial scar that prevents the spread of inflammation to adjacent healthy tissue. Controversial views are presented by various studies performed on the role of glial scar formation. Some studies suggest that reactive scar forming astrocytes act as a barrier for leukocytes through the blood brain barrier as observed in experimental acute encephalitis (EAE) (Voskuhl et al. 2009), thus preventing peripheral immune cells to enter the CNS. Various molecules released by astrocytes promote angiogenesis, interaction with other extracellular molecules regulate vascularisation and clearance of dying cells. Other studies suggest that they promote neurite growth; however, the scar formed also arrest the growth of axon in the vicinity of reactive astrocytes thus halting regenerative process after injury (Silver and Miller 2004). However, there is an active debate on the beneficial or detrimental effect of astrocyte activation and scar formation (Wee Yong 2010). The beneficial and detrimental aspect of astrocytes during neuroinflammation is shown in Fig. 3.2.

Neuroinflammation not only leads to activation of astrocytes but also induces astrocytic apoptosis. Loss of astrocytes has been reported in major depressive disorders, neuromyelitis optica and Rasmussen's encephalitis, whereas astrogliosis is observed in diseases like multiple sclerosis and HIV-1 dementia (Bien et al. 2005; Holley et al. 2003; Parratt and Prineas 2010; Ton and Xiong 2013).

3.3 Inflammation, Astrocytes and Neurological Disease

Inflammation and proinflammatory cytokines have been implicated in various neurological disorders, mood disorders and in declined cognitive function (Xanthos and Sandkuhler 2014). Inflammatory response induced by astrocytes is important mediator of neuronal loss in brain pathology. Some of the neuroinflammatory diseases are discussed below in brief:

3.3.1 Alzheimer's Disease

Alzheimer's disease (AD) is a chronic neurological disease and is a leading cause of dementia, generated due to deposition of neurotoxic peptide β amyloid (Aβ) in brain. Formation of neuritic plaque and neurofibrillary tangles occurs subsequent to Aβ deposition and acts as a pathological hallmark of the disease. Neuroinflammation

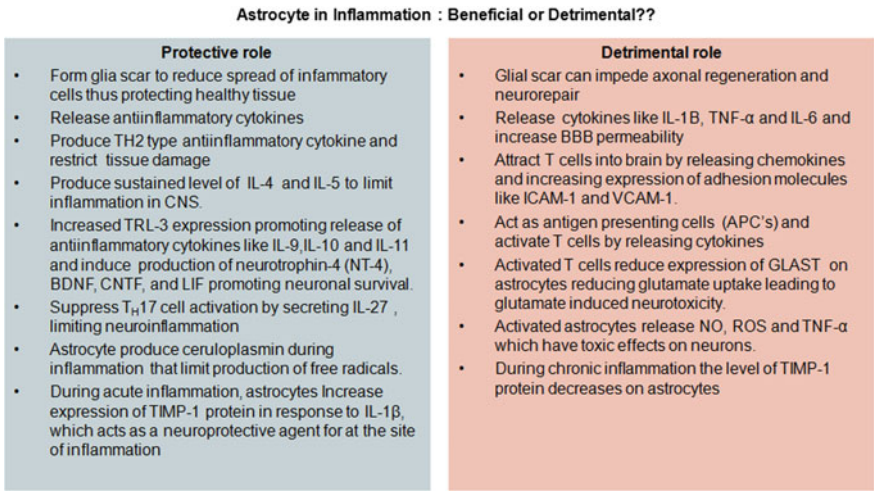

Fig. 3.2 Dual role of astrocytes during neuroinflammation

occurs in the AD brain as a consequence of astrocytes and microglia activation. Dementia due to neuronal loss is a major consequence of the disease but the importance of astrocytes in its pathogenesis cannot be ignored as they play a key role in accelerating neuronal apoptosis during disease progression. During Alzheimer's disease robust changes in astrocytes morphology and astrocytes activation occur leading to reactive astrocytes (Fu et al. 2015). Aβ activates astrocytes and induces release of cytokines and chemokines like (C-C motif) ligand 2 (CCL2) and RANTES that act as a chemoattractant for circulating microglia and macrophage. These microglia and macrophage secrete various cytokines generating ROS when they come in close proximity to plaque thus contributing to neurodegenerative process of AD (Johnstone et al. 1999). Reactive astrocytes also induce secretion of proinflammatory cytokines like IL-1β, IL-17 and IP-10 which accelerates Aβ-induced neurotoxicity via caspase-3 activation leading to tau phosphorylation and truncation in neurons (Garwood et al. 2011; Zheng et al. 2002). Deregulation of calcium signalling and calcium homeostasis in astrocytes is another important factor underlying Aβ-mediated neuronal loss (Lim et al. 2014). Thus astrocytes can mediate indirect neuronal death during Alzheimer's disease, however, Aβ25-35 also leads to astrocytes cell death via increased calcium mobilization from endoplasmic reticulum (Oseki et al. 2014).

3.3.2 Huntington Disease

Huntington disease (HD) is an autosomal dominant disorder which arises due to expansion of CAG repeats in Huntington (Htt) gene and causes neurodegeneration of striatal neurons. The resultant mutant form (mHtt) aggregates in astrocytes and

neurons affecting brain function. The major neuronal loss occurs in cortex and striatum giving rise to cognitive decline and movement disorders ultimately leading to death of the affected individual (Kim et al. 2014; Nana et al. 2014). During Huntington disease, astrocytes participate in chronic inflammation by prolonged activation of NF-κB pathway leading to increased release of proinflammatory cytokines from astrocytes resulting in neuronal damage (Hsiao et al. 2013). Disturbance in astrocytes-mediated potassium homeostasis also contributes to disease pathogenesis. In HD patients and HD mouse model functional expression of Kir4.1 channel is reduced which results in increased K^+ concentration in the extracellular space and increased excitability of medium spiny striatal neuron (Tong et al. 2014). Augmented glutamate production and its release from astrocytes through calcium dependent exocytosis, decreased expression of GS (which converts glutamate to glutamine) in astrocytes, along with decrease in glutamate uptake due to reduced expression of glial glutamate transporter EAAT2 (GLT-1) culminates in HD progression (Lee et al. 2013; Lievens et al. 2001). Conversely neuroprotective functions of astrocytes are also observed in vitro in Q111 striatal cells and in vivo model of HD using R6/1 mice, where it was shown that glial conditioned media protects striatal and nigrostriatal dopaminergic neurons much more effectively than neurotrophic factors (Perucho et al. 2013; Ruiz et al. 2012; Zheng et al. 2002).

3.3.3 Parkinson Disease

Parkinson disease (PD) is caused by degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) causing characteristic movement disorders. Active microglia and to a lesser extent active astrocytes are found at a site of neuronal loss. Increase immunoreactivity to GFAP and increase in number of astrocytes has been observed in some PD cases; however, the neuronal loss was more in those sites having fewer GFAP expressing astrocytes. Increased levels of proinflammatory cytokines like TNF-α, IL-1β, IL-6 and decreased neurotrophins has been reported in patients with PD (Nagatsu et al. 2000). In 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) model of Parkinson disease, astrocytes activation with marked upregulation of GFAP occurs after neuronal death. However, unlike deleterious effect of microglial activation, activation of astrocytes provides neuroprotection in PD, as documented in a study (Ishida et al. 2006), proteases activated receptor (PAR-1) expression increased in SNpc which provided neuroprotection against noxious stimuli-induced death of dopaminergic neurons. Another mechanism for neuroprotection is scavenging of reactive oxygen species (ROS) released by damaged or dying neurons and neutralizing the deleterious effects of activated microglia. Local activation of astrocytes in substantia nigra also protects dopaminergic neurons from deleterious effect of 6-hydroxy dopamine (Saura et al. 2003).

Astrocytic response to some of the neurodegenerative diseases is summarized in Table 3.2.

Table 3.2 Astrocyte response during various neurodegenerative diseases

Neurodegenerative disease	Cellular response	References
Alzheimer's disease	Aβ-induced neurotoxicity and tau phosphorylation	Garwood et al. (2011) and Phillips et al. (2014)
Huntington disease	Decreased Kir4.1 channels, loss of astrocytic GABA release Increased Ca^{2+} dependent glutamate release Dysregulation of corticostriatal ascorbic acid release and glutamate uptake Increased p65 mediated inflammatory response Activation of P2X7R on astrocytes leading to neuronal damage	Hsiao et al. (2013), Lee et al. (2013), Rebec (2013), Tong et al. (2014) and Wojtowicz et al. (2013)
Parkinson disease	P2X7R mediated synaptotoxicity, gliosis and neurotoxicity, increase in proinflammatory cytokines, oxidative stress	Carmo et al. (2014) and More et al. (2013)
Autism	Impaired processing and trafficking of Na(+)/H(+) exchanger Formation of astrocyte mega–domain Alteration of astrocytes and Wnt/β-catenin signalling	Cao et al. (2012), Ilie et al. (2014), Kondapalli et al. (2013) and Mitterauer (2013)
Demyelinating disease (MS)	Demyelination and degeneration by promoting inflammation, damage of oligodendrocytes and axons, and glial scarring, remyelination by promoting oligodendrocyte survival, proliferation and maturation. Activation of P2X7R on microglia/macrophage releasing cytokines leading to cell death and ATP release	Claycomb et al. (2013), Nagatsu et al. (2000)
Epilepsy	Decrease in EAAT2 on astrocytes, increased adenosine kinase, reduced glutamine synthetase, modulation of AMPA and NMDA receptor	Kong et al. (2012), Lopes et al. (2013), Theofilas et al. (2011) and van der Hel et al. (2005)
Japanese encephalitis (JEV)	Alters astrocyte and neuronal differentiation of NSC, increase MMP-9 in astrocytes thus causing inflammation, increase RANTES gene expression in astrocytes, induction of proinflammatory cytokines and proteins in astrocytes	Ariff et al. (2013), Chen et al. (2011), Das et al. (2008), Mishra et al. (2008), Tung et al. (2010) and Yang et al. (2012)
Prions disease	Close relationship between PrPc and GFAP hyperimmunoreactivity, loss of PKCδ positive Purkinje neurons, increased αβ crystalline	Belluzzi and Sacchi (1988), Hernandez et al. (2014) and Wang et al. (2013)
Neuropathic pain	Induction of CXCL1 and CXCR2 in astrocytes and neurons JNK induced MCP-1 production in spinal cord astrocytes Up regulation of Cx 43 in astrocytes	Chen et al. (2014), During et al. (1977), Gao et al. (2010), Moon et al. (2014) and Zheng et al. (2002)

3.3.4 HIV-Associated Neurological Disorder

HIV-associated neurocognitive disorder (HAND) is a phenomenon that leads to cognitive, motor and behavioural deficits in approximately 50 % of individuals suffering from AIDS. Central nervous system infection by HIV-1 is characterized by activation of microglial cells, formation of multinucleated giant cells, myelin pallor, breaching of blood brain barrier, astrocyte dysfunction and neuronal loss (Elbirt et al. 2015; Pant et al. 2012).

Using antiretroviral therapy almost complete eradication of systemic HIV-1 infection is made possible; however, the virus still persists in immunologically protected CNS. This is an area of major concern. In this chapter, we have attempted to highlight the role of astrocytes in HIV-1 infection culminating in neuroAIDS.

The CNS allows the persistence of HIV-1 virus despite highly effective antiretroviral therapy. Cells-like microglia, macrophages and astrocytes act as a reservoir of the infection in the brain and later on contribute to chronic inflammation in the infected brain, even in absence of viral load (Churchill and Nath 2013). CNS is immunologically protected site for the virus. Glial cells release several viral and cellular factors which either directly affect neurons by acting on their membrane or act on the uninfected cells contributing to neuronal damage. Macrophages/microglia are believed to be key players/candidate in HIV infection. HIV virus enters these cells by binding to CD4 and CCR5 receptors (Soulie et al. 2012). However, in astrocytes it enters in a CD4 independent manner relying on the presence of other membrane receptors (Zhuang et al. 2014). HIV virus infects glial cells which release various soluble factors. The virus jeopardizes the function of neurons. Oligodendrocytes also get productively infected (Albright et al. 1996), but the viral tropism is severely restricted to microglia only. The pathological response of various glial cells to HIV infection is shown in Fig. 3.3.

3.3.4.1 Viral Reservoir in CNS

The main stumbling block in complete eradication of HIV is the persistence of virus in the brain. Even in presence of HAART the virus escapes from the attack of immune cells and hide in CD4+ T lymphocytes, haemopoietic progenitor cells and CNS. However, in advance stages of infection decrease in CD4+ T lymphocyte occurs and at that stage macrophages serve as a leading source of HIV infection in the CNS. In the CNS, cells-like macrophage/microglia and astrocytes act as reservoir for latent HIV infection. All these cells have a long life span thereby they act as a long term reservoir of HIV infection. This provides the virus with an excellent opportunity to reside and replicate within these cells and spread the infection to uninfected cells. Moreover, as the virus hides in these reservoirs, the antiretroviral drugs become ineffective over time because of incomplete suppression of viral replication, which may develop antiretroviral drug resistance that further compounds the problem of neuroAIDS.

The following account deals with the role of astrocytes in HIV-1 neuropathogenesis (Fig. 3.4), how the virus infects the astrocyte and what are the various

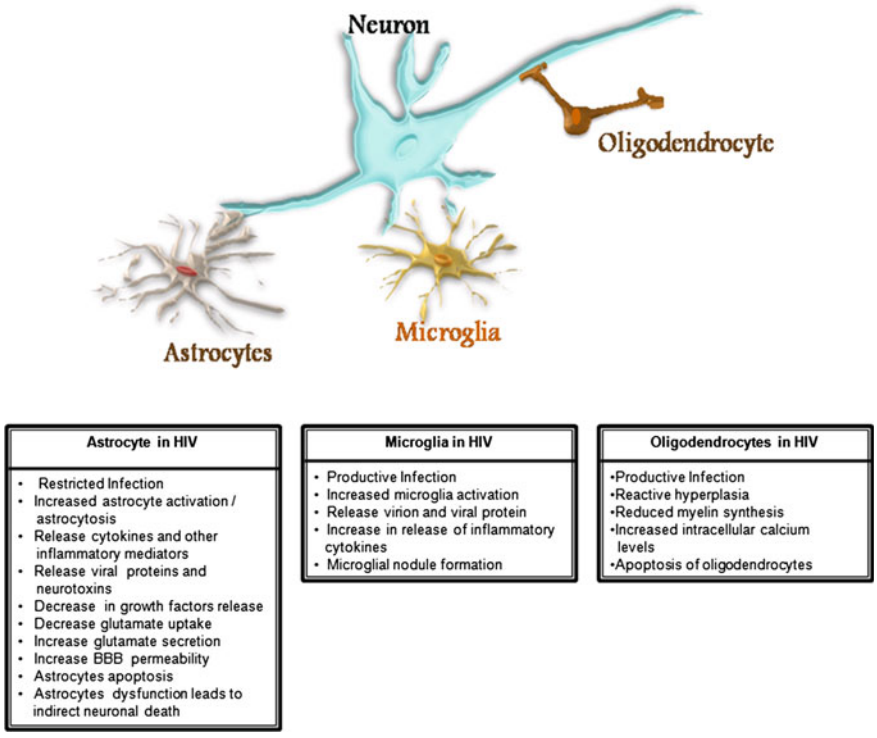

Fig. 3.3 Involvement of glial cells in HIV-1 infection: HIV-1 affects the different glial cells-like microglia, astrocytes and oligodendrocytes up to varying extent. The activated or infected glial cells release several viral factors, neurotoxins, cytokines and chemokines which ultimately contribute to indirect neuronal damage, a major pathological hallmark of HIV associate dementia

causes of viral latency or restricted infection in astrocytes. Finally, we will take a closer look on astrocyte response to virus infection which ultimately culminates into neuronal death.

3.3.4.2 Astrocyte Infection with the Virus

Post-mortem studies from HIV-infected brain tissue revealed that a small proportion of astrocytes is non-productively infected by the HIV that contributes to its neuropathogenesis (Gorry et al. 2003). Various HIV strains are permissive to astrocytes in vivo as well as in cultures of human foetal brain derived astrocytes and astrocytoma cell lines (Nath et al. 1995). However, highly sensitive techniques are required to detect HIV RNA and proviral DNA. About 19 % of GFAP+ cells in patients with HIV-1 associated dementia are found to be infected with the virus (Churchill et al. 2009). The frequency of astrocyte infection correlates with severity of HIV encephalitis and was more in cells surrounding perivascular macrophages. Using human foetal brain-derived astrocytes it was proposed that low level HIV

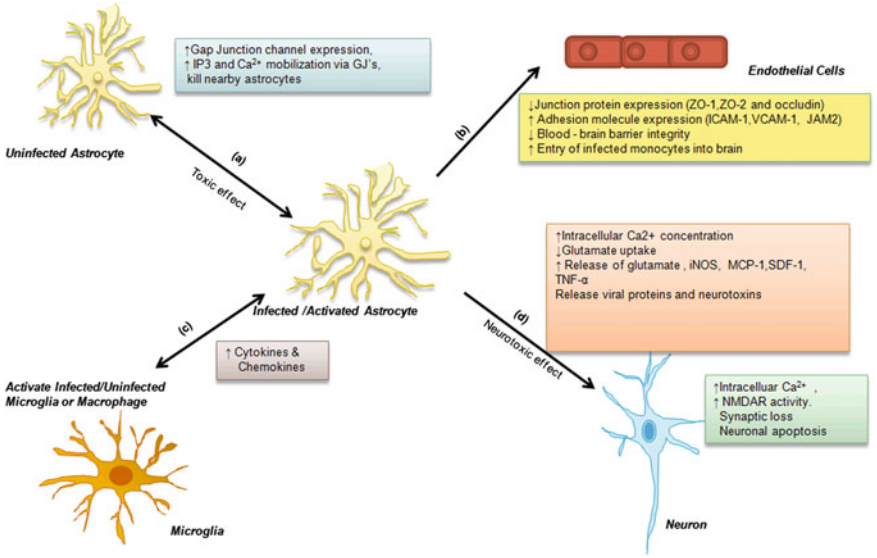

Fig. 3.4 Pathophysiological role of astrocytes (Cell-Cell Interaction) in HIV Neuropathogenesis: The extent of viral infection in astrocytes is very low. Despite the limited rate of infection astrocytes influence the physiological activities of nearby astrocytes, neurons and endothelial cells via several mechanisms. *a* HIV-1 infected/activated astrocytes send their toxic signal to nearby uninfected astrocytes via the gap junction channels in the form of diffusing IP_3 and Ca^{2+} waves. *b* The altered expression of gap junction channels is also responsible for decreased blood brain barrier integrity and permeability through modulation of tight junction protein and junctional adhesion molecules. The activated astrocytes release Monocyte Chemoattractant Protein (MCP-1) which helps in transmigration of infected monocytes into the brain through the blood brain barrier. *c* Astrocyte-microglia crosstalk profoundly affects the inflammatory environment in the brain through the release of various cytokines and chemokines. *d* The infected/activated astrocytes exert neurotoxic affects on the nearby neurons by releasing viral proteins and neurotoxins. The virus or the released viral proteins reduce the expression of glutamate transporters and increase the release of glutamate from the astrocytes. The excess glutamate activates the NMDAR activity on neurons increasing intracellular calcium subsequently causing synaptic loss and neuronal apoptosis

infection in astrocytes occurs in three stages which were also confirmed by HIV-infected autopsy brain tissue (Messam and Major 2000; Takahashi et al. 1996). These stages are as follows:

Productive phase: In this stage astrocytes produce the infectious viral progeny in a cytopathic manner. Production of viral structural and regulatory proteins occurs efficiently.

Latent phase: Loss of mRNA of structural protein followed by loss of viral regulatory proteins except Nef.

Reactivation phase: Occurs due to treatment of astrocytes with cytokines or culturing astrocytes with CD4+ cells. It resembles the productive phase, however, to a lesser extent. In this phase the predominant mRNA transcripts are Tat, Rev and Nef (Tornatore et al. 1994).

3.3.4.3 Route for HIV Entry in Astrocytes

Astrocytes lack cell surface CD4 expression, however, they posses CXCR4, CCR5 and other co-receptors that are required for a successful HIV-1 infection in addition to CD4 receptors. Some strains of HIV-1 have been shown to infect CD4 negative cells via CXCR4 and CCR5 co-receptors (Edinger et al. 1997; Picard et al. 1997). The CD4 independent entry of virus in the brain is also shown in new non-human primate model of R5 SHIV-induced encephalitis in which the virus infects microglia in a CD4 independent way and even CD4 negative astrocytes were shown to be infected by the virus (Zhuang et al. 2014). However, the CXCR4 and CCR5 co-receptors do not play a significant role in mediating HIV infection in astrocytes. It is well documented that astrocytes are preferentially infected by T-tropic strain of HIV-1; however, the M-tropic strain also infects astrocytes, though less efficiently. HIV-1 enters astrocytes in a CD4+ independent manner with an alternate mode using galactosyl ceramide receptor; as antibodies against GalC inhibits viral internalization and infection in CD4 negative cell lines (Harouse et al. 1991a, b). However, in human foetal astrocytes a CD4 and cerebroside independent pathway occurs which requires the presence of surface molecules that act as receptor for HIV-1 infection (Hao et al. 1997). Several studies have indicated efficient viral (M-tropic and T-tropic strain) entry through receptor mediated endocytosis using astrocyte membrane receptors (Chauhan et al. 2014). HIV–gp120 protein acts as a ligand for membrane protein having molecular weight of 65 kDa and hence named p65 as a receptor for HIV (Hao and Lyman 1999). In these studies, HIV virions were recognized to be present in clathrin coated pits suggesting clathrin to be associated with the endocytic pathway. Another study suggests surface molecule of 260 kDa to be a receptor for gp-120 binding as antibody to this molecule can inhibit astrocyte infection but the antibodies against galactocerebroside and CD4 receptor had no effect (Ma et al. 1994). It was hypothesized that it may be possible that 260 kDa may be a tetramer complex of 65 kDa molecule. Involvement of human mannose receptor has also been identified in HIV-1 infection of astrocytes and suggests that HIV-1 interaction with these receptors play an important role in HIV-1 neuropathogenesis (Liu et al. 2004). Cell to cell contact between HIV-infected CD4 + cells and CD4 negative astrocytes is also another possible mechanism for HIV infection, whereby astrocyte spread the infectious virus to CD4+ cells without de novo production using CD81 vesicles (Clarke et al. 2006; Gray et al. 2014).

Thus astrocytes act as a source of virus dissemination and persistence in the brain. Moreover, HIV itself has the capability to infect CD4 negative cells via envelope protein (Env) and then interact with cells-like astrocytes in CD4 independent manner (Zhuang et al. 2014). A recent report showed that HIV-1 infects astrocyte naturally through endocytosis via Rab protein, independent of CD4, CXCR4 and CD11a co-receptors. It causes low level infection that leads to persistent and minimally productive infection in astrocytes (Chauhan et al. 2014). Another study by Gray et al. demonstrated that astrocytes harbour virus for a short term period of 72 h and during this period the virus resides in vesicles and suggests that vesicles may be responsible as entry site for the virus in astrocytes. They also

Table 3.3 Route of entry of HIV virus in astrocytes

• CD4+ independent
• Receptor mediated endocytosis
• Galactosyl Ceramide receptor
• Mannose receptor
• Cell to cell contact between $CD4^+$ T cells and $CD4^-$ astrocytes
• Direct interaction by viral envelope protein (Env)

demonstrate that astrocyte protect the virus and can transfer the infection to T cells, thus helping in propagation of viral infection (Gray et al. 2014) (Table 3.3).

3.3.4.4 Limited Viral Replication in Astrocytes (Latent Infection)

Chronically infected astrocytes show very low level of viral replication as compared to fibroblasts and T lymphocyte cells. Limited viral replication in astrocytes results from the low level of viral entry, transcription, viral protein processing, and virion maturation (Schweighardt and Atwood 2001). The virus thus remains in a latent state in the astrocytes. One of the mechanisms for low transcription of the viral transcript is the epigenetic silencing of HIV-1 LTR activity through the class I HDAC's and histone methyl transferase (HMT) (Narasipura et al. 2014).

Astrocytes produce very low amount of virus due to inefficient translation of HIV structural proteins gag, env, and nef. However, the expression of Tat and Rev proteins occurs efficiently (Gorry et al. 1999). Low level expression of Nef protein, despite high levels of mRNA, blocks the translation of multiple spliced HIV mRNA in astrocytes and contributes to persistent and restricted HIV replication in astrocytes (Gorry et al. 1998). During persistent viral infection astrocytes express Nef and Rev, predominantly Nef; but no viral structural protein. However, when persistent state of infection in astrocytes is reactivated by cytokines like TNF-α and IL-1β, i.e. when low viral replication occurs in astrocytes, they start expressing Tat and Rev also along with Nef protein. These studies suggest that viral persistence occurs due to accumulation of viral regulatory protein over viral structural protein. Furthermore, via negative regulatory element (NRE) Nef also mediates suppression of exogenous HIV-LTR activity thus restricting virus production (Brack-Werner et al. 1992; Ludvigsen et al. 1996), Thus in absence of virus production, astrocytes may contribute to the HIV pathogenesis by production of viral regulatory protein.

Restricted HIV infection occurs not due to low expression of Rev, but perhaps due to block in Rev-Rev response element (RRE) function (Neumann et al. 1995). Altered expression of Rev cellular cofactor DDX1 creates unfavourable environment for Rev function in astrocytes and contributes to restricted replication in these cells (Fang et al. 2005). It also allows the sequestration of Rev towards cytoplasm. These studies suggest that diminished function of Rev is a hallmark of limited virus production in astrocytes. Rev stimulates synthesis of HIV structural proteins in astrocytes with only 10 % efficiency of that in non-glial cells (Table 3.4).

Table 3.4 Reasons for limited viral production/replication in astrocytes

• Inefficient translation of HIV structural proteins gag, env, and nef
• Nef mediated suppression of exogenous HIV-LTR activity via negative regulatory element (NRE)
• Block in Rev RRE function and sequestration of Rev toward cytoplasm

3.3.4.5 Astrocyte Response to HIV-1 Infection

During acute stages of HIV-1 infection astrocytes and other glial cells also exert some neuroprotective mechanism. For example HIV-1 infected astrocytes express tissue inhibitor of metalloproteinase-1 (TIMP-1) which protects the neurons from apoptosis. It acts via inhibiting the opening on mPTP and modulating the Bcl-2 family of proteins (Ashutosh et al. 2012). Upon gp120 exposure, the astrocytes up regulate their antioxidant defence mechanisms by increasing the expression of the nuclear factor erythroid-derived 2-related factor 2 (Nrf2) thus exhibiting a protective effect on neurons. But, on chronic inflammation the astrocyte protective mechanism fails and dysfunction of astrocytes then leads to neuronal apoptosis. Astrocytes respond to HIV-1 infection by astrogliosis and undergo morphological changes along with increase in levels of GFAP (Repunte-Canonigo et al. 2014) which also occurs via viral proteins and factors secreted by infected macrophages. It is one of the major pathological hallmarks of HIV-infected brain. Early HIV infection in astrocytes augments telomerase activity and telomerase length, whereas during advanced stages HIV telomerase activity reduces to the level of uninfected cells which is one of the reason of increased GFAP expression showing astrocytes activation which further leads to neuronal damage (Ojeda et al. 2014). In astrocytes the entry of the virus is limited and also the astrocytes are minimal viral productive cells thus not contributing to increase viral load during HIV-1 infection. However, they cause neuronal damage via release of several viral proteins, neurotoxins and cytokines/chemokines.

The exact mechanism of how the astrocytes cause the indirect death of neurons is not very well understood and needs further exploration. According to the literature some of the mechanisms which may contribute to the astrocyte induced neuronal injury are as follows:

Glutamate-Induced Excitotoxicity

Failure of maintaining the extracellular glutamate concentration by astrocyte is one of the major contributors of neuronal damage. Under physiological conditions neurons release the excitatory neurotransmitter glutamate during neuronal activity which acts on post synaptic neuron via glutamate receptors. The excess of the glutamate is taken up by astrocytes through glutamate transporters EAAT1 and EAAT2 which then convert it to glutamine in the presence of enzyme glutamine synthase, thus maintaining glutamate homeostasis. However, during HIV-1 infection the virus or the viral proteins reduced the expression of EAAT1 and EAAT2 on astrocytes (Wang et al. 2003; Zhou et al. 2004). The excess glutamate then binds to

NMDAR receptor on neurons and increases the intracellular calcium ion concentration. Increased calcium leads to further release of glutamate from astrocytes thus adding on to the glutamate concentration in an autocrine manner. Thus, increased glutamate releases from neurons and decreased glutamate uptake by astrocytes collectively increases the extracellular glutamate concentration and causes glutamate-induced excitotoxicity leading to neuronal apoptosis (Kaul et al. 2001).

Spread of Toxic Signals via Gap Junction Channels

In the CNS astrocytes connect to one another or to nearby neurons via gap junction channels. The gap junction channel and the extracellular ATP signalling are the two major signalling modes by which the astrocytes propagate the signal to the distant astrocytes in the form of calcium waves. It is documented that the expression of these gap junctions especially connexin 43 get enhanced during HIV infection. During HIV-1 pathogenicity the virus protects the infected astrocytes from apoptosis; however, it propagates the neurotoxic signals to uninfected astrocytes and neurons via gap junction channels. Dysregulation of IP3 and Ca^{2+} is found to be responsible for bystander killing of uninfected cells (Eugenin and Berman 2013). It opens connexin 43 hemichannel promoting dysregulated secretion of dickkopf-1 protein (DKK1) affecting stability of neuronal process (Orellana et al. 2014). Blocking the gap junction channel has been found to reduce neuronal apoptosis during HIV neuropathogenesis. The astrocytic gap junction also reduces the integrity of blood brain barrier thus allowing the infected cells to enter the brain adding on to the neuroinflammation and neurotoxicity.

Purinergic Receptors

Purinergic receptors are ligand gated or metabotropic receptors which get activated upon binding of ATP or its analogs to the receptors (Burnstock 2013; Tewari and Seth 2015). Different purinergic receptors vary in their efficiency for ligand binding. Recent data related to purinergic receptors and HIV infection suggest that when HIV envelope protein interacts with the CD4+ target cells (PBMC's), the cell releases ATP via pannexin hemichannels. The released ATP then binds to P2Y2 receptor and facilitate the HIV infection by activating proline-rich tyrosine kinase 2 (Pyk2) (Seror et al. 2011). Another study suggests that purinergic receptors are also required for HIV infection of primary human macrophage (Hazleton et al. 2012). They suggested that the binding of HIV to macrophage leads to release of ATP. ATP further binds to and activates the purinergic receptors present on cell surface in an autocrine manner to help in viral entry and replication. It was found that activation of different purinergic receptors was responsible for viral entry (P2X1) and viral replication (P2X1, P2X7 and P2Y1) within these immune cells. The role of released ATP from macrophages is not only limited to the population of macrophages but it also exerts detrimental effects on the neurons. The ATP released from macrophages acts on purinergic receptors on the neuron and leads to excessive release of glutamate from the neuron thus affecting the glutamatergic signalling and reducing the spine density on neurons. Recent study from our lab has also shown the involvement of purinergic receptor in HIV-1 induced direct and indirect

neuronal damage. We have found that HIV viral protein Tat leads to neuronal apoptosis via P2X7R as blocking the P2X7R on astrocytes or directly on neurons by its pharmacological antagonists or using siRNA approach reversed the neurotoxic effect of Tat. In addition to the role of P2X7R in astrocyte induced indirect neuronal death, we have also found that P2X7R on astrocytes also regulates the release of Tat-induced MCP-1 release. MCP-1 is a major neuroinflammatory biomarker in the brain of HIV-1 infected patients thus suggesting that P2X7R present on astrocytes acts as one of the contributor to neuroinflammation in HAND (Tewari et al. 2015).

Wnt/β Catenin Signalling

Analysis of spatial relationship between astrocytes and neuron suggests that with increasing severity of HIV-associated dementia astrocytes interact more with surviving interneurons as compared to surviving pyramidal neurons in the superior frontal gyrus (Roberts et al. 2013). Tat B inhibits Wnt/β catenin signalling in astrocytes through its dicysteine motif and enhances HIV replication in infected astrocytes. Simultaneously it also dysregulates the neighbouring uninfected cells, contributing to viral pathogenesis. In contrast to this Tat C, due to absence of dicysteine motif was unable to suppress Wnt signalling (Henderson et al. 2012). Thus, basal level of HIV transcription in astrocytes was affected by modulation of the β-catenin/TCF-4 axis, which may drive low level/persistent HIV in astrocytes contributing to brain inflammation (Aldhous and Anderson 1990).

Release of Proinflammatory Cytokines and Chemokines from Astrocytes

CNS inflammation is a major problem in HAD which is caused by imbalance of various cytokines/chemokines released by glial cells (Tavazzi et al. 2014). Astrocytes respond to the cytokines released by the infected microglial/macrophage and in turn also release other proinflammatory cytokines which further leads to increase in inflammation in the brain. The proinflammatory cytokines released from astrocytes include IL-8, TNF-α and IL-1β. In addition to this various chemokines and their receptors were also up regulated in patients of HIV with or without encephalitis. The role of the upregulated chemokines are elegantly reviewed by Gonzalez-Scarano and Martin-Garcia (2005). Few of these cytokines and chemokines are discussed below in brief.

CXCL-8

CXCL8 (Interleukin 8 or IL-8) levels are high in CSF of patients with HAD. It is released by macrophages, microglia or astrocytes. Immune-activated macrophages release IL-1β and TNF-α which induced the production of CXCL8 from human astrocytes in a MAPK dependent pathway (Zheng et al. 2008). CXCL8 also promotes the productive virus infection in macrophage and microglia through CXCR1 and CXCR2 receptors (Mamik and Ghorpade 2014).

Stromal Derived Factor (SDF-1) and C-C Motif Ligand 2 (CCL2)
Exogenous addition of HIV-1 Tat induces CCL2 from monocytes-derived macrophages (MDM) and astrocytes (Conant et al. 1998; Lim and Garzino-Demo 2000; Mengozzi et al. 1999). Increased expression of inflammatory cytokines like TNF-α in HIV-1 infected macrophages also enhances CCL2 release from nearby astrocytes (Muratori et al. 2010). The IL-1β released from macrophage also regulates the release of stromal derive factor-1 (SDF-1) and CCL2 from astrocytes and enhances the proliferation, differentiation and migration of neural progenitor cells (Wu et al. 2012). The neural progenitor cells then migrate towards the site of IL-1 β injection. These chemokines also attract inflammatory cells into the brain parenchyma that complicates the brain environment by triggering an inflammatory response.

CCL5
In the hippocampus viral protein R (Vpr) released from astrocytes acts as a neurotoxin and impairs hippocampal dependent learning (Torres and Noel 2014). HIV viral protein Tat and Vpr induce proinflammatory cytokine CCL5 in astrocytes in a PI3 K and MAPK dependent manner (Gangwani et al. 2013; Nookala et al. 2013). CCL5 attracts monocytes to the brain parenchyma and is one of the mechanisms leading to HIV induced neurotoxicity.

Nitric Oxide Synthase (NOS) and Arachidonic Acid (AA)
Viral proteins like gp-120 induce cPLA2 and nNOS, however cPLA2 derived-arachidonic acid (AA) from astrocytes decline nNOS and enhance iNOS leading to NO and IL-1 β release through NF-κB activation which could contribute to neuronal death (Persichini et al. 2014). Another study suggests that treatment of metabolite of arachidonic acid, leukotriene C4 (LTC4), an eicosanoid to astrocytes help in transmigration of infected CD4+ T cells across blood brain barrier by release of chemokine CX3CL1/fractalkine, which supports in HIV-1 disease progression (Bertin et al. 2014).

3.4 Conclusions

A critically important area of neuroscience research today is to understand the role of glia, particularly astrocytes in its relation to function of neurons in human brain. Unfortunately, astrocytes were overlooked as researchers focused on neurons as key components of the CNS. Only recently, astrocytes have gathered the due attention, and their role has been duly recognized in normal CNS and brain pathology. Glial cells-like astrocytes are in limelight because of recent pioneer discoveries of their function in brain information processing, neuronal activity and synaptic plasticity. Currently, astrocytes are believed to be indispensible for optimal neuronal function. In fact there is a dynamic relationship between astrocytes and neurons that is modulated through an active neuron-glia crosstalk which is critical for physiological brain function. Glial cells are of particular interest due to their ability to respond

to insult in brain. Astrocytes and microglial cells are the only cell types capable to tackle an event of bacterial or viral infection or injury. They do so by mounting an inflammatory response that is initially controlled and beneficial of the host cells but under uncontrolled conditions may lead to neuroinflammation that may culminate in neuronal injury. Neuroinflammation in brain is mainly due to the activation of the immune cells-like microglial, though the contribution of astrocytes cannot be considered less important. At the early stages of infection the astrocytes serve to protect the neurons from the noxious stimuli, however, on persistent action of the virus or due to chronic infection in a later stage, these cells fail to combat the harmful effect of the virus and become dysfunctional. Astrocyte dysfunction disturbs the homeostasis mechanisms for proper neuronal functioning ultimately leading to neuronal injury or neuronal apoptosis. Neuroinflammation has been implicated in several neurodegenerative disorders and claimed to be one of the possible therapeutic targets. As a follow-up of these findings, anti-inflammatory drugs have been tested for their neuroprotective abilities in clinical trials with modest success. Hence it is necessary to direct sincere efforts in the field of glial biology with special emphasis on understanding their role in neuroinflammation. Perhaps in near future neuroprotection will be attempted via "gliaprotective" strategies which may be possibly more rewarding as astrocytes are pivotal to neuronal health and function.

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References

Abbott NJ (2002) Astrocyte-endothelial interactions and blood-brain barrier permeability. J Anat 200(6):629–638

Albright AV, Strizki J, Harouse JM, Lavi E, O'Connor M, Gonzalez-Scarano F (1996) HIV-1 infection of cultured human adult oligodendrocytes. Virology 217(1):211–219

Aldhous P, Anderson A (1990) Soviet accident. Under a cloud. Nature 347(6292):411

Andriezen WL (1893) The neuroglia elements in the human brain. Br Med J 2(1700):227–230

Anlauf E, Derouiche A (2013) Glutamine synthetase as an astrocytic marker: its cell type and vesicle localization. Front Endocrinol (Lausanne) 4:144

Ariff IM, Thounaojam MC, Das S, Basu A (2013) Japanese encephalitis virus infection alters both neuronal and astrocytic differentiation of neural stem/progenitor cells. J Neuroimmune Pharmacol 8(3):664–676

Ashutosh Chao C, Borgmann K, Brew K, Ghorpade A (2012) Tissue inhibitor of metalloproteinases-1 protects human neurons from staurosporine and HIV-1-induced apoptosis: mechanisms and relevance to HIV-1-associated dementia. Cell Death Dis 3:e332

Barry D, McDermott K (2005) Differentiation of radial glia from radial precursor cells and transformation into astrocytes in the developing rat spinal cord. Glia 50(3):187–197

Belluzzi O, Sacchi O (1988) The interactions between potassium and sodium currents in generating action potentials in the rat sympathetic neurone. J Physiol 397:127–147

Bertin J, Jalaguier P, Barat C, Roy MA, Tremblay MJ (2014) Exposure of human astrocytes to leukotriene C4 promotes a CX3CL1/fractalkine-mediated transmigration of HIV-1-infected CD4(+) T cells across an in vitro blood-brain barrier model. Virology 454–455:128–138

Bien CG, Granata T, Antozzi C, Cross JH, Dulac O, Kurthen M, Lassmann H, Mantegazza R, Villemure JG, Spreafico R, Elger CE (2005) Pathogenesis, diagnosis and treatment of Rasmussen encephalitis: a European consensus statement. Brain 128(Pt 3):454–471

Bolton MM, Eroglu C (2009) Look who is weaving the neural web: glial control of synapse formation. Curr Opin Neurobiol 19(5):491–497

Brack-Werner R, Kleinschmidt A, Ludvigsen A, Mellert W, Neumann M, Herrmann R, Khim MC, Burny A, Muller-Lantzsch N, Stavrou D et al (1992) Infection of human brain cells by HIV-1: restricted virus production in chronically infected human glial cell lines. AIDS 6(3):273–285

Burnstock G (2013) Introduction to purinergic signalling in the brain. Adv Exp Med Biol 986:1–12

Cahoy JD, Emery B, Kaushal A, Foo LC, Zamanian JL, Christopherson KS, Xing Y, Lubischer JL, Krieg PA, Krupenko SA, Thompson WJ, Barres BA (2008) A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. J Neurosci 28(1):264–278

Cao F, Yin A, Wen G, Sheikh AM, Tauqeer Z, Malik M, Nagori A, Schirripa M, Schirripa F, Merz G, Brown WT, Li X (2012) Alteration of astrocytes and Wnt/beta-catenin signaling in the frontal cortex of autistic subjects. J Neuroinflammation 9(1):223

Carmo MR, Menezes AP, Nunes AC, Pliassova A, Rolo AP, Palmeira CM, Cunha RA, Canas PM, Andrade GM (2014) The P2X7 receptor antagonist Brilliant Blue G attenuates contralateral rotations in a rat model of Parkinsonism through a combined control of synaptotoxicity, neurotoxicity and gliosis. Neuropharmacology 81:142–152

Chauhan A, Mehla R, Vijayakumar TS, Handy I (2014) Endocytosis-mediated HIV-1 entry and its significance in the elusive behavior of the virus in astrocytes. Virology 456–457:1–19

Chen CJ, Ou YC, Chang CY, Pan HC, Liao SL, Raung SL, Chen SY (2011) TNF-α and IL-1β mediate Japanese encephalitis virus-induced RANTES gene expression in astrocytes. Neurochem Int 58(2):234–242

Chen G, Park CK, Xie RG, Berta T, Nedergaard M, Ji RR (2014) Connexin-43 induces chemokine release from spinal cord astrocytes to maintain late-phase neuropathic pain in mice. Brain 137 (Pt 8):2193–2209

Choi SS, Lee HJ, Lim I, Satoh J, Kim SU (2014) Human astrocytes: secretome profiles of cytokines and chemokines. PLoS ONE 9(4):e92325

Churchill M, Nath A (2013) Where does HIV hide? A focus on the central nervous system. Curr Opin HIV AIDS 8(3):165–169

Churchill MJ, Wesselingh SL, Cowley D, Pardo CA, McArthur JC, Brew BJ, Gorry PR (2009) Extensive astrocyte infection is prominent in human immunodeficiency virus-associated dementia. Ann Neurol 66(2):253–258

Clarke JN, Lake JA, Burrell CJ, Wesselingh SL, Gorry PR, Li P (2006) Novel pathway of human immunodeficiency virus type 1 uptake and release in astrocytes. Virology 348(1):141–155

Claycomb KI, Johnson KM, Winokur PN, Sacino AV, Crocker SJ (2013) Astrocyte regulation of CNS inflammation and remyelination. Brain Sci 3(3):1109–1127

Cohen JE, Fields RD (2008) Activity-dependent neuron-glial signaling by ATP and leukemia-inhibitory factor promotes hippocampal glial cell development. Neuron Glia Biol 4(1):43–55

Conant K, Garzino-Demo A, Nath A, McArthur JC, Halliday W, Power C, Gallo RC, Major EO (1998) Induction of monocyte chemoattractant protein-1 in HIV-1 Tat-stimulated astrocytes and elevation in AIDS dementia. Proc Natl Acad Sci USA 95(6):3117–3121

Das S, Mishra MK, Ghosh J, Basu A (2008) Japanese Encephalitis Virus infection induces IL-18 and IL-1β in microglia and astrocytes: correlation with in vitro cytokine responsiveness of glial cells and subsequent neuronal death. J Neuroimmunol 195(1–2):60–72

Diamond MC, Scheibel AB, Murphy GM Jr, Harvey T (1985) On the brain of a scientist: Albert Einstein. Exp Neurol 88(1):198–204

Doetsch F (2003) The glial identity of neural stem cells. Nat Neurosci 6(11):1127–1134

During R, Wilken HP, Straube E, Schmidt H (1977) Bacteriological studies of the human amniotic fluid in repeated amniocenteses. Zentralbl Gynakol 99(21):1299–1304

Edinger AL, Amedee A, Miller K, Doranz BJ, Endres M, Sharron M, Samson M, Lu ZH, Clements JE, Murphey-Corb M, Peiper SC, Parmentier M, Broder CC, Doms RW (1997) Differential utilization of CCR5 by macrophage and T cell tropic simian immunodeficiency virus strains. Proc Natl Acad Sci USA 94(8):4005–4010

Elbirt D, Mahlab-Guri K, Bezalel-Rosenberg S, Gill H, Attali M, Asher I (2015) HIV-associated neurocognitive disorders (HAND). Isr Med Assoc J 17(1):54–59

Eugenin EA, Berman JW (2013) Cytochrome C dysregulation induced by HIV infection of astrocytes results in bystander apoptosis of uninfected astrocytes by an IP3 and calcium-dependent mechanism. J Neurochem 127(5):644–651

Fang J, Acheampong E, Dave R, Wang F, Mukhtar M, Pomerantz RJ (2005) The RNA helicase DDX1 is involved in restricted HIV-1 Rev function in human astrocytes. Virology 336(2): 299–307

Fok-Seang J, Miller RH (1992) Astrocyte precursors in neonatal rat spinal cord cultures. J Neurosci 12(7):2751–2764

Fu W, Shi D, Westaway D, Jhamandas JH (2015) Bioenergetic mechanisms in astrocytes may contribute to amyloid plaque deposition and toxicity. J Biol Chem

Gangwani MR, Noel RJ Jr, Shah A, Rivera-Amill V, Kumar A (2013) Human immunodeficiency virus type 1 viral protein R (Vpr) induces CCL5 expression in astrocytes via PI3 K and MAPK signaling pathways. J Neuroinflammation 10:136

Gao YJ, Zhang L, Ji RR (2010) Spinal injection of TNF-α-activated astrocytes produces persistent pain symptom mechanical allodynia by releasing monocyte chemoattractant protein-1. Glia 58 (15):1871–1880

Gard AL, Burrell MR, Pfeiffer SE, Rudge JS, Williams WC 2nd (1995) Astroglial control of oligodendrocyte survival mediated by PDGF and leukemia inhibitory factor-like protein. Development 121(7):2187–2197

Garwood CJ, Pooler AM, Atherton J, Hanger DP, Noble W (2011) Astrocytes are important mediators of Aβ-induced neurotoxicity and tau phosphorylation in primary culture. Cell Death Dis 2:e167

Gonzalez-Scarano F, Martin-Garcia J (2005) The neuropathogenesis of AIDS. Nat Rev Immunol 5(1):69–81

Gorry P, Purcell D, Howard J, McPhee D (1998) Restricted HIV-1 infection of human astrocytes: potential role of nef in the regulation of virus replication. J Neurovirol 4(4):377–386

Gorry PR, Howard JL, Churchill MJ, Anderson JL, Cunningham A, Adrian D, McPhee DA, Purcell DF (1999) Diminished production of human immunodeficiency virus type 1 in astrocytes results from inefficient translation of gag, env, and nef mRNAs despite efficient expression of Tat and Rev. J Virol 73(1):352–361

Gorry PR, Ong C, Thorpe J, Bannwarth S, Thompson KA, Gatignol A, Vesselingh SL, Purcell DF (2003) Astrocyte infection by HIV-1: mechanisms of restricted virus replication, and role in the pathogenesis of HIV-1-associated dementia. Curr HIV Res 1(4):463–473

Gray LR, Turville SG, Hitchen TL, Cheng WJ, Ellett AM, Salimi H, Roche MJ, Wesselingh SL, Gorry PR, Churchill MJ (2014) HIV-1 entry and trans-infection of astrocytes involves CD81 vesicles. PLoS ONE 9(2):e90620

Hao HN, Lyman WD (1999) HIV infection of fetal human astrocytes: the potential role of a receptor-mediated endocytic pathway. Brain Res 823(1–2):24–32

Hao HN, Chiu FC, Losev L, Weidenheim KM, Rashbaum WK, Lyman WD (1997) HIV infection of human fetal neural cells is mediated by gp120 binding to a cell membrane-associated molecule that is not CD4 nor galactocerebroside. Brain Res 764(1–2):149–157

Harouse JM, Bhat S, Spitalnik SL, Laughlin M, Stefano K, Silberberg DH, Gonzalez-Scarano F (1991a) Inhibition of entry of HIV-1 in neural cell lines by antibodies against galactosyl ceramide. Science 253(5017):320–323

Harouse JM, Laughlin MA, Pletcher C, Friedman HM, Gonzalez-Scarano F (1991b) Entry of human immunodeficiency virus-1 into glial cells proceeds via an alternate, efficient pathway. J Leukoc Biol 49(6):605–609

Hazleton JE, Berman JW, Eugenin EA (2012) Purinergic receptors are required for HIV-1 infection of primary human macrophages. J Immunol 188(9):4488–4495

Henderson LJ, Sharma A, Monaco MC, Major EO, Al-Harthi L (2012) Human immunodeficiency virus type 1 (HIV-1) transactivator of transcription through its intact core and cysteine-rich domains inhibits Wnt/β-catenin signaling in astrocytes: relevance to HIV neuropathogenesis. J Neurosci 32(46):16306–16313

Hernandez RS, Sarasa R, Toledano A, Badiola JJ, Monzon M (2014) Morphological approach to assess the involvement of astrocytes in prion propagation. Cell Tissue Res 358(1):57–63

Holley JE, Gveric D, Newcombe J, Cuzner ML, Gutowski NJ (2003) Astrocyte characterization in the multiple sclerosis glial scar. Neuropathol Appl Neurobiol 29(5):434–444

Hsiao HY, Chen YC, Chen HM, Tu PH, Chern Y (2013) A critical role of astrocyte-mediated nuclear factor-κB-dependent inflammation in Huntington's disease. Hum Mol Genet 22(9): 1826–1842

Ilie A, Weinstein E, Boucher A, McKinney RA, Orlowski J (2014) Impaired posttranslational processing and trafficking of an endosomal Na+/H+ exchanger NHE6 mutant (Delta(370)WST (372)) associated with X-linked intellectual disability and autism. Neurochem Int 73:192–203

Ishida Y, Nagai A, Kobayashi S, Kim SU (2006) Upregulation of protease-activated receptor-1 in astrocytes in Parkinson disease: astrocyte-mediated neuroprotection through increased levels of glutathione peroxidase. J Neuropathol Exp Neurol 65(1):66–77

Johnstone M, Gearing AJ, Miller KM (1999) A central role for astrocytes in the inflammatory response to β-amyloid; chemokines, cytokines and reactive oxygen species are produced. J Neuroimmunol 93(1–2):182–193

Kaul M, Garden GA, Lipton SA (2001) Pathways to neuronal injury and apoptosis in HIV-associated dementia. Nature 410(6831):988–994

Kessaris N, Pringle N, Richardson WD (2008) Specification of CNS glia from neural stem cells in the embryonic neuroepithelium. Philos Trans R Soc Lond B Biol Sci 363(1489):71–85

Kim HA, Jiang L, Madsen H, Parish CL, Massalas J, Smardencas A, O'Leary C, Gantois I, O'Tuathaigh C, Waddington JL, Ehrlich ME, Lawrence AJ, Drago J (2014) Resolving pathobiological mechanisms relating to Huntington disease: gait, balance, and involuntary movements in mice with targeted ablation of striatal D1 dopamine receptor cells. Neurobiol Dis 62:323–337

Kondapalli KC, Hack A, Schushan M, Landau M, Ben-Tal N, Rao R (2013) Functional evaluation of autism-associated mutations in NHE9. Nat Commun 4:2510

Kong Q, Takahashi K, Schulte D, Stouffer N, Lin Y, Lin CL (2012) Increased glial glutamate transporter EAAT2 expression reduces epileptogenic processes following pilocarpine-induced status epilepticus. Neurobiol Dis 47(2):145–154

Lee W, Reyes RC, Gottipati MK, Lewis K, Lesort M, Parpura V, Gray M (2013) Enhanced Ca(2 +)-dependent glutamate release from astrocytes of the BACHD Huntington's disease mouse model. Neurobiol Dis 58:192–199

Lievens JC, Woodman B, Mahal A, Spasic-Boscovic O, Samuel D, Kerkerian-Le Goff L, Bates GP (2001) Impaired glutamate uptake in the R6 Huntington's disease transgenic mice. Neurobiol Dis 8(5):807–821

Lim SP, Garzino-Demo A (2000) The human immunodeficiency virus type 1 Tat protein up-regulates the promoter activity of the beta-chemokine monocyte chemoattractant protein 1

in the human astrocytoma cell line U-87 MG: role of SP-1, AP-1, and NF-κB consensus sites. J Virol 74(4):1632–1640

Lim D, Ronco V, Grolla AA, Verkhratsky A, Genazzani AA (2014) Glial calcium signalling in Alzheimer's disease. Rev Physiol Biochem Pharmacol 167:45–65

Liu Y, Liu H, Kim BO, Gattone VH, Li J, Nath A, Blum J, He JJ (2004) CD4-independent infection of astrocytes by human immunodeficiency virus type 1: requirement for the human mannose receptor. J Virol 78(8):4120–4133

Lopes MW, Soares FM, de Mello N, Nunes JC, Cajado AG, de Brito D, de Cordova FM, da Cunha RM, Walz R, Leal RB (2013) Time-dependent modulation of AMPA receptor phosphorylation and mRNA expression of NMDA receptors and glial glutamate transporters in the rat hippocampus and cerebral cortex in a pilocarpine model of epilepsy. Exp Brain Res 226 (2):153–163

Ludvigsen A, Werner T, Gimbel W, Erfle V, Brack-Werner R (1996) Down-modulation of HIV-1 LTR activity by an extra-LTR nef gene fragment. Virology 216(1):245–251

Ma M, Geiger JD, Nath A (1994) Characterization of a novel binding site for the human immunodeficiency virus type 1 envelope protein gp120 on human fetal astrocytes. J Virol 68 (10):6824–6828

Mamik MK, Ghorpade A (2014) Chemokine CXCL8 promotes HIV-1 replication in human monocyte-derived macrophages and primary microglia via nuclear factor-κB pathway. PLoS ONE 9(3):e92145

Mengozzi M, De Filippi C, Transidico P, Biswas P, Cota M, Ghezzi S, Vicenzi E, Mantovani A, Sozzani S, Poli G (1999) Human immunodeficiency virus replication induces monocyte chemotactic protein-1 in human macrophages and U937 promonocytic cells. Blood 93 (6):1851–1857

Messam CA, Major EO (2000) Stages of restricted HIV-1 infection in astrocyte cultures derived from human fetal brain tissue. J Neurovirol 6(Suppl 1):S90–S94

Mishra MK, Kumawat KL, Basu A (2008) Japanese encephalitis virus differentially modulates the induction of multiple pro-inflammatory mediators in human astrocytoma and astroglioma cell-lines. Cell Biol Int 32(12):1506–1513

Mitterauer BJ (2013) Astrocyte mega-domain hypothesis of the autistic savantism. Med Hypotheses 80(1):17–22

Moon JY, Roh DH, Yoon SY, Choi SR, Kwon SG, Choi HS, Kang SY, Han HJ, Beitz AJ, Oh SB, Lee JH (2014) sigma1 receptors activate astrocytes via p38 MAPK phosphorylation leading to the development of mechanical allodynia in a mouse model of neuropathic pain. Br J Pharmacol 171(24):5881–5897

More SV, Kumar H, Kim IS, Song SY, Choi DK (2013) Cellular and molecular mediators of neuroinflammation in the pathogenesis of Parkinson's disease. Mediators Inflamm 2013:952375

Muratori C, Mangino G, Affabris E, Federico M (2010) Astrocytes contacting HIV-1-infected macrophages increase the release of CCL2 in response to the HIV-1-dependent enhancement of membrane-associated TNFα in macrophages. Glia 58(16):1893–1904

Nagatsu T, Mogi M, Ichinose H, Togari A (2000) Changes in cytokines and neurotrophins in Parkinson's disease. J Neural Transm Suppl (60):277–290

Nana AL, Kim EH, Thu DC, Oorschot DE, Tippett LJ, Hogg VM, Synek BJ, Roxburgh R, Waldvogel HJ, Faull RL (2014) Widespread heterogeneous neuronal loss across the cerebral cortex in Huntington's disease. J Huntingtons Dis 3(1):45–64

Narasipura SD, Kim S, Al-Harthi L (2014) Epigenetic regulation of HIV-1 latency in astrocytes. J Virol 88(5):3031–3038

Nath A, Hartloper V, Furer M, Fowke KR (1995) Infection of human fetal astrocytes with HIV-1: viral tropism and the role of cell to cell contact in viral transmission. J Neuropathol Exp Neurol 54(3):320–330

Neumann M, Felber BK, Kleinschmidt A, Froese B, Erfle V, Pavlakis GN, Brack-Werner R (1995) Restriction of human immunodeficiency virus type 1 production in a human

astrocytoma cell line is associated with a cellular block in Rev function. J Virol 69(4):2159–2167

Nookala AR, Shah A, Noel RJ, Kumar A (2013) HIV-1 Tat-mediated induction of CCL5 in astrocytes involves NF-κB, AP-1, C/EBPα and C/EBPγ transcription factors and JAK, PI3K/Akt and p38 MAPK signaling pathways. PLoS ONE 8(11):e78855

Oberheim NA, Goldman SA, Nedergaard M (2012) Heterogeneity of astrocytic form and function. Methods Mol Biol 814:23–45

Ojeda D, Lopez-Costa JJ, Sede M, Lopez EM, Berria MI, Quarleri J (2014) Increased in vitro glial fibrillary acidic protein expression, telomerase activity, and telomere length after productive human immunodeficiency virus-1 infection in murine astrocytes. J Neurosci Res 92(2):267–274

Orellana JA, Saez JC, Bennett MV, Berman JW, Morgello S, Eugenin EA (2014) HIV increases the release of dickkopf-1 protein from human astrocytes by a Cx43 hemichannel-dependent mechanism. J Neurochem 128(5):752–763

Oseki KT, Monteforte PT, Pereira GJ, Hirata H, Ureshino RP, Bincoletto C, Hsu YT, Smaili SS (2014) Apoptosis induced by Abeta25–35 peptide is Ca(2+)—IP3 signaling-dependent in murine astrocytes. Eur J Neurosci 40(3):2471–2478

Pant M, Garg P, Seth P (2012) Central Nervous System Infection by HIV-1: special emphasis to NeuroAIDS in India. Proc Natl Acad Sci USA 82(1):81–94

Parratt JD, Prineas JW (2010) Neuromyelitis optica: a demyelinating disease characterized by acute destruction and regeneration of perivascular astrocytes. Mult Scler 16(10):1156–1172

Perea G, Navarrete M, Araque A (2009) Tripartite synapses: astrocytes process and control synaptic information. Trends Neurosci 32(8):421–431

Persichini T, Mastrantonio R, Del Matto S, Palomba L, Cantoni O, Colasanti M (2014) The role of arachidonic acid in the regulation of nitric oxide synthase isoforms by HIV gp120 protein in astroglial cells. Free Radic Biol Med 74:14–20

Perucho J, Casarejos MJ, Gomez A, Ruiz C, Fernandez-Estevez MA, Munoz MP, de Yebenes JG, Mena MA (2013) Striatal infusion of glial conditioned medium diminishes huntingt in pathology in r6/1 mice. PLoS ONE 8(9):e73120

Pfrieger FW, Barres BA (1997) Synaptic efficacy enhanced by glial cells in vitro. Science 277 (5332):1684–1687

Phillips EC, Croft CL, Kurbatskaya K, O'Neill MJ, Hutton ML, Hanger DP, Garwood CJ, Noble W (2014) Astrocytes and neuroinflammation in Alzheimer's disease. Biochem Soc Trans 42(5):1321–1325

Picard L, Simmons G, Power CA, Meyer A, Weiss RA, Clapham PR (1997) Multiple extracellular domains of CCR-5 contribute to human immunodeficiency virus type 1 entry and fusion. J Virol 71(7):5003–5011

Rebec GV (2013) Dysregulation of corticostriatal ascorbate release and glutamate uptake in transgenic models of Huntington's disease. Antioxid Redox Signal 19(17):2115–2128

Repunte-Canonigo V, Lefebvre C, George O, Kawamura T, Morales M, Koob GF, Califano A, Masliah E, Sanna PP (2014) Gene expression changes consistent with neuroAIDS and impaired working memory in HIV-1 transgenic rats. Mol Neurodegener 9:26

Roberts ES, Chana G, Nguyen TB, Perera G, Landau S, Rabe-Hesketh S, Glass JD, McArthur J, Everall IP (2013) The spatial relationship between neurons and astrocytes in HIV-associated dementia. J Neurovirol 19(2):123–130

Robertson JM (2014) Astrocytes and the evolution of the human brain. Med Hypotheses 82 (2):236–239

Ruiz C, Casarejos MJ, Gomez A, Solano R, de Yebenes JG, Mena MA (2012) Protection by glia-conditioned medium in a cell model of Huntington disease. PLoS Curr 4:e4fbca54a2028b

Saura J, Pares M, Bove J, Pezzi S, Alberch J, Marin C, Tolosa E, Marti MJ (2003) Intranigral infusion of interleukin-1β activates astrocytes and protects from subsequent 6-hydroxydopamine neurotoxicity. J Neurochem 85(3):651–661

Schweighardt B, Atwood WJ (2001) HIV type 1 infection of human astrocytes is restricted by inefficient viral entry. AIDS Res Hum Retroviruses 17(12):1133–1142

Seror C, Melki MT, Subra F, Raza SQ, Bras M, Saidi H, Nardacci R, Voisin L, Paoletti A, Law F, Martins I, Amendola A, Abdul-Sater AA, Ciccosanti F, Delelis O, Niedergang F, Thierry S, Said-Sadier N, Lamaze C, Metivier D, Estaquier J, Fimia GM, Falasca L, Casetti R, Modjtahedi N, Kanellopoulos J, Mouscadet JF, Ojcius DM, Piacentini M, Gougeon ML, Kroemer G, Perfettini JL (2011) Extracellular ATP acts on P2Y2 purinergic receptors to facilitate HIV-1 infection. J Exp Med 208(9):1823–1834

Serrano-Pozo A, Gomez-Isla T, Growdon JH, Frosch MP, Hyman BT (2013) A phenotypic change but not proliferation underlies glial responses in Alzheimer disease. Am J Pathol 182(6): 2332–2344

Silver J, Miller JH (2004) Regeneration beyond the glial scar. Nat Rev Neurosci 5(2):146–156

Sofroniew MV (2009) Molecular dissection of reactive astrogliosis and glial scar formation. Trends Neurosci 32(12):638–647

Somjen GG (1988) Nervenkitt: notes on the history of the concept of neuroglia. Glia 1(1):2–9

Soulie C, Calvez V, Marcelin AG (2012) Coreceptor usage in different reservoirs. Curr Opin HIV AIDS 7(5):450–455

Takahashi K, Wesselingh SL, Griffin DE, McArthur JC, Johnson RT, Glass JD (1996) Localization of HIV-1 in human brain using polymerase chain reaction/in situ hybridization and immunocytochemistry. Ann Neurol 39(6):705–711

Tavazzi E, Morrison D, Sullivan P, Morgello S, Fischer T (2014) Brain inflammation is a common feature of HIV-infected patients without HIV encephalitis or productive brain infection. Curr HIV Res 12(2):97–110

Tewari M, Seth P (2015) Emerging role of P2X7 receptors in CNS health and disease. Ageing Res Rev 24(Pt B):328–342

Tewari M, Monika Varghse RK, Menon M, Seth P (2015) Astrocytes mediate HIV-1 Tat-induced neuronal damage via ligand-gated ion channel P2X7R. J Neurochem 132(4):464–476

Theofilas P, Brar S, Stewart KA, Shen HY, Sandau US, Poulsen D, Boison D (2011) Adenosine kinase as a target for therapeutic antisense strategies in epilepsy. Epilepsia 52(3):589–601

Ton H, Xiong H (2013) Astrocyte dysfunctions and HIV-1 neurotoxicity. J AIDS Clin Res 4 (11):255

Tong X, Ao Y, Faas GC, Nwaobi SE, Xu J, Haustein MD, Anderson MA, Mody I, Olsen ML, Sofroniew MV, Khakh BS (2014) Astrocyte Kir4.1 ion channel deficits contribute to neuronal dysfunction in Huntington's disease model mice. Nat Neurosci 17(5):694–703

Tornatore C, Meyers K, Atwood W, Conant K, Major E (1994) Temporal patterns of human immunodeficiency virus type 1 transcripts in human fetal astrocytes. J Virol 68(1):93–102

Torres L, Noel RJ Jr (2014) Astrocytic expression of HIV-1 viral protein R in the hippocampus causes chromatolysis, synaptic loss and memory impairment. J Neuroinflammation 11:53

Tung WH, Tsai HW, Lee IT, Hsieh HL, Chen WJ, Chen YL, Yang CM (2010) Japanese encephalitis virus induces matrix metalloproteinase-9 in rat brain astrocytes via NF-κB signalling dependent on MAPKs and reactive oxygen species. Br J Pharmacol 161(7):1566–1583

van der Hel WS, Notenboom RG, Bos IW, van Rijen PC, van Veelen CW, de Graan PN (2005) Reduced glutamine synthetase in hippocampal areas with neuron loss in temporal lobe epilepsy. Neurology 64(2):326–333

Voskuhl RR, Peterson RS, Song B, Ao Y, Morales LB, Tiwari-Woodruff S, Sofroniew MV (2009) Reactive astrocytes form scar-like perivascular barriers to leukocytes during adaptive immune inflammation of the CNS. J Neurosci 29(37):11511–11522

Walz W, Lang MK (1998) Immunocytochemical evidence for a distinct GFAP-negative subpopulation of astrocytes in the adult rat hippocampus. Neurosci Lett 257(3):127–130

Wang Z, Pekarskaya O, Bencheikh M, Chao W, Gelbard HA, Ghorpade A, Rothstein JD, Volsky DJ (2003) Reduced expression of glutamate transporter EAAT2 and impaired glutamate transport in human primary astrocytes exposed to HIV-1 or gp120. Virology 312 (1):60–73

Wang K, Zhang J, Xu Y, Ren K, Xie WL, Yan YE, Zhang BY, Shi Q, Liu Y, Dong XP (2013) Abnormally upregulated αB-crystallin was highly coincidental with the astrogliosis in the

brains of scrapie-infected hamsters and human patients with prion diseases. J Mol Neurosci 51 (3):734–748

Wee Yong V (2010) Inflammation in neurological disorders: a help or a hindrance? Neuroscientist 16(4):408–420

Wojtowicz AM, Dvorzhak A, Semtner M, Grantyn R (2013) Reduced tonic inhibition in striatal output neurons from Huntington mice due to loss of astrocytic GABA release through GAT-3. Front Neural Circuits 7:188

Wu Y, Chen Q, Peng H, Dou H, Zhou Y, Huang Y, Zheng JC (2012) Directed migration of human neural progenitor cells to interleukin-1β is promoted by chemokines stromal cell-derived factor-1 and monocyte chemotactic factor-1 in mouse brains. Transl Neurodegener 1(1):15

Xanthos DN, Sandkuhler J (2014) Neurogenic neuroinflammation: inflammatory CNS reactions in response to neuronal activity. Nat Rev Neurosci 15(1):43–53

Yang CM, Lin CC, Lee IT, Lin YH, Yang CM, Chen WJ, Jou MJ, Hsiao LD (2012) Japanese encephalitis virus induces matrix metalloproteinase-9 expression via a ROS/c-Src/PDGFR/PI3K/Akt/MAPKs-dependent AP-1 pathway in rat brain astrocytes. J Neuroinflammation 9:12

Zhang Y, Barres BA (2013) A smarter mouse with human astrocytes. BioEssays 35(10):876–880

Zheng WH, Bastianetto S, Mennicken F, Ma W, Kar S (2002) Amyloid β peptide induces tau phosphorylation and loss of cholinergic neurons in rat primary septal cultures. Neuroscience 115(1):201–211

Zheng JC, Huang Y, Tang K, Cui M, Niemann D, Lopez A, Morgello S, Chen S (2008) HIV-1-infected and/or immune-activated macrophages regulate astrocyte CXCL8 production through IL-1β and TNF-α: involvement of mitogen-activated protein kinases and protein kinase R. J Neuroimmunol 200(1–2):100–110

Zhou BY, Liu Y, Kim B, Xiao Y, He JJ (2004) Astrocyte activation and dysfunction and neuron death by HIV-1 Tat expression in astrocytes. Mol Cell Neurosci 27(3):296–305

Zhuang K, Leda AR, Tsai L, Knight H, Harbison C, Gettie A, Blanchard J, Westmoreland S, Cheng-Mayer C (2014) Emergence of CD4 independence envelopes and astrocyte infection in R5 simian-human immunodeficiency virus model of encephalitis. J Virol 88(15):8407–8420

Infections and Inflammation in the Brain and Spinal Cord: A Dangerous Liaison

4

Kallol Dutta, Sourish Ghosh and Anirban Basu

Abstract

Unicellular microorganisms developed on Earth approximately 3–4 billion years ago, and since the evolution of modern man (Homo sapiens) about 200,000 years ago, there been a close interaction between them which has not always been beneficial for the host. Diseases resulting from microbial infections have for long been a bane of human society and with the discovery of viruses and prions, the array of infectious agents has further widened. An infectious agent may target either specific or multiple cell types, organs, or organ systems. As a response to the infections, the body 'fights back' with its own set of defenders, i.e., the immune system. In this chapter, we focus on the various types of infections that can affect our central nervous system (CNS), arguably the most complicated organization of matter that we have the knowledge about, and the immune responses against them. The CNS had been long considered to be 'immune-privileged' due to its apparent separation from the rest of the body by specialized barriers. However, these barriers have been found to be dynamic in nature, regulating the flow of material across them. Also, the cells in the brain are themselves equipped with various mechanisms to detect the presence of the infectious agents and respond accordingly to contain or neutralize the threat posed by them. The response mechanism often results in a condition termed as inflammation, which in itself is a complex process involving multiple mediators. Inflammation is often referred as 'double-edged' sword as, if un-controlled, it results in severe damage to the host itself. In a non-regenerating organ system

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such as the CNS this has detrimental ramifications that are commonly termed as neurodegeneration. Thus, in this chapter we have strived to provide the reader not only on the different types of pathogens infecting human CNS but also the immune response associated with them. More specifically, we have tried to provide information about how these pathogens are detected/recognized by cells of the CNS, how the cells respond following the detection, and how is the response regulated (if there is any regulation at all). Even though we have separated the response against bacteria, viruses, fungi, parasites, and prions in different sections, the readers will no doubt notice a certain degree of overlap in the mechanism of response of these different types of pathogens indicating the plasticity of the immune system. However, there is some uniqueness associated with each pathogen infection which makes the immune systems task even more difficult. In this chapter, we have strived to incorporate multitude of information in a concise manner; however, we do stress that this is by no means all-encompassing. Hence, the readers are encouraged to follow-up any particular point of interest from the cited publications.

4.1 The Brain and the Barriers

With about 86 billion neurons (Herculano-Houzel 2009) and innumerous glial cells, the brain–spinal cord unit, is arguably the most complex organ system of the human body. The cellular content of the CNS is varied. The key cell-type is the neuron although this only constitutes around 5 % of the cellular total and can vary between regions of the brain. In addition to neurons, there are the glial cells that support neurons through a range of functional phenotypes. Astrocytes assist in sustaining neuronal metabolism and neurotransmission. Oligodendrocytes have elongated processes that surround axons and produce myelin, which effectively insulates axonal processes and enables efficient electrical transmission along axons. Finally, microglia provide an immune function within the CNS, acting as the resident macrophage population removing dead cells, and are particularly important in the development of the fetal brain.

Protected by the calvarium, dura and blood–brain barrier (BBB), or the blood–spinal cord barrier (BSCB), the brain or the central nervous system (CNS) as a whole, is quite well guarded. The BBB is a dynamic multicellular interface of glial and vascular cells that tightly restricts the movement of solutes and cells in the circulation into the CNS parenchyma. The BBB, that is present over 99 % of the brain vasculature, also protects the CNS from potential pathogens or other harmful substances while regulating transport of essential molecules and maintaining a stable environment. It is composed of a network of vessels that form structural and chemical barriers between the brain and systemic circulations. The neurons, the extracellular matrix, and non-neuronal cells including astrocytes, pericytes, and

vascular endothelial cells function as a neurovascular unit to regulate BBB permeability and maintain the integrity and function of the CNS. The BSCB is the functional equivalent of the BBB in the sense of providing a specialized microenvironment for the cellular constituents of the spinal cord. Even if intuitively the BSCB could be considered as the morphological extension of the BBB into the spinal cord, evidence suggests that this is not so. The BSCB shares the same principal building blocks with the BBB; nevertheless, it seems that morphological and functional differences may exist between them (Fig. 4.1). However, there are

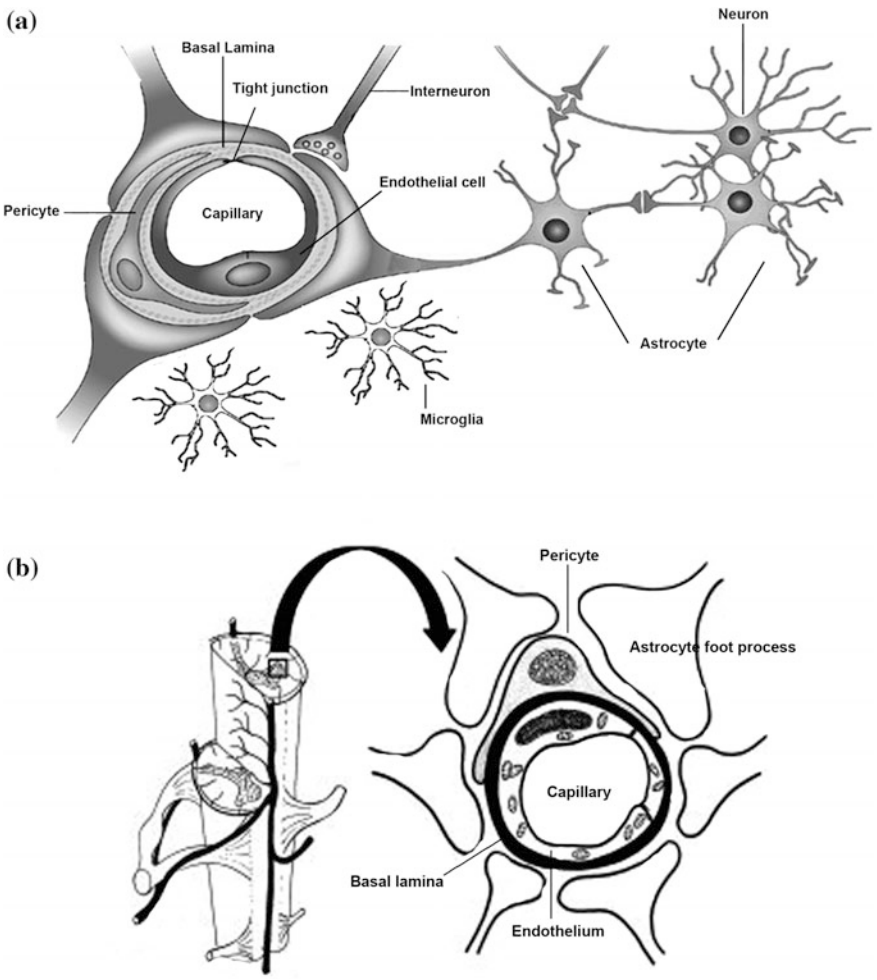


Fig. 4.1 Structural comparison of the BBB and BSCB. The blood brain barrier (**a**) and blood-spinal cord barrier (**b**) shares the same principal building blocks but may differ morphologically or functionally. There are glycogen deposits only on BSCB microvessels; the BSCB also has decreased tight junction (ZO-1, occludin) and adherence junction protein (VE-cadherin, β-catenin) expression than the BBB

regions in the CNS, where the BBB is not well defined. These regions (commonly termed as the circumventricular organs see Box 1) facilitate the passage of larger molecules into the CNS that would normally be screened by the BBB. The downside of this is that these regions allow unhindered access to certain pathogens into the CNS, as we see later in this chapter.

Box 1: Circumventricular Organs (CVOs)

- Pineal body.
- Neurohypophysis (posterior pituitary).
- Area postrema.
- Subfornical organ.
- Vascular organ of the lamina terminalis.
- Median eminence.

The cerebrospinal fluid (CSF) that covers the brain and the spinal cord is a good indicator of infection in the CNS. The CSF, previously regarded as an ultrafiltrate of plasma, is, in fact, actively produced by the secretory epithelium of the choroid plexus. CSF circulates from the ventricles through the subarachnoid space, which is located between the arachnoid and the pial membranes, and is mainly resorbed into venous blood through the arachnoid villi, which are "outpouchings" of the arachnoid membrane that extend into the venous sinuses of the cerebral hemispheres. Formation and absorption of CSF are extensive processes—the human CSF volume turns over approximately four times each day (Strazielle and Ghersi-Egea 2000). Components of the CSF are major indicators of neuroinflammatory reactions. The normal ranges of these components are shown in Tables 4.1 and 4.2 give an idea how these components are altered in disease conditions.

In the past, CNS was considered an immune-privileged site, a term first coined by Peter Medawar, resulting from the observation that allografts placed in certain locations, such as the eye or brain was not rejected by the immune system with the rapidity observed in other organs (Barker and Billingham 1977). The cause of this privilege has been attributed almost exclusively to the BBB. In addition, there are no lymphatic vessels within the parenchyma of the brain that would provide a conduit for antigen presenting dendritic-cells to move directly to lymphoid tissue. However, it would be wrong to assume that the absence of comparable cells and structures found in the periphery represents an absence of these functions. Numerous studies have now identified the multiple mechanisms of immune surveillance that support the CNS, initiating both protective and damaging immune responses. Likewise there are now defined routes by which immune cells can enter the CNS, particularly in response to infection (Ransohoff et al. 2003). Within the

Table 4.1 Normal CSF values of importance in infectious diseases of the nervous system (Hasbun 2014)

Parameter	Adult	Term infants	Premature infants
Cell count (per cubic millimeter)	<5	9[a]	9[a]
Percent polymorphonuclear lymphocytes	0[b]	61[b]	57[b]
Protein (mg/dL) (lumber)			
Mean	30	90	115
Range	9–58	20–170	65–150
Glucose (mg/dL)			
Mean	62	52	50
Range	45–80	34–119	24–63
CSF blood-glucose ratio			
Mean	0.6	0.81	0.74
Range	0.5–0.8	0.44–2.4	0.55–1.55

[a]Represents mean value. The range of cell counts in normal neonates is 0–32 cells/mm^3 and in premature infants it is 0–29 cells/mm^3

[b]Rare polymorphonuclear lymphocytes may be seen in cytocentrifuged samples of CSF from normal adults if CSF leucocyte count falls below 4 cells/mm^3 or less even if protein or glucose levels are normal

Table 4.2 CSF components in CNS infections

	Purulent meningitis (acute bacterial)	Viral meningo-encephalitis	Granulomatous meningitis (tuberculosis, fungal meningitis, sarcoid, syphilis, listeria, brucella, etc.)
WBC's	More than 1000/cu mm	Less than 500/cu mm	Less than 200/cu mm
Protein	High	Normal or slightly elevated	High
Glucose	Low (often less than 20 mg%)	Normal	Low (rarely as low as in bacterial meningitis)

Adapted from: http://www.dartmouth.edu/~dons/figures/chapt_25/Table_25-5.htm

CNS the immune mechanisms are mainly coordinated by the glial cells but neurons themselves were classically believed to be non-immunogenic. However, recent studies are producing results which indicate that following pathogenic (or antigenic) challenge, neurons also activate responses that coordinate defense against the insult and facilitate antigen clearance. It has been shown that neurons possess functional pattern recognition receptors (PRRs) (Peltier et al. 2010) which are necessary for pathogen detection and activation of a protein signaling cascade that ultimately leads to the generation of immune (innate) responses. The appropriate and timely orchestration of these events is critical in responding to viral infection particularly in the early chemokine and cytokine signals that trigger the innate immune response.

Specific anatomical features seem to have altered the nature of immune and inflammatory responses in the CNS. Firstly, as the CNS is encased in rigid bone and is covered by an inelastic dural lining, the total volume of the CNS is not flexible. An increase in the volume of extracellular fluid, as commonly observed with inflammatory swelling, would increase tissue pressure, and subsequently oppose arterial influx, thereby threatening secondary ischaemic damage. Secondly, the function of the CNS depends on the viability of neurons, which are mainly post-mitotic and non-regenerating. Given these constraints, it is perhaps predictable that the CNS has a limited capacity for inflammatory and immune reactivity (Perry and Andersson 1992). In addition to the absence of tissue lymphatics, there is a low level of expression of MHC class II molecules in the intact human brain (Ebner et al. 2013) and it is restricted to reactive microglia and phagocytic macrophages cells with limited capacity for antigen presentation to naive cells; no resident dendritic-cell population has been detected in the brain parenchyma. It is not surprising, therefore, that immune and inflammatory responses in the CNS are different from those in other internal organs. Thus, the claim that the CNS is a site of immune privilege has now been modified, and it is now proposed that the CNS is a site of selective and modified immune reactivity.

4.2 Meningitis, Encephalitis, Abscesses and Myelitis—Outcome of Central Nervous System Infections

The organisms that are involved in CNS infection could be bacterial, viral, fungal or parasitic in nature. Additionally, prions represent an unusual class of infectious agent that can damage the brain. A role for innate immunity in inflammation of CNS is being increasingly evidenced. Cells of the CNS such as neurons, astrocytes, and microglia along with pattern recognition receptors, cytokines, chemokines, complement, peripheral immune cells, and signal pathways form the basis for neuroinflammation. Local synthesis of a number of innate immune humoral factors within CNS offers an opportunity for therapeutic intervention. Furthermore, excessive activation of immune system is thought to be destructive to tissues whereas, simultaneously, it opens up possibilities to harness this activation in a controlled manner to obtain desired therapeutic or preventive strategies in CNS diseases. A detailed understanding of the processes and mechanisms involved in the etiopathogenesis of CNS diseases as well as normal functioning of CNS immunity is essential. Infections of CNS usually result in meningitis, encephalitis, and/or abscesses.

In order to better understand meningitis, one must first have the knowledge about the structures surrounding the brain (see Box 2). Beneath the inner surface of the skull, the brain is surrounded by a membranous covering known as the meninges. A fluid known as cerebrospinal fluid (CSF) circulates around the brain and serves to cushion the brain against injury. Meningitis is an inflammation of the meninges due to infection (see Box 3). It occurs when a foreign pathogen invades

the subarachnoid space and populates the CSF. The foreign microorganisms can either be bacteria or viruses. Accordingly, meningitis can be classified as either bacterial or viral or in rare cases may result due to fungal infections. Generally, bacterial meningitis is more dangerous than the viral form and can constitute a medical emergency. Bacterial meningitis is an infection of the pia and arachnoid and adjacent cerebrospinal fluid. The outer arachnoid serves as a barrier to the spread of infection, but involvement of the subdural space can occur, resulting in a subdural empyema. This complication is more common in children than adults. The most common organisms involved are *Hemophilus influenza, Neisseria meningitides* (Meningococcus), and *Streptococcus pneumoniae*. Patients present with fever, headache, seizures, altered consciousness, and neck stiffness.

Box 2: The Meninges

The meninges consist of three layers: the dura mater, the arachnoid mater, and the pia mater

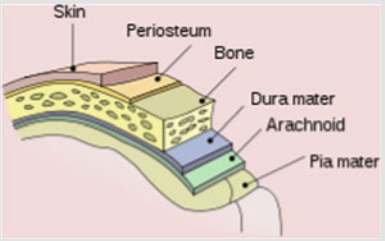


Image from: http://en.wikipedia.org/wiki/Meninges

Box 3: Complications of Meningitis

- Communicating hydrocephalus
- Loculated CSF collections
- Subdural effusion/empyema
- Cerebral infraction
- Dural sinus thrombophlebitis

Encephalitis refers to a diffuse parenchymal inflammation of the brain that occurs when a virus directly infects the brain or when a virus, vaccine, or something else triggers inflammation. The spinal cord may also be involved, resulting in a disorder called encephalomyelitis. Encephalitis due to bacterial infection is usually as part of bacterial meningitis (meningoencephalitis). Protozoa, causing cerebral toxoplasmosis, or causing cerebral malaria, can also infect the brain and cause

encephalitis. Symptoms of encephalitis start as nausea, vomiting, diarrhea, or abdominal pain and may gradually develop into flu-like features such as cough, fever, a sore throat, a runny nose, swollen lymph nodes, and sensorial impairment is an essential feature of encephalitis. In severe cases there could be personality changes or confusion, seizures, paralysis or numbness, and sleepiness that can progress to coma and death. Acute encephalitis of the non-herpetic type presents with signs and symptoms similar to meningitis, but with the added features of any combination of convulsions, delirium, altered consciousness, aphasia, hemiparesis, ataxia, ocular palsies, and facial weakness.

Post-infectious encephalitis, also known as acute disseminated encephalomyelitis (ADEM), is an acute demyelinating disease thought to represent an immune-mediated complication of infection, rather than a direct viral infection of the CNS. The clinical presentation is one of confusion, seizures, headaches, and fever. Ataxia may occur. Spinal cord involvement may lead to paraplegia or quadriplegia. The most common viruses implicated are measles and chicken pox. It occasionally is seen after vaccination for rabies or smallpox or following nondescript respiratory infections. MR demonstrates lesions in the white matter of the cerebrum, cerebellum and brainstem, often while CT is normal or non-diagnostic. The lesions may be patchy and involve the subcortical white matter. Involvement of the deep gray matter has also been reported. Recovery following the encephalitis is varied. Many people come through the illness with little or no difficulties, yet a plethora of manifestations may affect other cases as shown in Fig. 4.2.

A brain abscess is a circumscribed region of infection within the substance of the brain. The abscess is initially characterized by an area of necrotic brain tissue surrounded by a zone of cerebritis (local inflammation of brain cells). As the abscess develops, the necrotic area becomes filled with pus and a ring of cells surrounds the area. A mature abscess is characterized by an encapsulated necrotic puss-filled region of brain tissue, surrounded by an area of cerebritis.

A brain abscess forms as the result of the spread of an infection into brain tissue from elsewhere. There are three possible origins of this infection:

1. An abscess most commonly arises via the direct extension into the skull of a local infection in the paranasal sinuses or in the middle ear. In India it is mostly secondary to middle ear infection, while in the west it is generally an extension of sinusitis.
2. Microorganisms can also be spread by the blood during a systemic infection. In this case, bacteria are carried to the site of abscess from a distant source, typically the lungs, mouth, or heart valves. Under these circumstances, there may be multiple abscesses in the brain.
3. Lastly, a brain abscess can result from a compound head trauma. An infection can arise from a wound penetrating the skull. In this case inoculation with bacteria occurs from infected bone fragments or debris from the penetrating instrument.

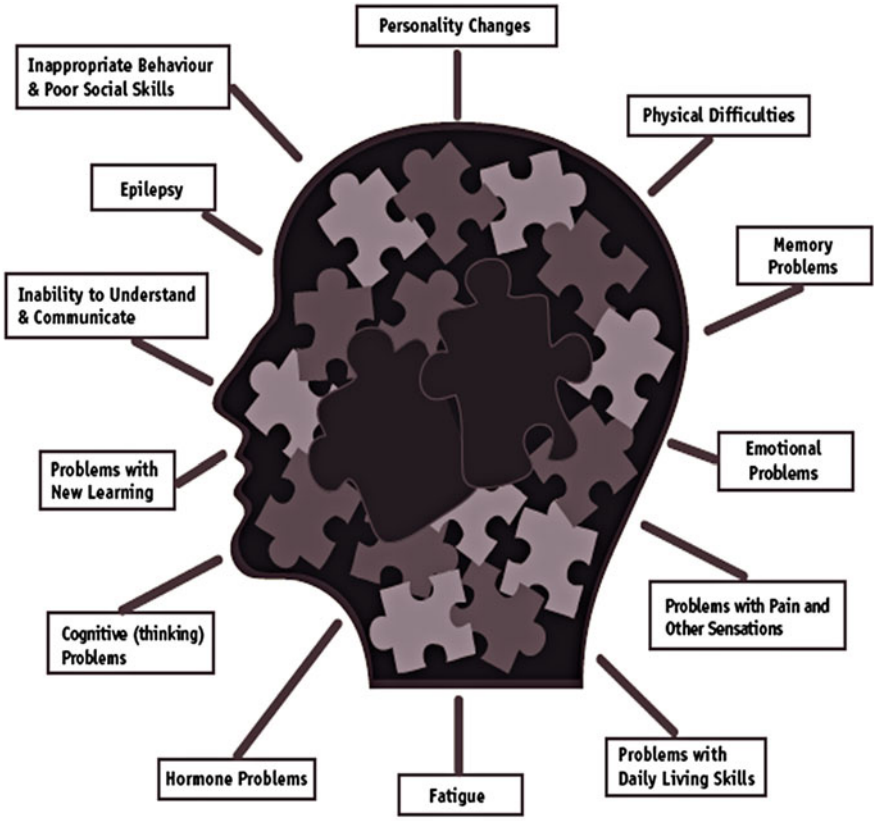


Fig. 4.2 Long-term problems associated with encephalitis. Encephalitis affects each person differently depending on the brain regions affected and the type of encephalitis, in addition to an individual's support system and access to treatment. The long term effects are mostly cognitive impairment affecting attention, memory, language, problem solving, decision making, planning, and organization apart from actual physical problems, mostly characterized by motor dysfunctions

Symptoms resulting from a brain abscess depend on the size and the location of the infection. Only 50 % of patients with a brain abscess present with a fever and, when present, fever is often chronic low-grade. A brain abscess usually presents with symptoms typical of any space-occupying mass within the substance of the brain, i.e., increased intracranial pressure, seizures, and a focal neurological deficit. The commonly observed deficits include weakness on one side of the body (hemiparesis), impaired speech production (dysphasia), visual field deficits, and an inability to smoothly coordinate muscle movements, such as during walking (ataxia).

The term myelitis refers to inflammation of the spinal cord. Transverse myelitis is a neurological disorder caused by inflammation across spinal cord at one level, or a segment. Inflammation can damage or destroy myelin leaving a scar that interrupt communications between in the spinal cord and the rest of the body. This inflammation can be due to infections or as autoimmune disorder. Suspected infectious agents causing myelitis include varicella zoster, herpes simplex, cytomegalovirus, Epstein-Barr, influenza, echovirus, HIV, hepatitis A, and rubella. Bacterial skin infections, middle-ear infections (otitis media), and Mycoplasma pneumoniae (bacterial pneumonia) have also been associated with the condition (Cree 2014).

4.3 Bacterial Infections of the CNS

Bacterial infections of the CNS are life-threatening conditions resulting commonly in meningitis, with a high mortality rate. Highly immunogenic substances such as cell wall fragments, peptidoglycans, or lipoteichoic acid (from Gram-positive bacteria) or lipopolysaccharide (from Gram-negative bacteria) are released post-replication of these microorganisms resulting in a severe inflammatory response in he host—(Sellner et al. 2010). There are several pathogens that can cause bacterial meningitis and age seems to be an important determinant for some types of bacterial infection as shown in Table 4.3.

Apart from the ones listed in the Table 4.3, there are other bacteria that also have the capacity to infect the CNS causing meningitis or encephalomeningitis. CNS tuberculosis (most prominently caused by Mycobacterium tuberculosis) is one of the most devastating clinical manifestations of tuberculosis (TB). CNS involvement is noted in 5–10 % of extrapulmonary TB cases, and accounts for approximately 1 % of all TB cases (Cherian and Thomas 2011). It carries a high mortality and a distressing level of neurological morbidity, and disproportionately afflicts children and immune-compromised subjects such as individuals suffering from human immunodeficiency virus (HIV) infections (Woldeamanuel and Girma 2014). CNS tuberculosis manifests itself primarily as tuberculous meningitis and less commonly

Table 4.3 Common causes of bacterial meningitis across various age groups

Age groups	Causes
Newborns	Group B *Streptococcus, Escherichia coli, Listeria monocytogenes*
Infants and children	*Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae* type b
Adolescents and young adults	*Neisseria meningitidis, Streptococcus pneumoniae*
Older adults	*Streptococcus pneumoniae, Neisseria meningitidis, Listeria monocytogenes*

Accessed and adapted from: http://www.cdc.gov/meningitis/bacterial.html

as tubercular encephalitis, intracranial tuberculoma, or a tuberculous brain abscess (see Box 4). It has been suggested that CNS tuberculosis develops in two stages. Initially small tuberculous lesions (Rich's foci) develop in the CNS, either during the stage of bacteraemia of the primary tuberculous infection or shortly afterwards. These initial tuberculous lesions may be in the meninges, the subpial or subependymal surface of the brain or the spinal cord, and may remain dormant for years after initial infection. The location of these foci and the capacity to control them ultimately determine which form of CNS tuberculosis occurs. Rupture or growth of one or more of these small tuberculous lesions produces development of CNS tuberculosis which manifests itself primarily as tuberculous meningitis (TBM) and less commonly as tubercular encephalitis, intracranial tuberculoma, or a tuberculous brain abscess (Rich and McCordock 1933; Berger 1994; Rom and Garay 2004).

Box 4: Classification of CNS Tuberculosis (Adapted From Cherian and Thomas 2011)

Intracranial

- Tuberculous meningitis (TBM)
- Tuberculous encephalopathy
- Tuberculous vasculopathy
- CNS tuberculoma (single or multiple)
- Tuberculous Brain Abscess

Spinal

- Pott's spine and Pott's paraplegia
- Non-osseous spinal tuberculoma
- Spinal meningitis

Staphylococcal meningitis is rare, can be community- or hospital-acquired, and usually results in severe disease. Staphylococcal meningitis is caused by either *Staphylococcus aureus* or *Staphylococcus epidermidis* with reported cases of *S. aureus*-associated meningitis and brain abscesses increased in recent years (Pedersen et al. 2006). *S. aureus* has been demonstrated to efficiently adhere to and invade human brain microvascular endothelial cells by means of lipoteichoic acid, thereby gaining access to the CNS (Sheen et al. 2010). *Borrelia burgdorferi*, the cause of **Lyme disease**, is a tick-borne spirochete associated with chronic meningitis or meningoencephalitis, and cranial or peripheral neuropathy. Meningitis occurs in the earlier stages of disease from direct spirochetal invasion of the CSF. Multifocal white matter lesions from the encephalitis can mimic multiple sclerosis

(Bockenstedt and Wormser 2014). **Sarcoidosis** is a granulomatous inflammatory disease of unknown etiology affecting multiple organs in the body, but mostly the lungs and lymph glands. It has been suggested that bacteria of a variety called cell wall deficient (CWD), or L-Forms, or coccoid forms may be involved in the development of this disease (Cantwell 1982; Almenoff et al. 1996; Saidha et al. 2012). However, it is probable that microbes are a likely trigger (but not as an infection) in a genetically predisposed individual and that this initial event culminates in the sarcoidosis granulomatous response (du Bois et al. 2003). In approximately 5 % of cases, the CNS is involved as a granulomatous infiltration of the meninges and underlying parenchyma, most notably at the base of the brain. It may also affect cranial or peripheral nerves as isolated disease. Cranial nerve palsies, chronic meningitis, and hypothalamic—pituitary dysfunction are frequent manifestations. A particularly interesting form of meningeal sarcoid results in thick meningeal plaques, often over the convexities mimicking meningiomas.

4.3.1 Bacterial Entry into the CNS

Colonization of the mucosal membranes is an important feature of most pathogens causing community-acquired meningitis. After colonization, bacteremia allows the microbes to reach the BBB and enter the CNS. A high load of bacteria circulating in the blood is thought to be necessary for the invasion of the CNS, and is therefore a risk factor for developing meningitis. Bacteria can cross the BBB either by transcellular migration or para-cellular migration and/or by "hitch-hiking" inside infected macrophages. Using transcellular migration, pathogens can cross the BBB without any evidence of intercellular tight-junction disruption or detection of microorganisms between endothelial cells (Kim 2008). *Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Escherichia coli,* and *Neisseria meningitidis* reach the CNS through this mechanism. The paracellular traversal mechanism involves the penetration of the pathogen between barrier cells, with or without evidence of tight-junction disruption. Borrelia sp and Treponema pallidum cross the BBB through this mechanism. Other pathogens can cross the BBB using macrophages as 'Trojan horses' (Lopez-Castejon et al. 2012). *Mycobacterium tuberculosis*, residing in the phagosomes of macrophages, (Bobadilla et al. 2013) may utilize the cell as a Trojan horse to cross the BBB; however, it has also been shown in animal models that free mycobacteria enter the CNS hematogenously and that cellular carriers may not be required for this transport (Wu et al. 2000a, b). Further proof of this came by infecting an in vitro model of human BBB with *M. tuberculosis* that showed host cell actin cytoskeletal rearrangements were necessary for successful dissemination of the bacteria into the CNS (Jain et al. 2006). Post transport into the CNS *M. tuberculosis* have been reported to preferentially infect microglia (Spanos et al. 2015) but astrocytes (Rock et al. 2005) and neurons (Randall et al. 2014) are also potential targets.

Bacterial pathogen-associated molecular patterns (PAMPs; such as peptidoglycans, cell wall fragments, lipopolysaccharides, and lipoteichoic acid), can be recognized by cells in CNS following interactions with various pattern recognition

receptors (PRRs). Toll-like receptors (TLRs) are evolutionarily conserved receptors present in multiple cell types. In the CNS also different types of TLRs are expressed subserving specific needs. In general, all TLRs (except TLR3) transduce their signal through the MyD88 pathway. (Ichiyama et al. 2002). The end result of this pathway is the activation of MAP kinases or NF-κB, resulting in initiation of transcription of multiple genes associated with inflammation (Tato and Hunter 2002; Koedel et al. 2000; Kastenbauer et al. 2004).

Another group of intracytoplasmic PRRs is the NLR (NOD-like) family, with 23 proteins present in the human genome (Creagh and O'Neill 2006). The NLRs have a tripartite structure with an N terminus containing an effector domain, that is, either Pyrin or apoptotic speck-containing (ASC) protein, with a procaspase-1 recruitment domain (CARD), a central nucleotide binding domain (NACHT), and C terminal region composed of leucine-rich repeats that are responsible for detecting pathogens and autoregulation. The crucial step in NLR function is oligomerization of the NACHT domain to form an inflammasome, a structure composed of NLRP, and ASC containing a CARD-processing procaspase-1. TLR activation has an important role in the capacity of the inflammasome to process PAMPs such as LPS. The expression of NLR by BBB and CNS cell is not well documented, but the NLR (NOD2) is expressed by microglia and astrocytes in response to *N. meningitidis* (Chauhan et al. 2009). Activation of the NRLP3 inflammasome by bacteria, RNA, DNA, and ATP initiates transcription of IL-1α, IL-18, TNF-α, and chemokines for neutrophil chemoattraction and also mediates cleavage of pro-IL-1β into IL-1β. The same cytokines are elevated in CSF from bacterial meningitis cases and correlate with neutrophil numbers and clinical outcome. Furthermore, low levels of NLR increased the severity of sepsis and mortality in a number of bacterial infections, thereby emphasizing the importance of the inflammasome for regulating the severity of inflammation in bacterial meningitis (Osawa et al. 2011). IL-1β has been reported to stimulates microglia and subsequently robustly induces Krüppel-like factor 4(Klf4) via PI3 K/Akt pathway which positively regulates the production of endogenous IL-1β as well as other pro-inflammatory markers, cyclooxygenase-2, monocyte chemoattractant protein-1 and IL-6. Klf4 also negatively regulates the expression of inducible nitric oxide synthase, thereby playing a key role in regulating the immunomodulatory activities of microglia (Kaushik et al. 2010, 2012, 2013). Immune response against *S. aure*us, *L. monocytogenes, Klebsiella pneumoniae,* and *E. coli* have been shown o be mediated via NLRP3 (Davis et al. 2011).

Box 5: Expression of TLRs in CNS Cell Types

Microglia expresses *all TLRs* identified to date,
Astrocytes express *TLR 2, 3*, 5, and *9*,
Neurons express *TLR 3, 7, 8,* and *9* and
Oligodendrocytes express *TLR 2* and *3*.

Macrophages present in the meninges express glycoproteins SRA-1 (CD204) and SRA-2, both of which detect pathogens using polyanions on the cell wall instead of PAMPs (Mukhopadhyay et al. 2006). These SRAs also regulate the antipathogen inflammatory response through interaction with TLR2 and TLR4 by reducing NO and inflammatory cytokine synthesis, together with increased clearance of apoptotic cells by stimulating macrophages expressing CD36 (Baranova et al. 2008). Dendritic cell-specific intercellular adhesion molecule grabbing nonintegrin (DC-SIGN) is a known PRR expressed by dendritic cells as part of the innate immunity for the recognition of *M. tuberculosis* (Tailleux et al. 2003). DG-SIGN induction has also been reported from stimulated microglia (Lambert et al. 2008) and thus may be involved in microbial response post *M. tuberculosis* infection.

The meninges, choroid plexus, and ependyma express CD14 (a multifunctional scavenger receptor, that detects Gram-negative bacteria endotoxins in CSF and serum by interacting with TLR4, increasing inflammatory cytokines (Guo et al. 2009) and promoting phagocytosis of apoptotic neutrophils in the CSF to reduce the severity of tissue inflammation (Lacroix et al. 1998). At the CVO, circulating endotoxin may come into direct contact with cells of the CNS (Laflamme et al. 2001). Microglia expressing both TLR4 and CD14 are present in the CVO and upon sensing the presence of endotoxinupregulate proinflammatory mediators and TLR2 gene expression throughout the brain parenchyma as an attempt to kick-start a rapid CNS-wide neutrophil-independent antipathogen inflammatory response, before pathogens actually enter the CSF (Rivest 2009). In response to this, polymorphonuclear leukocytes cross the BBB by binding to selectins E and P along with IL-8. TNF-α, one of the secreated proinflammatory cytokine, induces production of adhesion molecules ICAM-1 and ICAM-2, which further facilitates extravasation of the leukocyte along chemoattractant concentration gradients. These leukocytes work to eliminate the invading pathogen through a rapid and robust production of reactive oxygen species (ROS). They release high amounts of superoxide anion (O_2^-) and nitric oxide (NO), generating peroxynitrite ($ONOO^-$) (Klein et al. 2006). These molecules mediate cell death by membrane peroxidation, breakdown of protein structure, DNA damage, and subsequent activation of poly (ADP)-ribose polymerase (PARP) leading to energy depletion.

4.3.2 Bacterial Virulence Factors

Inflammatory response in the host varies according to the various bacterial virulence factors (Scheld et al. 2002; Gerber and Nau 2010) such as exo- or endotoxins, cell surface proteins that mediate bacterial attachment, cell surface glycoproteins that protect a bacterium, and enzymes that may contribute to the pathogenicity of the bacterium. Components of bacterial cell wall have been reported to trigger inflammatory response in the host and mediate caspase-dependent apoptosis by activating the p53 pathway. Peptidoglycans and lipotechoic acids from Gram +ve bacterial cell wall is recognized in CNS by TLR2. The capsule of pathogens such as

S. pneumoniae, N. meningitidis, E. coli K1, and *H. influenzae* prevents phagocytosis by inhibiting binding with iC3b (a phagocytosis stimulating complement factor) and Fc, that stimulate receptor-mediated phagocytosis and thus acts as virulence factors (Jonsson et al. 1985; Gilsdorf et al. 2004; Mitchell et al. 2004; Mitchell and Mitchell 2010; Raymond 2012). *Listeria monocytogenes* is another pathogen which is capable of escaping from cellular phagosomes and its lipoteichoic acids are recognized by TLR2 with the help of CD14 and TLR6 (Flo et al. 2000; Janot et al. 2008) and its protein flagellin is recognized by TLR5 (Hayashi et al. 2001).

The virulence of some other bacteria is based on the production of enzymes such as coagulase, proteolytic enzymes, hyaluronidase, neuraminidase, and catalase. Hemolysin and cytolysin from Streptococcus have the ability to cause inflammatory activation, and these same enzymes produced by *S. agalactiae* induce chemokines and ICAM-1 in brain microvascular endothelium cells (Mitchell et al. 2004). Pneumolysin (recognized by TLR4) and H_2O_2 are produced and released by pneumococcus, which causes mitochondrial damage and subsequent neuronal death by caspase-independent pathway involving apoptosis-inducing factor (AIF) (Braun et al. 2002). Endotoxins such as lipopolysacchride are produced by Gram-negative bacteria such as *E. coli* and *Neisseria meningitides* which is recognized by TLR4 and causes damage to the microvascular endothelial cells that constitute the BBB in the human brain (Khan et al. 2012). *Streptococcus pyogenes*, a Gram-positive bacteria associated with brain abscess, produces a wide array of virulence factors including M protein, fibronectin-binding protein (Protein F), and lipoteichoic acid for adherence; hyaluronic acid capsule as an immunological disguise and to inhibit phagocytosis; invasions such as streptokinase, streptodornase (DNase B), hyaluronidase, and streptolysins and other exotoxins (Cole et al. 2011).

The virulence factors of *M. tuberculosis* are varied and complex. This bacterium does not have classical virulence factors like those which are the major causes of diseases due to other bacterial pathogens. Instead, its virulence can be defined by the factors that are important for the progression of tuberculosis. Be its special mechanism for cell entry, or it slow rate of generation, or its lipid-rich cell wall, all of these contribute to the virulence of this highly pathogenic microorganism. Added to this are the mechanisms that they possess to escape phagolysosomal killing, either by involving its adenylate cyclase enzyme that intoxicates the internalizing cells (Agarwal et al. 2009) and/or by detoxification of oxygen radicals that are produced intracellularly in response to infection. A detailed description of *M. tuberculosis* virulence factors is beyond the scope of this chapter, but interested readers could get more information from a recent review article by Forrellad and collegues (Forrellad et al. 2013).

4.3.3 Role of the Complement System in Bacterial Meningitis

The importance of the complement (C) system in bacterial meningitis can be gauzed from the fact that persons who are genetically deficient of functioning complement system are predisposed to such infections (Skattum et al. 2011). Cells

of the meninges, ependyma, and choroid plexus are exposed constantly to the CSF and express all the components of classical C system (Roos et al. 2004). C1q is the first component of the C system and functions as PRR capable of detecting PAMPs and activating the classical pathway (Gasque 2004). Mannose-binding lectin binds to mannose groups on bacteria, and activates C through mannose-binding lectin-associated serine proteases (Eisen and Minchinton 2003). Activation of the C pathway through C1q binds to LPS, IgG, DNA, and RNA, whereas pathogen binding directly to C3b activates alternative C pathways (Arlaud et al. 2002; Blom et al. 2009). The final common pathway for both classical and alternative C pathways is the generation of (C5-9) membrane attack complex, which is capable of lysing bacteria (Gasque 2004). The formation of opsonins C3b and iC3b also targets pathogens to promote phagocytosis by A integrin receptors CR3 and CR4 present in the meninges, choroid plexus (Kolmer) cells, and microglia (Singhrao et al. 1999). Regulation of C activation prevents self-destruction and is the responsibility of Cregs (C1 esterase inhibitor, CD46, CD55, CD35, and factor H) expressed by glia, neurons, and BBB endothelium. Complement regulators reduce C activation by pathogens by inhibiting the cytolytic membrane attack complex and anaphylatoxin receptor (C3aR and C5aR) generation. The overall effect is to reduce inflammatory cytokine expression and neutrophil infiltration. Importantly, the Cregs are also expressed by endothelial, choroid plexus, and ependymal cells in contact with the CSF and are strategically placed to regulate C activation and intraventricular inflammation after detection of systemic and intraventricular bacteria (Gasque et al. 1998).

4.3.4 Immunoregulation in CNS Following Bacterial Infections

So far we have seen that the CNS innate immune response relies upon the resident cells expressing both phagocytic and scavenger receptors capable of distinguishing "self" (host) from "nonself" (neurotoxic proteins, pathogens, apoptotic cells) and so reduce bystander injury. Neurons and glia also express "death signals" to initiate apoptosis in damaged neurons and inflammatory cells, transforming them into "safe targets" for rapid clearance from the CNS by glial cells expressing phagocytic receptors (Elward and Gasque 2003). If apoptotic cells remain undetected and not cleared from inflamed tissues, they will undergo lysis with the release of neurotoxic enzymes, contributing to secondary host tissue necrosis. The components of the C pathway facilitate pathogen and apoptotic cell phagocytosis, as well as inflammatory cell migration into areas of tissue damage (Griffiths et al. 2009). The regulation of the destructive arm of the "double-edged sword" is vital and relies upon serpins (self-defence proteins), regulators of complement activation (Cregs) and various "don't eat me" signals termed neuroimmunoregulatory molecules (NIRegs). NIRegs act at the cellular level (microglia, macrophages, BBB, and endothelium) to regulate brain inflammation. The range of NIRegs regulating microglia activity is

expanding and includes CD200 (and its receptor CD200R), the integrin CD47 with its receptor CD172, together with the semaphorin Sema 3A and CD22.

During a bacterial infection, the BBB endothelium increases expression of cyclic AMP, in response to overactivation of inflammatory genes, increased endothelial IFN-γ, TNF, NO expression, resulting in a downregulation of endothelial inflammatory cytokines. This is mediated by the expression of suppressors of cytokine signaling proteins 1 and 3 (SOCS 1 and 3) (Qin et al. 2012). As a NIReg, SOCS inhibit microglial and macrophage responses to both pathogens and LPS by regulating the JAK/STAT signaling pathway, resulting in reduced NO, IL-1α, and IFN-γ levels and stabilizing the BBB through cadherin at interendothelial cell junctions (Hernangomez et al. 2014).

CD200 is a 41–47 kd surface molecule and a member of the Ig supergene (IgSF) family characterized by two IgSF domains that represent the most commonly found domain type in the leukocyte membrane. The presence of two IgSF domains suggests that this molecule is related to cell adhesion and regulation. As a glycoprotein, CD200 is located on the membrane of myeloid cells, cerebellar neurons, retinal neurons, and vascular endothelium. The counter receptor to CD200, CD200R, also contains two IgSF domains and is expressed by myeloid cells and rodent brain microglia (Broderick et al. 2002; Koning et al. 2009). The presence of the NIReg CD200 provides a "don't eat me" signal and, on binding to CD200R, reduces microglial activation (Pietila et al. 2012). Mesenchymal stem cell expression of CD200 is responsible for inhibiting macrophage TNF expression, an effect mediated through the CD200-CD200R pathway, providing a mechanism to increase their survival by inhibiting host microglial phagocytosis (Pietila et al. 2012).

Another member of the IgSF protein family, CD47 is constitutively expressed by endothelium, neurons, macrophages, and dendritic cells (Hoarau et al. 2011). The counter receptor for CD47 is signal regulatory protein SIRP alpha (CD172), a plasma membrane protein expressed by myeloid cells and neurons (Brown and Frazier 2001). The interaction between CD47 on a host cell with a myeloid cell expressing CD172a recruits the tyrosine phosphatases SHP-1 and SHIP-2 resulting in the downregulation of macrophage phagocytosis, the prevention of neutrophils migrating across the BBB, an increase of anti-inflammatory TGF-β, expression and a reduction of interferon α levels, all contributing to the reduction of the severity of any inflammatory response (de Vries et al. 2002). CD47 also interacts with a further counter receptor, thrombospondin TSP, expressed by microglia, astrocytes, and smooth muscle cells. Mouse models of endotoxemia or other bacterial infections have shown that CD47-CD172a are available at a constant level in the CNS, but if the coreceptor level falls, this initiates an regulated inflammatory response, including phagocytosis of host cells (Okazawa et al. 2005). Neutrophil migration into inflamed tissue is inhibited by PAMPs activating TLR2; this response is fine tuned by the NIReg CD47 by either stabilizing TLR2 expression at the cell surface or acting as a neutrophil microbial sensor (Chin et al. 2009).

Sialic acid-binding immunoglobulin-like lectins (Siglecs) are NIRegs expressed by host cells and not bacteria, providing a marker helping to distinguish self (sialic acid-positive) from non-self (no sialic acid, as for pathogens and apoptotic cells).

The importance of Siglecs as a regulator of brain inflammation in BM is emphasized by the number of common CNS meningitis pathogens (*N. meningitidis, Haemophilus influenzae*, group B Streptococcus, and *E. coli*) that express sialic acid, incorporated into their cell walls. This represents a molecular mimic because the sialic is a "self" or "don't eat me" signal and is detected by a host Siglec receptor (Macauley et al. 2014).

In bacterial meningitis, brain hypoxia, cell necrosis, and ischemic infarction increase the release of DAMPs into the extracellular space. DAMPs (damage-associated molecular patterns) also known as alarmins, are molecules released by stressed cells undergoing necrosis that act as endogenous danger signals to promote and exacerbate the inflammatory response. The best known neurotoxic DAMPs are high mobility group box-1 (HMGB1), heat shock proteins, and ATP. These exacerbate the CNS inflammation by activating TLR and NLR pathways (Kigerl et al. 2014). Thrombomodulin (CD141) is an anticoagulant and represents a NIReg located strategically on endothelial cell surfaces and is expressed by microglia. Its N-terminal lectin-like domain binds to HMBG-1 and prevents subsequent binding to TLRs, reducing the activation of PMN and severity of inflammation (Abeyama et al. 2005; Yu et al. 2006).

4.4 Viral Infections of the Human CNS

It is a fact that the number of people affected by viral infections to the CNS each year is greater than all bacterial, fungal, and protozoal infections combined (Romero and Newland 2003). The magnitude of viral diseases in humans is difficult to explain within the limits of this chapter; however, in the following sections, we shall endeavor to provide a succinct account of the patho-physiological processes involved.

4.4.1 Entry of Viruses into CNS

Mechanisms of viral entry into the CNS do not differ greatly from those that have already been discussed for bacterial infections of CNS. A comprehensive list of viruses that can cause CNS infections can be found in Table 4.4. Air-borne viruses (measles and mumps) or the ones that gain entry through the oral route (human enteroviruses) are able to move quickly past mucosal epithelial barriers and establish infection in oropharyngeal or lymphoid tissues in small intestine (Reuter and Schneider-Schaulies 2010; Rhoades et al. 2011; Bale 2014). Arboviruses are taken up by Langerhans cells post intradermal entry following insect bites, and then migrate to the draining lymph node (Johnston et al. 1996; Wu et al. 2000a, b). Once in secondary lymphoid tissues, viruses are often shed into the blood stream, resulting in systemic infection. Some viruses directly infect vascular endothelial cells of BBB, which allow direct passage into the CNS (Chaudhuri 2000). A key

Table 4.4 Classification and entry routes of viruses that can cause CNS infections

Genome	Virus family	Specifics	Viruses	CNS entry
dsDNA	Adenoviridae		MAV-1	BBB and BMVECs
	Herpesviridae	Alpha herpesvirus	HSV-1, HSV-2, VZV, PRV, and BHV	Sensory nerve endings and ORN
		Beta herpesvirus	HCMV	BBB and BMVECs
		Gamma herpesvirus	EBV	BBB and BMVECs
	Polyomaviridae		JCV	BBB and BMVECs
dsRNA	Reoviridae	Mouse model	T3	BBB; peripheral nerve?
(+)ssRNA	Coronaviridae	Mouse model	MHV	Peripheral nerve and ORN
	Flaviviridae	Hepacivirus	HCV	BBB and BMVECs
		Zoonotic	WNV and JEV	Peripheral nerve, BBB, ORN, and BMVECs
	Picornaviridae	Enterovirus	Poliovirus and EV71	NMJs and BBB
		Mouse model	TMEV	BBB
	Togaviridae	Zoonotic	CHIKV	ORN?
		Mouse model	SINV	ORN
(–)ssRNA	Arenaviridae	Zoonotic	LCMV	BBB
	Bornaviridae	Zoonotic	BDV	ORN
	Bunyaviridae	Zoonotic	LACV	ORN and BBB?
	Orthomyxoviridae	Zoonotic	Influenza A	Peripheral nerve and ORN
	Paramyxoviridae	Morbilivirus	MV	BBB
		Rubulavirus	MuV	BBB
		Henipavirus (zoonotic)	HeV and Nipah	BBB and ORN?
	Rhabdoviridae	Zoonotic	RabV and VSV and Chandipura virus	NMJs and ORN
ssRNA-RT	Retroviridae	Lentivirus	HIV and HTLV	BBB

Adapted from Koyuncu et al. (2013)

determinant of BBB function is the proper assembly of tight-junctions (TJs) and adherens junctions (AJs) between brain microvascular endothelial cells (BMECs). Neurotropic viral infections have been shown to alter junction protein expression and function in several ways, including direct mechanisms via viral proteins and downstream immune-mediated regulation of junction integrity. The effects of retroviruses on BBB junctions have been the most extensively characterized and several retroviruses, including HIV-1, human T-lymphotropic virus (HTLV-1),

simian immunodeficiency virus (SIV), and feline immunodeficiency virus (FIV) have been shown to diminish expression of BBB TJ proteins in vitro and in vivo (Miller et al. 2012). The HIV-1 protein Tat has been shown in numerous studies to activate BBB endothelium, decrease TJ protein expression, and degrade junctions via MMP-9 and RhoA-mediated cleavage of the TJ protein occludin (Strazza et al. 2011). Similarly, the HIV-1 virion envelope protein gp120 has been shown to enhance BBB permeability by decreasing expression of several TJ proteins, including claudin-5 and Zo-1, and by inducing proteosome-mediated degradation of Zo-1 and Zo-2 (Kanmogne et al. 2005; Nakamuta et al. 2008; Louboutin et al. 2010). In addition to viral proteins, reports also suggests that elevated CCL2 levels during HIV-1 infection also possibly contribute to BBB breakdown and junction disruption via multiple mechanisms, including disruption of AJs in brain endothelium via phosphorylation and sequestration of β-catenin (Roberts et al. 2012). Other studies have linked CCL2 to endothelial barrier disruption in the context of mouse adenovirus and Dengue virus infections as well (Lee et al. 2006; Gralinski et al. 2009). Apart from retroviruses, interest in the impact of flaviviruses on BBB junctions is also well documented (Mishra et al. 2009; Verma et al. 2009; Suen et al. 2014). However, in many cases of flaviviral infections, the BBB breakdown may occur more indirectly through the action of inducible inflammatory mediators, as opposed to directly via viral proteins (Chen et al. 2014; Li et al. 2015). Indeed, inflammatory cytokines and chemokine expression via multiple cellular sources has been linked to suppression of BBB junction protein expression and degradation of existing junctions during many viral infections, including studies using retroviruses, RabV-1, MAV-1, and others (Gralinski et al. 2009; Chai et al. 2014, 2015).

Additionally, there are areas of the CNS such as the CVOs that are not completely protected by the BBB and serve as entry points for several viruses (Wolinsky et al. 1974; van Den Pol et al. 1999; Preuss et al. 2009; Wuerfel et al. 2010). Infected hematopoietic cells are also used as 'Trojan horses' to transport virus into the CNS via the blood supply (Kim et al. 2003; Tabor-Godwin et al. 2010; Bielefeldt-Ohmann et al. 2012; Meier et al. 2012). The picornavirus enterovirus 71 (EV71) (Solomon et al. 2010) and a ubiquitous human polyomavirus, JC virus (JCV) (Boothpur and Brennan 2010), can also infiltrate into the CNS by the Trojan horse mode of entry. In the case of JCV infection, infiltration of infected B-cells into the CNS in immune suppressed patients can result in the infection of oligodendrocytes (the myelin producing cells) and astrocytes, leading to a fatal inflammatory disease in the brain called progressive multifocal leukoencephalopathy (PML). Finally, systemic viral infection can lead to inflammation-induced breakdown of the BBB, allowing viruses to literally slip through the cracks into the CNS (Conant et al. 2012; Savarin et al. 2013; Williams et al. 2013; Chai et al. 2014; Johnson et al. 2014).

However, some viruses also have another unique way of infecting the CNS wherein they infect and migrate through peripheral nerves. After entry into the host through a bite from infected animal, (rabies) or after ingestion (polio), the virus initially infects myocytes and mucosal epithelial cells, respectively. Neuromuscular

junctions (NMJs) are specialized synapses between muscles and motor neurons that facilitate and control muscle movement. NMJs can be gateways for many viruses to spread into the CNS. Most motor neurons have their cell bodies in the spinal cord, which, in turn, are in synaptic contact with motor centers in the brain. Poliovirus and RabV infections spread into the CNS through NMJs. While RabV particles enter the NMJ directly after a bite from infected animal, poliovirus particles reach the NMJ by a more circuitous route (Racaniello 2006). Later, both of these viruses use these peripheral motor neurons to make their way into the CNS (Ohka et al. 2012; Gluska et al. 2014). Herpes simplex virus (HSV)-1 initially infects keratinocytes before migrating to peripheral sensory neurons (Price 1986). HSV-1 has also been proposed to reach the CNS via olfactory sensory neurons whose dendrites are directly exposed to airways in the nose (Mori et al. 2005) and in experimental model of the disease optic route of infection is also possible (Garner and LaVail 1999). Nipah virus, influenza virus, and rabies virus have also been shown to enter the CNS via olfactory nerves (Lafay et al. 1991; Munster et al. 2012; van Riel et al. 2015). However, it is important to note that while some viruses have a preference for the hematogenous or peripheral nerve route to enter CNS, other viruses are able to take advantage of both (Swanson and McGavern 2015). Once viruses gain access to the CNS, they spread to various regions (see Table 4.5) and the ensuing immune response combines to shape the resulting disease and inflammation.

4.4.2 Viral Recognition in the CNS

The innate immune system is the host's first line of defence against invading pathogens. Important components of innate immune system including the macrophages, dendritic cells, natural killer cells, mast cells, neutrophils, and the C system play complementary roles in limiting viral replication and dissemination, as well as in initiation of adaptive immune response. Cellular components of innate immune system limit viral infection either by direct phagocytic activity or by releasing type I interferon and inflammatory mediators after sensing various viral components. Sensing of these viral components is mainly achieved through highly conserved germline encoded family of proteins—the pattern recognition receptors (PRRs). Viral PAMPs (pathogen-associated molecular patterns) that are detected by these PRRs include genomic DNA, single-stranded RNA (ssRNA), double-stranded RNA (dsRNA), RNA with 5′-triphosphate ends, and viral proteins.

Several TLRs have been demonstrated to specifically recognize viral motifs, including TLRs 2, 3, 7, 8, and 9. While TLR2 is best known to bind a variety of microbial cell wall component (bacterial lipoproteins, peptidoglycans, and lipoteichoic acid or yeast cell wall zymosan), it can also recognize as yet unidentified, viral motifs (Cai et al. 2012). In contrast, endosomal TLRs such as TLR3 recognize viral double-stranded RNA (dsRNA) and its synthetic analog, poly(I:C) (polyinosine-deoxycytidylic acid), while TLR7 and TLR8 mediate responses to GU-rich single-stranded RNA (ssRNA) produced in virus-infected cells. TLR4 is well known for its response to LPS but has been reported to be involved in

Table 4.5 CNS regions (or cells) affected by viruses that cause meningitis and/or encephalitis

Cortical neurons	Alphaviruses Bunyaviruses Herpes simplex virus (HSV) Japanese encephalitis virus (JEV) Measles virus St. Louis encephalitis virus (SLEV) Tick-borne encephalitis virus (TBEV) West Nile encephalitis virus (WNV)
Microglia	Human immunodeficiency virus (HIV)
Oligodendrocytes	John Cunningham virus (JCV)
Thalamus	Human enteroviruses Rabies virus (RabV) West Nile encephalitis virus
Hippocampus	Human enteroviruses Rabies virus West Nile encephalitis virus
Brainstem	Human enteroviruses Rabies virus West Nile encephalitis virus
Cerebellum	Human enteroviruses West Nile encephalitis virus
Ependyma/choroid plexus	Cytomegalovirus (CMV) Human enteroviruses Lymphocytic choriomeningitis virus (LCMV) Mumps
Meninges/perivascular	Human enteroviruses Human immunodeficiency virus Japanese encephalitis virus Lymphocytic choriomeningitis virus Measles virus Mumps virus Nipah virus
Motor neurons	Human enteroviruses Japanese encephalitis virus Rabies virus West Nile encephalitis virus Tick-borne encephalitis virus

generating immune response against HIV (Suh et al. 2009). In non-neuronal cell types HIV glycoprotein gp120 has been reported to activate TLR4 pathway (Hernandez et al. 2012; Nazli et al. 2013).

Finally, TLR9 detects viral DNA with unmethylated CpG motifs (Kawai and Akira 2011). Signaling downstream of TLRs relies on the recruitment of TIR adaptor proteins MyD88, MyD88 adaptor like/TIR domain-containing adaptor protein (Mal/TIRAP), Toll/IL-1 receptor domain-containing adaptor inducing IFN-β (TRIF) and TRIF-related adaptor molecule (TRAM) (O'Neill and Bowie 2007). MyD88, the prototypical member of the TIR group, is utilized by all TLRs

(except TLR3, which uses TRIF). Engagement of TLRs triggers TIR adaptor recruitment to activate IkB kinases (IKK)s such as TANK binding kinase 1 (TBK1) and IKKb resulting in the activation of NFkB and IRF family members culminating in the expression of genes whose products (interferon) help in viral elimination. Interferons activate the upregulation of a large number of proteins that act to control infection at the cellular level and attract immune effector cells. Three mechanisms of direct inhibition of virus replication have been identified. Activation of protein kinase R (PKR) in response to double-stranded RNA (as in virus replication intermediates) which inhibits eukaryotic translational factor 2 that in turn restricts synthesis of viral proteins. Activation of 2′5′ oligoadenylate synthetase (OAS), which activates RNase L and in turn degrades viral RNA. Finally, the Mx family of proteins are activated that target nucleocapsids.

Sterile alpha and TIR motif-containing protein (SARM) is the fifth member of the TIR adaptor protein family that also comprises MyD88, Mal/TIRAP, TRIF, and TRAM. In mice, SARM is expressed mainly in the CNS where it appears to have TLR- independent functions even though it contains a TIR domain (Kim et al. 2007). SARM has been reported to contribute to the pro-inflammatory response to VSV in the CNS by mediating cytokine production by neurons, and this process was shown to be dependent on the presence of microglia, indicating an important role for cell communication in the antiviral functions of SARM in the CNS. Interestingly, mice lacking SARM show reduced inflammation and improved survival in response to the virus (Hou et al. 2013). Therefore, SARM in this case can be regarded as a mediator of immunopathology during VSV infection where the absence of SARM reduces the inflammatory response to the virus, thus improving survival. It was recently reported that SARM mediates apoptosis in neurons in response to La Crosse virus, a member of the bunyavirus family and a leading cause of pediatric encephalitis. In neurons SARM localizes to mitochondria, binds ATP synthase following viral infection, and leads to the production of ROS, resulting in oxidative stress and apoptosis (Mukherjee et al. 2013).

WNV recognitions have been reported to occur via TLR3 in the CNS. WNV infection leads to a TLR-dependent inflammatory response, which is involved in brain penetration of the virus and neuronal injury (Wang et al. 2004). However, this has been contested and studies by others have shown that on the contrary WNV is recognized by RIG-1 and MDA5 (Daffis et al. 2008). This could be plausible as WNV's closest cousin, the JEV, has been shown by us to be recognized by RIG-1 and thereby activating its downstream cascade of inflammatory events (Nazmi et al. 2011, 2012). Interestingly, ablating RIG-1 or its downstream adapter did not completely block antiviral innate immune responses leading us to believe multiple recognition pathways could exist for the same virus. Concomitantly, we later found out that TLR7 was also involved in detecting the viral PAMP and generating antiviral defense mechanism (Nazmi et al. 2014). Also, another recent study using TLR3 knockout mice showed increased susceptibility to JEV along with severe CNS inflammation characterized by early infiltration of inflammatory CD11b+Ly-6Chigh monocytes along with profoundly increased viral burden, proinflammatory cytokine/chemokine expression as well as BBB permeability (Han et al. 2014).

More recently though, immune response against a mutant strain of WNV has been shown to be mediated via TLR7 (Xie et al. 2013). Another interesting case where a single virus is recognized by 2 different PRR is found in case of TMEV. The single-stranded RNA genome of TMEV is believed to bind to TLR7 and its double-stranded replication intermediate to TLR3 on the endosomes and lysosomes of host cells (Hause et al. 2007). TLR3 present on astrocytes has been reported to sense HSV-2 infection immediately after entry into the CNS, possibly preventing HSV from spreading beyond the neurons mediating entry into the CNS (Reinert et al. 2012). On the contrary, reactivation of HSV-1 at the CNS would likely induce and activate TLR2 and TLR4 receptors directly through interaction of astrocytes with the pathogen and also indirectly by endogenous ligands produced locally, such as serum amyloid protein, potentiating the neuroinflammatory response (Villalba et al. 2012). Neurovirulence of Langat virus (LGTV), a ssRNA tick-borne flavivirus, has been reported to be TLR7 mediated. It was observed that TLR7 is not essential in controlling LGTV pathogenesis, but it is important in controlling virus infection in neurons in the CNS, possibly by regulating neuroinflammatory responses (Baker et al. 2013). Similar observations were also made in case of a polytropic retrovirus infection. TLR7 was found to be necessary for the early production of certain cytokines and chemokines and was also involved in the early activation of astrocytes. However, it was not necessary for cytokine production and astrocyte activation at later stages of infection and did not alter viral pathogenesis or viral replication in the brain. This suggested that other PRRs may be able to compensate for the lack of TLR7 during retrovirus infection in the CNS (Lewis et al. 2008). Ablation studies have also revealed that immune response against RabV may also be mediated via TLR7 (Li et al. 2011). The recently characterized endosomal TLR, TLR13, is expressed in mice but not humans and requires MyD88 for signaling. This TLR was shown to sense vesicular stomatitis virus (VSV) (Shi et al. 2011).

4.4.3 Inflammation in CNS Following Viral Infection

Post detection/recognition of the viral PAMPs by PRRs present in the CNS, the innate immune responses are activated triggering the production of type I interferons, a key element in controlling virus replication and spread (Randall and Goodbourn 2008). Type 2 interferons are produced exclusively by lymphoid cells, which are absent early in infection but contribute to later control of pathogens. While the CNS has unique immunological status, there is increasing evidence that there is a vigorous innate immune response to viral infection of cells within it (Savarin and Bergmann 2008). For example, TLRs are selectively upregulated in the brain in response to infection with different viruses (McKimmie et al. 2005). Microglia, respond to either activation stimuli or direct viral infection to release an array of cyto/chemokines and other mediators that are primarily responsible for the generation of the inflammatory milieu within the CNS (Das Sarma 2014). Astrocytes also play a critical role in regulating CNS inflammation by chemical cross-talk with microglia (Shih et al. 2006; Farina et al. 2007), but interestingly, they may also

serve as viral reservoirs in CNS (Thompson et al. 2011; Sips et al. 2012; Palus et al. 2014). Neurons can produce a range of innate immune-associated proteins including type 1 interferons in response to infection with rabies virus (Prehaud et al. 2005), Theiler's virus, La Crosse virus (a member of the bunyavirus family) (Delhaye et al. 2006) and JEV (Nazmi et al. 2011, 2012, 2014).

Both virus-infected and uninfected glial cells, predominantly astrocytes, provide the early inflammatory signals (Lane et al. 2000). Pro-inflammatory cytokines, IFN-γ, and TNF-α, are markedly increased in CNS tissues during HSV-1 infection in the brain and microglia have been shown to respond to HSV-1 by secreting pro-inflammatory and chemotactic molecules such as TNF-α, IL-1β, IL-6, IL-12, CCL7, CCL8, CCL9, CXCL1, CXCL2, CXCL4, and CXCL5 (Lokensgard et al. 2001). Human microglia have also been shown to respond to RNA viruses including WNV by producing cytokines and chemokines (Cheeran et al. 2005). Elevated levels of IL-1β are readily detectable in neural tissue from WNV encephalitis patients and cultured human glia produce this potent inflammatory cytokine in response to WNV challenge (van Marle et al. 2007). In case of JEV infection also, such responses have been observed (Ghoshal et al. 2007). Enhanced or sustained viral replication is associated with raised levels of TNF-α and IL-6 although TNF-α does not seem to play direct antiviral roles; rather its effects are concentrated on disrupting the BBB (Wang et al. 2004). MHV infection induces a robust CNS inflammatory response comprising both the innate and adaptive immune components. CNS infection is initially presented by fast, active and coordinated expression of matrix metalloproteinases (MMPs), chemokines, a tissue inhibitor of MMPs (TIMP-1) and proinflammatory cytokines viz. IL-1α, Il-1β, TNF-α, IL-6, and IL-12 primarily in microglia and astrocytes (Bergmann et al. 2006).

Activated peripheral lymphocytes contribute in upregulating adhesion molecules and chemokine receptors, both facilitating the entry of circulating lymphocytes into the CNS. Together, these molecules facilitate BBB disruption and attract innate immune effectors, which further enhance the expression of inflammatory factors. Once the CNS integrity is compromised, MMP expression ushers tissue influx of inflammatory cells, activation of cytokine secretion and CNS damage (Yong et al. 2001). In neurotropic canine distemper virus infection, a morbillivirus related to human measles virus, induction of MMP-2 expression in astrocytes and MMP-9 in neurons was noted (Khuth et al. 2001). Apoptotic neurons induce microglial cells to release neuroprotective molecules, such as anti-inflammatory cytokines and growth factors, while inhibiting synthesis of nitric oxide (NO) and pro-inflammatory cytokines (Minghetti et al. 2005). Paramyxoviruses, measles virus (MV) and mumps virus (MuV) can also lead to serious CNS infections. Primary MV and MuV infections begin in the upper respiratory tract, and infection of lymphoid tissue causes viremia and spread to other tissues. MuV is highly neurotropic and can result in high incidence of acute encephalopathy in children. Elevated levels of multiple cytokines were detected in cerebrospinal fluids of children diagnosed with MuV-associated acute encephalopathy (Watanabe et al. 2013). Unlike MuV, MV infection spreads to the CNS in approximately 0.1 % of the cases, causing several types of devastating

neurological diseases, such as fatal subacute sclerosing panencephalitis, which manifests weeks to years after infection (Buchanan and Bonthius 2012). Cumulatively, these observations therefore indicate a molecular cross-talk that is apparent between glial cells and neurons such that stressed neurons under a viral attack can protect themselves from further damage by activated microglia.

MicroRNAs (miRNAs) are an abundant class of small noncoding RNA molecules that play an important role in the regulation of gene expression at the post-transcriptional level. Altered expression of several miRNAs has been associated with various conditions that result in neuroinflammation (Guedes et al. 2013). Several studies suggest a role for miRNAs as modulators of M1 and M2 polarization in microglia. MiR-155, broadly considered a proinflammatory miRNA, was one of the first miRNAs to be directly linked to the M1 phenotype. This miRNA was shown to be upregulated in microglia in response to several proinflammatory stimuli, with consequent targeting of microglial anti-inflammatory events such as the suppressor of cytokine signaling 1 (SOCS-1), leading to the upregulation of several inflammatory mediators characteristic of the M1 phenotype, including the inducible nitrogen synthase (iNOS), IL-6, and TNF-α (Cardoso et al. 2012). Thus, it is absolutely plausible that miRNAs would also be involved in regulation of CNS inflammation following viral infections. In case of HCMV infection it has been reported that miR-21 attenuates the viral replication in neural cells by targeting a cell cycle regulator Cdc25a (Fu et al. 2015). In case of HIV infections, microglia-mediated oxidative damage induced by the viral Tat protein has been shown to be mediated by miR-17 (Jadhav et al. 2014) and in case of in vitro SIV infections, several miRNAs have been found to inhibit the viral replication (Sisk et al. 2013). In mouse model of rabies virus infection, miRNAs have been shown to play critical roles. Microarray analysis showed that miRNA expression becomes modulated in the brains of mice infected with rabies virus and functional analysis showed the differentially expressed miRNAs to be involved in many immune-related signaling pathways, such as the Jak-STAT signaling pathway, the MAPK signaling pathway, cytokine–cytokine receptor interactions, and Fc gamma R-mediated phagocytosis (Zhao et al. 2012). Finally, in case of JEV infections, miR155 has been shown to regulate microglia-mediated inflammation (Thounaojam et al. 2014) and down-regulate innate immune responses (Pareek et al. 2014). Interestingly, miR146a has also been recently shown to suppress cellular immune responses following JEV infection (Sharma et al. 2015).

Box 6: HIV Brain Infection

Neurological symptoms in 40 % of cases
At autopsy, 75–80 % of brains are involved

4.4.4 HIV-Associated Neurocognitive Disorder or Neuro-AIDS

Over 40 million people worldwide are infected by HIV, and, while it is most well known for its devastating effects on the immune system and the resulting acquired immunodeficiency syndrome (AIDS), it can also cause several neurological disorders, collectively known as AIDS dementia complex (ADC), or HIV-associated dementia (HAD) (see Box 6). Complications include encephalitis, behavioral changes, and a gradual decline in cognitive function, including trouble with concentration, memory, and attention. Milder cognitive complaints are common and are termed HIV-associated neurocognitive disorder (HAND) that are characterized by motor, and behavioral abnormalities (Kaul et al. 2005). Neuropsychologic testing can reveal subtle deficits even in the absence of symptoms. Infants, infected intra-utero with HIV, are asymptomatic at birth, presenting in time with developmental delay and recurrent infections. Later on at about 2–3 years of age, a progressive clinical syndrome evolves, manifested by seizures, motor deficits, acquired microcephaly, and behavioral and cognitive decline. However, in adults HIV can behave more insidiously than previously seen. In a recent study it has been shown that the virus can settle in infected person's brains as early as 4 months after infection. In turn, HIV in the brain can genetically mutate—differentiating itself from the type circulating in the blood—which means that certain drugs used to treat the virus may not work as well in the CNS as they do in other parts of the body. Over time, untreated HIV can cause negative neurological and mental-health effects, such as brain swelling and a form of dementia. A study examined 72 individuals in San Francisco—almost all adult males—who had recently tested positive for HIV. Samples of their blood and CSF were taken and paired and the results showed that HIV had invaded the CNS in over 70 % of the subjects within the first few months of infection. However, the more alarming observation was that during the second year of infection, the virus had started replicating itself in the CNS independently from viral populations in the blood in up to 25 % of these subjects. This process is known as compartmentalization, when a virus "sets up shop" in a discrete part of the body and begins to reproduce there on its own (Sturdevant et al. 2015).

Other AIDS-related disorders of the nervous system may be caused directly by the HIV virus, by certain cancers and opportunistic infections (illnesses caused by bacteria, fungi, and other viruses that would not otherwise affect people with healthy immune systems), or by toxic effects of the drugs used to treat symptoms. Moreover, neuro-AIDS disorders of unknown origin may be influenced by but are not caused directly by the virus. In Table 4.6 we have discussed these problems, briefly.

Table 4.6 Associated problems in Neuro-AIDS

CMV infection	• Major cause of non-Epstein-Barr virus infectious mononucleosis in patients with AIDS • Remain latent in host post infection but reactivates following immunosupression in AIDS (Cheung and Teich 1999) • Causes radiculopathy, a spinal cord syndrome characterized by lower extremity pain and weakness, spasticity, areflexia, urinary retention, and hypoesthesia (Miller et al. 1990) • Subacute encephalitis in conjunction with isolation of CMV from brain tissue or CSF has been reported (Hawley et al. 1983). (*refer to* Sect. 4.5 *for more information*)
CNS lymphomas	• Cancerous, diffuse, large-cell non-Hodgkin lymphoma of B-cell origin that usually occurs in the brain (rarely in the spinal cord) (Knowles 2003) • Almost always associated with the Epstein-Barr virus (belonging in the herpes family) (Corcoran et al. 2008)
CNS tuberculosis	• At least one-third of people living with HIV worldwide in 2013 were infected with TB; approximately 25 % of deaths among HIV-positive people are due to TB (http://www.who.int/mediacentre/factsheets/fs104/en/) • Immunosuppression increases susceptibility for acquiring or reactivating TB • may manifest as meningitis, tuberculoma, abscess, or other forms of disease. (*refer to* Sect. 4.3 *for more information*)
Cryptococcosis	• Cause of the most common life-threatening meningitis in AIDS • Responsible fungus is classified into a complex that contains two species (*Cryptococcus neoformans* and *C. gattii*) with eight major molecular types • The fungus first invades the lungs and spreads to the covering of the brain and spinal cord, causing inflammation (Antinori 2013). (*refer to* Sect. 4.6 *for more information*)
Neuropathy	• Late stages of infection could lead to Peripheral (Stavros and Simpson 2014) or Distal sensory polyneuropathy (Nicholas et al. 2007)
Progressive multifocal leukoencephalopathy	• Affects nearly 5 % of people with AIDS • Caused by the JC virus, which travels to the brain, infects multiple sites and causes demyelination • Post HAART rate of recovery is greater (Lima 2013)
Toxoplasma encephalitis, (*also called cerebral toxoplasmosis*)	• Caused by the parasite *Toxoplasma gondii* • Clinical CNS toxoplasmosis occurs in 3–15 % of patients with AIDS in the United States; 50–75 % of patients in some European countries and in Africa • Usually a complication of the late phase of the disease. (*refer to* Sect. 4.7 *for more information*)
Vacuolar myelopathy	• Chronic myelopathy associated with HIV infection • Occurs during the late stages of HIV infection, when $CD4^+$ lymphocyte counts are very low, often in conjunction with ADC, peripheral neuropathies, and opportunistic infections or malignancies (Anneken et al. 2006)

4.5 Fungal Infections of the Human CNS

Systemic mycoses (fungal infections of animals including humans) caused by primary or opportunistic fungal pathogens pose significant medical problems to public health, mainly due to the growing number of aging persons, and immunocompromised individuals who undergo solid organ transplantation and anticancer-chemotherapy, or have HIV infection. Although fungal infections contribute substantially to human morbidity and mortality, the impact of these diseases on human health is not widely appreciated. The following Table 4.7, adapted from a recent review article (Brown et al. 2012) gives an idea on the magnitude of the problem of mycoses in humans.

Table 4.7 Statistics of 10 most significant of invasive fungal infections

Disease (most common species)	Location	Estimated life-threatening infections/year at that location	Mortality rates (% in infected populations)
Opportunistic invasive mycoses			
Aspergillosis (*Aspergillus fumigatus*)	Worldwide	>200,000	30–95
Candidiasis (*Candida albicans*)	Worldwide	>400,000	46–75
Cryptococcosis (*Cryptococcus neoformans*)	Worldwide	>1,000,000	20–70
Mucormycosis (*Rhizopus oryzae*)	Worldwide	>10,000	30–90
Pneumocystis (*Pneumocystis jirovecii*)	Worldwide	>400,000	20–80
Endemic dimorphic mycoses			
Blastomycosis (*Blastomyces dermatitidis*)	Midwestern and Atlantic United States	~3000	<2–68
Coccidioidomycosis (*Coccidioides immitis*)	Southwestern United States	~25,000	<1–70
Histoplasmosis (*Histoplasma capsulatum*)	Midwestern United States	~25,000	28–50
Paracoccidioidomycosis (*Paracoccidioides brasiliensis*)	Brazil	~4000	5–27
Penicilliosis (*Penicillium marneffei*)	Southeast Asia	>8000	2–75

Table 4.8 Fungal infections and incidence of CNS involvements

Organism	CNS involvement (%)	Mortality (%)
Invasive candidiasis	3–64	11–67
Invasive aspergillosis	4–6	80–90
Cryptococosis	67–84	7–12
Histoplasmosis	5–20	20–40
Coccidioidomycosis	25	26
Blastomycosis	40	4.3–22
Zygomycosis	12	79–98
Dematiacious (*cladophialophora)*	100	71–74

More than hundred thousand fungal species are recognized by now and only a couple of hundreds are found to be pathogenic to humans. Fortunately, only about 10–15 % of pathological fungi usually produce systemic/CNS mycosis (Raman Sharma 2010). Table 4.8 gives a brief idea about the incidence of CNS involvement associated with invasive fungal infection (Kethireddy and Andes 2007). With the exception of *Candida albicans*, that is, present as a commensal on the human body, most fungal elements gain entry into the human body via the respiratory tract or through exposed wounds. The fungal pathogens reported till date to cause human CNS infections are *Cryptococcus neoformans*, *Coccidioides immitis*, *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis*, *Sporothrix schenckii*, *Penicillium marneffei*, Candida species, Aspergillus species, Zygomycetes, *Pseudallescheria boydii*, and those causing Phaeohyphomycosis and Hyalohyphomycosis (Romani 2011). Most of these organisms, with the exception of Candida species, are found in soil specifically where it is mixed with dead or decaying organic matter and animal or bird droppings. Fungal invasion of the CNS can cause one or more pathologies such as acute or chronic meningitis, abscesses or granuloma, encephalitis, stroke, parenchymal brain, or myelopathy (Jellinger et al. 2000; Baddley et al. 2002). A brief idea of the different fungi known to infect the human CNS is given in Box 7.

4.5.1 Immunopathogenesis of CNS Fungal Infections

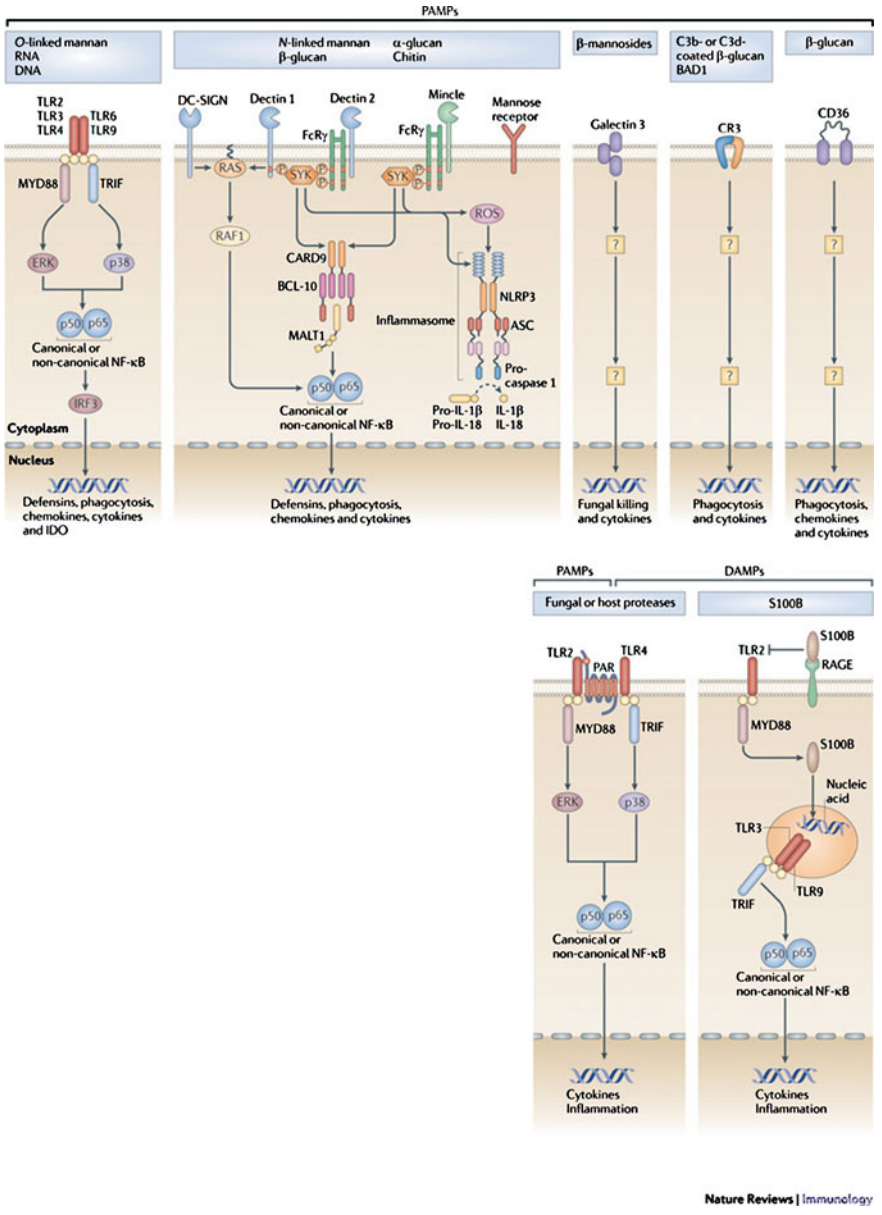
Immunopathogenesis of CNS post-fungal infections is not a very well-elucidated area of host–pathogen interaction studies given the rarity of occurrence of most of the infections. As in most cases the fungi are disseminated from the periphery, a general immune response against the invading pathogen is common.

Apart from the physical barriers and the constitutive defence mechanisms and opsonic recognition, pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs), C-type lectin receptors (CLRs) and the galectin family proteins (van de Veerdonk et al. 2008)—that could sense pathogen-associated molecular patterns (PAMPs) in fungi. Figure 4.3 gives a concise idea about the involvement of the

various PRRs in fungal pathogen recognition and the consequent downstream signaling cascades.

Reports about the role of TLRs in CNS fungal infections are limited. It is known that immune signaling by Candida albicans and Aspergillus fumigatus essentially occurs via TLR2, TLR4, and TLR9 downstream pathways that are implicated in different ways to control the infections. In addition, Aspergillus hyphae, unlike conidia and Candida hyphae and yeasts, seem to be sensed through TLR4, which indicates that TLRs discriminate between distinct fungal morphotypes (Romani 2011). Expression of TLRs (Hanke and Kielian 2011) and various galectin proteins (Sakaguchi and Okano 2012; Shin 2013; Zanetta 1998) in various cells types in the CNS is quite well elucidated. Recent studies have also indicated the presence of CLRs in the brain (Lech et al. 2012). Thus, it would not be too farfetched to imagine a critical role of these PRRs following a CNS invasion by any fungal pathogen.

Fungal infections of the CNS also evoke humoral and cellular responses as in bacterial infections with the possibility to enable the host to eliminate the pathogen. Activation of the resident brain cells by fungi combined with relative expression of immune-enhancing and immune-suppressing cytokines and chemokines which play a determinant role and partially explain the immunopathogenesis of CNS fungal infections. Activated resident brain cells such as microglia, astrocytes, and endothelial cells express major histocompatibility complex (MHC) Class I and Class II molecules and therefore act as antigen presenting cells. In addition, they express complement receptors, produce cytokines, chemokines, and molecules with antifungal activity, such as nitric oxide (NO) and are capable of phagocytosis. Microglia, acting as antigen presenting cells, stimulates T-cell proliferation and cytokine secretion, which in turn stimulate these semiprofessional phagocytes to ingest and more effectively kill invading fungi (Klein and Sato 2000). The precise mechanisms that explain the association of CNS fungal infection with the particular MHC molecules are unknown. However, several models have been proposed, including the direct involvement of human leukocyte antigen (HLA) molecules and the involvement of closely linked genes. In an immunocompetent host, during the initial immune response to a fungal pathogen, HLA molecules must bind to peptides derived from fungal proteins and the T-cell repertoire must include clones that can be activated by such HLA-bound peptides. Nevertheless, non-fulfillment of either of these requirements may render a host carrying a particular combination of HLA alleles more susceptible to certain infections than another who has a different combination of alleles. Especially in CNS involvement of paracoccidioidomycosis, the MHC molecules are not expressed in a constitutional way in the CNS, at least at the level found in the majority of other tissues. In addition, in situations with an immunological stimulation there is an increase of expression of these molecules (de Almeida et al. 2005).



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◀ **Fig. 4.3** Molecular events associated with recognition of fungal pathogens. Pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) that are present during fungal infections are recognized by pattern recognition receptors (PRRs). The major PRRs are Toll-like receptors (TLRs); C-type lectin receptors (CLRs; such as dectin 1 (also known as CLEC7A), dectin 2 (also known as CLEC6A), DC-specific ICAM3-grabbing non-integrin (DC-SIGN), mincle and the mannose receptor); galectin family proteins (such as galectin 3) and receptor for advanced glycation end-products (RAGE). TLRs and CLRs activate multiple intracellular pathways upon binding to specific fungal PAMPs, including β-glucans (especially β-(1,3)-glucans with varying numbers of β-(1,6) branches), chitin, mannans linked to proteins through N- or O-linkages, β-(1,2)-linked oligomannosides and fungal nucleic acids. These signals activate canonical or non-canonical nuclear factor-κB (NF-κB) and the NOD-, LRR- and pyrin domain-containing 3 (NLRP3) inflammasome, and this culminates in the production of defensins, chemokines, cytokines, reactive oxygen species (ROS) and indoleamine 2,3-dioxygenase (IDO). Complement receptor 3 (CR3) and members of the scavenger receptor family (such as CD36) mediate recognition of β-glucans and the fungal adhesin BAD1 (Blastomyces adhesion 1). After TLR activation, protease-activated receptors (PARs) sense proteolytic virulence factors and tissue injury and contribute to fungal recognition through a dual sensor system. In addition, the alarmin S100B, through the spatiotemporal integration of signals from TLRs and RAGE, allows the immune system to discriminate between pathogen-derived and endogenous danger signals. By forming complexes with various TLR2 ligands, S100B inhibits TLR2 through a paracrine epithelial cell- and neutrophil-mediated regulatory circuit, and this accounts for its anti-inflammatory activity. However, the ability of S100B to bind nucleic acids results in the activation of intracellular TLRs that signal through TIR domain-containing adaptor protein inducing IFNβ (TRIF; also known as TICAM1) and this eventually resolves damage-associated inflammation through transcriptional downregulation of S100B gene expression. ASC; apoptosis-associated speck-like protein containing a CARD; BCL-10, B cell lymphoma 10; CARD9, caspase recruitment domain-containing protein 9; ERK, extracellular signal-regulated kinase; FcRγ, Fc receptor γ-chain; IL, interleukin; IRF3, IFN-regulatory factor 3; MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; MYD88, myeloid differentiation primary response protein 88; SYK, spleen tyrosine kinase. Adapted by permission from Macmillan Publishers Ltd: (Romani L, Nature Reviews Immunology 11, 275–288 (April 2011) | doi:10.1038/nri2939), copyright (2011)

Box 7: Mycoses Affecting Human Central Nervous System

Cryptococcosis is the most common CNS fungal infection in AIDS, occurring in 8.7–13 % of patients. *Cryptococcus neoformans* has a peculiar propensity to affect individuals with cell mediated immunity, and it usually produces meningitis. The cryptococcal organisms may enter the brain via the VR spaces at the base of the brain. Proliferation of the organisms within the VR spaces produces gelatinous pseudocysts of variable size to give a mottled appearance on imaging studies. Meningeal enhancement is not often present unless a chronic inflammation has developed. A chronic relapsing infection can result in cryptococcal brain abscesses (Tien et al. 1991). **Coccidioidomycosis** (also known as Valley fever), caused by *Coccidioides,* begins as a primary pulmonary infection after inhalation of the organism. Most patients remain asymptomatic, and less than 0.2 % of primary infections disseminate. Occasionally, this fungus may reach the meninges, either by hematogenous spread or by direct extension from osteomyelitis of the skull or

vertebrae. Symptoms of chronic meningitis are most common (Mischel and Vinters 1995) but there are cases in which brain involvement occurs without meningitis; however this presentation is unusual (Mendel et al. 1994; Banuelos et al. 1996). Spinal arachnoiditis with obstructive hydrocephalus and cerebral vasculitis with infarcts have been reported. **Histoplasmosis** is an infection caused by the fungus *Histoplasma*. Humans can get histoplasmosis after inhaling the microscopic fungal spores from the air, often after participating in activities that disturb the soil. Most of the people infected have minimal symptoms, and dissemination occurs only rarely. When dissemination does occur, it has been estimated that between one tenth and one fourth of patients have CNS involvement. Although granulomas and other brain parenchymal lesions have been described, most patients with CNS lesions present with meningitis. Patients with histoplasma meningitis develop episodes of dizziness and tinnitus with gradually progressive confusion, nausea, and fever (Couch and Romyg 1977). Patients with **Blastomycosis** have subclinical disease, and dissemination occurs rarely. Disseminated blastomycosis is characterized by granulomatous or suppurating lesions (or both) of the lung, bone, and skin. In some series, blastomycosis has been reported to involve the brain in 6–33 % of disseminated cases. Although patients with CNS blastomycosis usually present with evidence of infection at other sites, occasionally meningitis is the initial presentation, without evidence of extraneural disease (Gonyea 1978; Kravitz et al. 1981). Although CSF cultures are rarely positive, chronic neutrophilic pleocytosis is a common finding in blastomycotic meningitis (Harley et al. 1994). In **Paracoccidioidomycosis,** the lung is the primary location for initial infection; a few patients have widely disseminated disease that involves the CNS, but rarely has the infection been reported to involve only the CNS. Meningitis is an unusual manifestation of infection but occurs occasionally in normal hosts (Dantas et al. 1990). The host response against this microorganism remains poorly understood. **Sporotrichosis** is an infection caused by a fungus called *Sporothrix schenckii*. Pulmonary disease from inhalation of spores is uncommon. Dissemination beyond the skin, lung, and joints is rare; only approximately a dozen cases of *Sporothrix* meningitis have been reported (Scott et al. 1987; Mahajan 2014). Most of the patients with meningitis do not have overt extraneural disease at presentation with diagnosis of this infection extremely slow and difficult. **Penicilliosis** caused by *Penicillium marneffei* infection has been emerging as a public health problem, especially among HIV-infected patients in the areas of endemicity in Southeast Asia, India, and China. Within these regions, *P. marneffei* infection is regarded as an AIDS-defining illness, and the severity of the disease depends on the immunological status of the infected individual (Vanittanakom et al. 2006; Le et al. 2010). Selected members of the other 225 *Penicillium* species are also reported to cause CNS disease. *Penicillium commune* was isolated from multiple brain and lung autopsy specimens from a patient with acute leukemia who was receiving

antibiotics and steroids (Huang and Harris 1963); *Penicillium chrysogenum* was isolated from CSF and brain biopsy samples of 2 nonimmunocompromised individuals with CNS symptoms (Lyratzopoulos et al. 2002; Kantarcioglu et al. 2004). An unidentified *Penicillium* species was isolated from multiple brain lesions of a patient with chronic liver disease at autopsy (Noritomi et al. 2005). **Candidiasis** is caused in humans when members of this species gain access to the blood stream and then the CNS, via contaminated intravenous procedures (del Pozo et al. 1998). Neonates, (Arisoy et al. 1994; Huttova et al. 1998a) neutropenic subjects (Huttova et al. 1998b), and patients recovering from major surgery (Casado et al. 1997; Sakaguchi and Okano 2012) are particularly susceptible to invasive candidiasis, including CNS involvement. Based on autopsy studies, *Candida* species are the most common fungi to invade the CNS (Parker et al. 1978; Mori and Ebe 1992; Liu et al. 2011; Shin 2013). *Candida* may cause meningitis (Buchs and Pfister 1983), ventriculitis (Jamjoom et al. 1992), or parenchymal lesions such as abscesses or granulomas. *C. albicans* is the species implicated in most CNS infections, but other species such as *Candida tropicalis, Candida lusitaniae,* and *Candida parapsilosis*, also occasionally produce CNS infection (Chadwick et al. 1980; Faix 1983; Sarma et al. 1993). **Aspergillosis** is an aggressive opportunistic fungal infection caused by organisms of *Aspergillus* species which gains entrance with inhalation of infected grains or dusts and results in primarily a pulmonary infection. Pathologic changes include a combination of suppuration and granulomas. Dissemination to the CNS may start as basal meningitis, but the organism readily invades vascular structures and extends into the brain parenchyma (Walsh et al. 2008). **Zygomycosis** results due to infection with fungi of the class *Zygomycetes* that are widespread in the environment. Infection is usually due to inhalation of spores. The genus *Rhizopus* is responsible for most infections caused by this group. CNS infection in compromised hosts can occur by direct extension from the paranasal sinuses through hematogenous spread such as illicit intravenous drug use or even by spread up nerve roots into the CNS (Skiada et al. 2009). ***Pseudallescheria boydii*** has emerged over recent years as the cause of fatal disseminated infections in individuals with neutropenia, AIDS, diabetes, renal failure, bone marrow or solid organ transplants, systemic lupus erythematous, and Crohn's disease; in those undergoing corticosteroid treatments; and in leukemia and lymphoma patients. Near-drowning incidents and natural disasters, such as the Indonesian tsunami in 2004, have shown *P. boydii* and the related species *Scedosporium apiospermum* and *Scedosporium aurantiacum* to be the causes of fatal CNS infections and pneumonia in immunocompetent victims who have aspirated polluted water (Thornton 2009). Presumably, this fungus penetrates through the cribriform plate during water immersion or establishes a pulmonary focus with later dissemination to the CNS, producing meningitis or brain abscesses (Kershaw et al. 1990; Hornbeek et al. 2012). **Phaeohyphomycosis** refers to infections

caused by one of several genera and species of pigmented fungi of the family Dematiaceae. Several fungal genera have been reported to affect people and other animals, including *Alternaria*, *Bipolaris*, *Cladophialophora (Xylohypha, Cladosporium)*, *Curvularia*, *Exophiala*, *Fonsecaea*, *Moniliella*, *Phialophora*, *Ramichloridium*, and *Scolecobasidium*. This group of fungi has occasionally caused CNS infection, and for certain species, it appears that there is some neurotropism. *Cladosporium trichoides,* also known as *Xylohypha bantiana* and renamed as *Cladophialophora bantianum,* is the most common isolate of this class of fungi found in CNS infections; the infection usually manifests as a brain abscess, although meningitis has been described (Heney et al. 1989; Osiyemi et al. 2001; Al-Tawfiq and Boukhamseen 2011; Jung and Kim 2014; Sood et al. 2014; Suri et al. 2014). Meningitis caused by other species of these "black molds" is also reported occasionally, and it has even been caused by contaminated corticosteroid injections around the spine (Chen et al. 2013; Chowdhary et al. 2014). **Hyalohyphomycosis** is infection caused by nonpigmented fungi (other than the genera *Aspergillus* or *Penicillium* or the class Zygomycetes) that in tissue form hyphal elements with hyaline or clear walls. Examples of genera causing hyalohyphomycosis in people and other animals include *Acremonium*, *Fusarium*, *Geotrichum*, *Paecilomyces*, *Pseudallescheria*, *Sagenomella*, *Phialosimplex*, *Geosmithia*, *Geomyces*, and *Scedosporium*. Hyalohyphomycosis is far less common than phaeohyphomycosis. In severely neutropenic patients, the soil saprophytes, *Fusarium*species, can produce CNS lesions. Because of similar histopathological features, *Fusarium* infection can be confused with aspergillosis unless cultures are performed. In the growing immunosuppressed population, *Trichosporon* infection, which usually involves only superficial skin or hair shafts, can disseminate to the brain. CNS infections with both *T. beigelii* (Surmont et al. 1990), and *Blastoschizomyces capitatus (Geotrichum capitatum)* (Girmenia et al. 1991) have been reported.

4.5.1.1 Role of Cyto/Chemokines Following CNS Infection by Fungi

The resident glial cells of the brain are responsible for the release of an array of cyto/chemokines, (immunoenhancer and immunosuppressant) (Licinio and Wong 1997). The actions of cytokines on the vasculature in the brain also may be of pathophysiological relevance. There is increasing evidence that a variety of cytokines such as interleukin (IL)-1α, IL-1β, IL-4, IL-6, IL-10, IL-12, IL-18, transforming growth factor-β1 (TGF-β1) and tumor necrosis factor-α (TNF-α) are constitutively expressed in the brain of animal models with CNS fungal infections. In addition, a variety of chemokines such as IL-8, macrophage inflammatory protein-1α (MIP-1α) and -1β (MIP-1β) and monocyte chemoattractant protein-1 (MCP-1) are also involved in the immunopathogenesis of CNS fungal infections

(Ashman et al. 1995). However, the cytokine/chemokine profile is not suggestive of a polarized Th1 or Th2 response and may simply indicate that CNS fungal infection is the result of an ineffective immune response; possibly due to an insufficient antifungal effector function of endogenous glial cells resulting from competing pro- and anti-inflammatory cytokines. A CNS-specific and TNF-α-dependent role for IL-6 and IL-1β in protection against cryptococcosis was suggested by findings with TNF/lymphotoxin-α-deficient mice (Blasi et al. 1995). In patients with AIDS and meningeal cryptococcosis as well as in experimental murine cryptococcal meningoencephalitis, cytokines IL-1α, IL-1β, IL-6, TNF-α, IFN-γ, IL-4, and IL-10 and molecules with bactericidal activity, such as NO and inducible nitric oxide synthase (iNOS) were induced above baseline levels, in the brain during the course of cryptococcal infection (Lortholary et al. 1999; Maffei et al. 2004). Interestingly, it was observed that the concomitant expression of TGF- β1, IL-4 and IL-10 was able to act as immunosuppresant, allowing the continuation of the infectious process. Additionally, although in the early stages of infection NO contributes to the killing of yeasts, the expression of iNOS by endogenous cells may have been modulated by the immunosuppressive cytokines or NO may cause immunosuppression itself, thereby permit progression of the infection. The paradoxical depression of iNOS may happen in the brain as a result of the neuroprotective action of microglia, expressing suppressive cytokines, such as TGF- β1, to a greater degree than proinflammatory cytokines IL-1 β, IL-6, IL-12, IFN-γ and TNF- α under natural conditions (Loddick et al. 1997).

IFN-γ has been reported to play an important role against *C. neoformans* CNS infections. IFN-γ was found to be essential for optimal growth inhibition in a murine model when *C. neoformans* was introduced directly into CNS. Protection mediated via IFN-γ is presumably due to the activation of effector cells already present at the site of infection or recruited to the site, as IFN-γ activates macrophages to better kill cryptococci (Mody et al. 1991; Buchanan and Doyle 2000).

4.5.1.2 Complement System and CNS Fungal Infections

In a variety of CNS diseases (either pathogen induced or neurodegenerative), the complement (C) system contributes to the inflammatory process and plays a central role in host defense against pathogens (Bonifati and Kishore 2007). A few reports are available concerning the role of C system in fungal infections of the brain (Speth et al. 2008). In cases of cerebral aspergillosis increased complement synthesis, a prerequisite for strengthened antifungal potency was visible in resident astrocytes, neurons, oligodendrocytes as well as in infiltrating macrophages after fungal infection. Surprisingly, microglia, although regarded as major immune cells, only marginally participated in synthesis of most complement proteins (Rambach et al. 2008). In in vitro models, *C. neoformans* has been shown to be an activator of the alternative complement pathway. Studies with complement deficient guinea pigs and mice indicate that the complement system plays an essential role in resistance to cryptococcosis. It is likely that the complement system contributes to host resistance by opsonization of the yeast to facilitate attachment and ingestion by

phagocytic cells, as well as by releasing chemotactic fragments of the complement cascade which contribute to the inflammatory response (Kozel 1993).

4.6 Parasitic Infections of the Human CNS

Protozoa and helminths are unique infectious agents that contribute significantly to human morbidity and mortality. Although these agents are referred to as parasites—implying a dependent way of life, they in this context do not differ from other pathogens like bacteria and viruses. Protozoa are single-cell organisms widely distributed in nature. Protozoal infections, though endemic to certain geographic regions for reasons of climate and availability of intermediate hosts to transmit them to man, are also seen outside their original geographical areas, probably facilitated by globalization including increase in international travel and migration of people from their native countries (Chimelli 2011).

Because of the variability of their size (50 μm–15 cm), metazoan (multicellular) parasites pose a unique problem for host immunity (Mulcahy et al. 2005). The helminthic species responsible for CNS disease are diverse. Each of the organisms has a complex life cycle involving human and nonhuman animal hosts in different stages of its development. The helminthic parasites that would be included in the flowing discussion have been grouped according to their class, i.e., as cestodes [tapeworms], nematodes [roundworms], or trematodes [flukes] as members of the same class share common features of development and often produce similar pathology in the CNS (see Table 4.9).

Table 4.9 List of parasitic infections of the CNS

Protozoan infections	Helminthic infections		
	Cestodes	Nematodes	Trematodes
Cerebral Malaria	*Cysticercosis*	*Disseminated Strongyloidiasis*	*Schistosomiasis*
Cerebral Toxoplasmosis	*Echinococcosis (Hydatid Disease) and Sparganosis*	*Trichinosis*	*Paragonimiasis and Fascioliasis*
Trypanosomiasis		*Eosinophilic Meningitis: Angiostrongyliasis, Gnathostomiasis, and Visceral Larva Migrans*	
Primary Amebic Meningoencephalitis and Granulomatous Amebic Encephalitis			
Cerebral Amebiasis due to Entamoeba histolytica			

Difficulty in combating protozoal diseases is attributed to an incomplete understanding of their pathogenesis and pathophysiology. In particular, it is not clear how protozoa cross the BBB, a key step in the development of CNS infections caused by these parasites. It is believed that at the cellular level, the strategies used by neuropathogenic protozoa to traverse across the blood–brain barrier include:

1. paracellular route by disrupting the tight junctions,
2. transcellular route while maintaining the integrity of the endothelial cell function,
3. by means of infected cells (Trojan horse mechanism) and/or
4. by inducing injury to the cerebral endothelium resulting in the disintegration of the blood–brain barrier (Elsheikha and Khan 2010).

Transcellular traversal involves penetration of protozoa through the brain microvascular endothelial cells (BMEC). This mode of invasion has been suggested for Trypanosoma spp. The paracellular route involves protozoa crossing of the BBB between the endothelial cells, by degrading the tight junction proteins. Several protozoa target the paracellular route including *Plasmodium falciprum*, Trypanosoma spp., *Toxoplasma gondii*, Acanthamoeba spp., Balamuthia spp., and Babesia spp. Leucocyte-facilitated entry into the CNS, using the Trojan horse mechanism, has been suggested for *T. gondii*. In addition, Acanthamoeba and Balamuthia produce BMEC death resulting in blood–brain barrier perturbations. In the aforementioned, the BMEC layer is the principal target of these protozoa. Therefore, mechanisms by which protozoal infections manipulate the BMEC structure and function is a topic of particular importance (Elsheikha and Khan 2010). Table 4.10 gives a complete idea of how different parasites gain access to the CNS.

Parasites can reach the CNS through CVOs via the bloodstream even without invading the brain parenchyma and/or prior to neuroinvasion. From the CVOs parasites can potentially affect neuronal functions at distinct brain sites, through humoral parasite–host interaction and/or axonal retrograde signaling. A detailed experimental model provided by *Trypanosoma brucei* showed, that the parasites reside for some time within the CVOs before crossing the BBB (Kristensson et al. 2010).

4.6.1 CNS Inflammation Due to Protozoal Infections

Every year, with over 500 million clinical cases, cerebral malaria is the most severe neurological complication of infection with Plasmodium falciparum. Cytokines and chemokines play a complex role in its pathogenesis and have both protective and harmful effects on the brain.

Parasite antigens released at schizogony trigger the release of both pro- and anti-inflammatory cytokines and a critical balance between these mediators is critical for parasite control (Idro et al. 2010). It has been reported that TNF-alpha, upregulates ICAM-1 expression on the cerebral vascular endothelium, thereby

Table 4.10 Portals of entry into the host and routes for spread to the CNS of selected human parasites

Parasite	Mode of entry	Route of spread to CNS	BBB interaction
Schistosoma spp.	Schistosoma spp. Skin, larval penetratio	Bloodstream as migrating worms or seeding of eggs	Egg embolization
Plasmodium spp.	Skin, mosquito bite	Bloodstream in erythrocytes	Infected erythrocytes attach to endothelia
Babesia	Skin, tick bite	Bloodstream	Infected erythrocytes attach to endothelia
Taenia solium	Intestine, larval penetration	Bloodstream	Lodge in small cerebral vessels
Toxocara canis	Intestine	Bloodstream	Choroid plexus; Cross BBB
Angiostrongylus cantonensis	Intestine	Bloodstream	Cross BBB
Gnathostoma spinigerum	Intestine	Along peripheral nerve roots	?
Acanthamoeba	Respiratory tract, skin	Bloodstream	Cross BBB
Balamuthia mandrillaris	Respiratory tract, skin	Bloodstream	Degrade and cross BBB
Trypanosoma brucei	Skin, tsetse fly bite	Bloodstream	Choroid plexus, CVOs Cross BBB similar to T cells
Toxoplasma gondii	Intestinal epithelia	Bloodstream in monocytes	Cross in infected monocytes
Trypanosoma cruzi	Skin, bug bite	Bloodstream	Cross BBB, probably within monocytes
Encephalitozoon cuniculi	Respiratory, intestinal tracts	Bloodstream	?
Naegleria fowleri	Nasal cavity	Olfactory route	No

Adapted from Kristensson et al. (2013)

increasing the cytoadhesion of the parasitized RBCs. Near areas of sequestration, there is increased local synthesis. The timing of this is important since early in disease, TNF may be protective but prolonged high levels contribute to complications (Hunt and Grau 2003) including dysregulation of synaptic transmission (strength, scaling and long-term potentiation) (Clark and Alleva 2009). Several other cytokines and chemokines are important and in particular, IL-1b, IL-6, and IL-10 (John et al. 2008), but low levels of the chemokine RANTES is independently associated with mortality (John et al. 2006). The role of nitric oxide (NO) is controversial. The association between NO activity and inducible nitric oxide synthase with pathogenesis has been inconsistent (Anstey et al. 1996; Cramer et al. 2005). Other inflammatory products such as the metabolites of the kynurenine pathway—quinolinic (NMDA receptor agonist and an excitotoxin) and kynurenic

acid (NMDA receptor antagonist)—may also be important in pathogenesis. Quinolinic acid causes seizures in animal models of brain disease while kynurenic acid is generally thought of as neuroprotective. Excitation by quinolinic acid may contribute to convulsions in cerebral malaria. In children, there are graded increases in cerebrospinal fluid concentration across outcome groups of increasing severity. Because of the role of NMDA receptors in modulating neurotransmission and as agonists, high levels of quinolinic acid may have long-term deleterious effects on cognitive functions (Dobbie et al. 2000; Medana et al. 2002). In mouse model of the disease, an accumulation of activated/effector CD8+ lymphocytes has been observed that have a duplicitous role in malaria infection—both helping to clear the parasite from the liver and blood and in orchestrating the damaging neuro-inflammation seen in cerebral malaria (Lamb et al. 2006).

Toxoplasmosis is caused by the parasite Toxoplasma gondii that can infect humans in 3 different ways

(a) by ingesting *T. gondii* tissue cysts (containing bradyzoites) present in the undercooked meat (especially lamb and pork) of infected animals;
(b) by ingesting highly infectious oocysts (containing sporozoites) present in water, garden soil, children's sandboxes, etc., contaminated by infected cat feces; or
(c) through congenital transplacental transmission of rapidly replicating tachyzoites from mothers who become infected during pregnancy (e.g., by changing the cat litter) and pass the infection to the fetus. Infected fetuses have a high incidence (almost 50 %) of CNS involvement. Early infection before 20 weeks of pregnancy is associated with severe, persistent neurologic abnormalities, whereas late infection after 30 weeks is rarely associated with deficits (Carruthers and Suzuki 2007).

Cerebral toxoplasmosis is an opportunistic infection which typically affects patients with HIV/AIDS, and is the most common cause of cerebral abscess in these patients, but in immunocompetent patients, acute encephalitis is rare. After proliferation of tachyzoites in various organs during the acute stage, the parasite forms cysts preferentially in the brain and establishes a chronic infection, which is a balance between host immunity and the parasite's evasion of the immune response. A variety of brain cells, including microglia, astrocytes, and neurons, can be infected (Jones et al. 1986; Chao et al. 1993; Fischer et al. 1997b; Halonen et al. 1998). In these cells TLR11 has been reported to be involved in recognition of the parasite in an experimental model of the disease, leading to generation of downstream immune responses (Atmaca et al. 2014). Among the cytokines produced in response to *T. gondii* infection, IFN-γ, which is released by T-cells that infiltrate into the brain following infection, is the most important (Schluter et al. 1995). In addition to IFN-γ, infection with *T. gondii* induces a variety of other proinflammatory (e.g., IL-1 and TNF-α) and anti-inflammatory (e.g., IL-10 and TGF-β) cytokines by microglia, astrocytes, and neurons (Fischer et al. 1997a; Schluter et al. 1997, 2001). These cytokines appear to play an important role in regulating the

resistance of hosts against *T. gondii* infection in the brain. Other than T cells B cells (Schluter et al. 1998), NK cells (Schluter et al. 1995), macrophages (Suzuki et al. 2005), and dendritic cells (Fischer et al. 2000) also infiltrate into the brain after infection.

Human African **trypanosomiasis** (sleeping sickness; HAT), caused by protozoan parasites of the genus Trypanosoma comes in two variants: East African (caused by *Trypanosoma brucei* rhodesiense) and West African (caused by *Trypanosoma brucei* gambiense). It has emerged over the last few decades as a major threat to human health in Africa (currently occurring in 36 countries in sub-Saharan Africa with about 60 million people at risk of developing the disease) (Kennedy 2004). The clinical course of this disease has two stages. In the first stage, the parasite is found in the peripheral circulation, but it has not yet invaded the CNS. Once the parasite crosses the BBB and infects the CNS, the disease enters the second stage. In T. b. rhodesiense a few weeks after infection, the parasite invades the CNS and eventually causes mental deterioration and other neurologic problems leading to death within a few months. However, in case of T. b. gambiense after 1–2 years, following infection, there is evidence of CNS involvement, with personality changes, daytime sleepiness with nighttime sleep disturbance, and progressive confusion. Other neurologic signs, such as partial paralysis or problems with balance or walking may occur, as well as hormonal imbalances. The course of untreated infection rarely lasts longer than 6–7 years and more often kills in about 3 years. The pathologic substrate of late-stage sleeping sickness is a meningoencephalitis in which cellular proliferation occurs in the leptomeninges, and a diffuse perivascular white matter infiltration consisting of lymphocytes, plasma cells, and macrophages is prominent. The perivascular cuffs and adjacent parenchyma contain markedly activated astrocytes and macrophages, and the white matter contains pathognomonic morular or Mott cells, which are thought to be modified plasma cells containing eosinophilic inclusions comprising of IgM (Adams et al. 1986).

Alteration of cytokine levels has been detected in patients with CNS sleeping sickness. Elevated IL-10 levels were detected in both the plasma and CSF in both early- and late-stage rhodesiense disease and total, but not free, plasma TNF-α level were also higher in late-stage disease. However, the source of IL-10 elevation is unclear. Similar studies in patients with gambiense infection have also reported elevations of CSF IL-10 levels in late-stage disease, as well as a rise in IL-6 and IL-8 (MacLean et al. 2001; Lejon et al. 2002). Other abnormalities which have been reported in patients with CNS HAT include very high CSF levels of prostaglandin D2 in CSF (Pentreath 1995), which may be related to the marked somnolence, and raised blood and CSF endotoxin levels that may also contribute to the CNS pathology (Pentreath 1989). In a mouse model of HAT it has been reported that astrocytes are activated 14–21 days after infection and prior to the development of the inflammatory response (Hunter et al. 1992), and those transcripts for several cytokines such as TNF-α, IL-1, IL-4, IL-6, and IFN-γ can be detected in the brain at this time (Hunter et al. 1991). Thus, it seems that early astrocyte activation is likely to be critical regulator in generating the CNS inflammatory response. There is also evidence for the role of various chemokines such as macrophage inflammatory

protein (MIP)-2, RANTES, and MIP-1α produced by astrocytes, microglia, and T cells early in the CNS infection in a rat model (Sharafeldin et al. 2000).

Another form of human trypanosomiasis called Chagas disease is caused by the parasite Trypanosoma cruzi, which is transmitted to animals and people by insect vectors that are found only in the Americas; hence, this disease is also referred to as **American trypanosomiasis**. CNS involvement is rare, however not unknown in immunocompromised people (Leiguarda et al. 1990; Pittella 2009). During the acute phase, amastigotes are rarely found, but inflammatory infiltrates are scattered throughout the CNS. Moreover, peripheral lymphocytes and antibodies recognizing neural components were described, suggesting the participation of the immune system in the genesis of neural lesions. In a mouse model, the disease inflammatory infiltrates were observed during the acute phase that did not correlate with the presence of detectable *T. cruzi* antigens. Infiltrates consisted mainly of CD8+ lymphocytes, although macrophages and a few CD4+ cells were also observed. In the chronic stage of infection, although neuropathies were a common finding, only mild inflammatory infiltrates were detected. The results suggested that the presence of CNS inflammatory infiltrates is not directly related to the presence of parasite antigens, which in turn indicates that encephalitis resolves during the acute phase of Chagas' disease (Silva et al. 1999).

Amebic meningoencephalitis, an extremely rare and sporadic CNS (CNS) infection, is caused by free-living amoebae; specifically, Naegleria fowleri (Cermeno et al. 2006) and Balamuthia mandrillari (Bakardjiev et al. 2003) as well as species of Acanthamoeba and Sappinia (Marciano-Cabral 2009). These free-living amoebas are found in stagnant fresh water pools and can infect humans swimming in the pools via the nasal route. The infection is nearly always fatal likely because of the difficulty of diagnosis and poor to marginal response of patients to therapy. **Granulomatous amebic encephalitis** (GAE) is equally rare, and usually fatal infection of the CNS is caused by Acanthamoeba species or Balamuthia mandrillaris. It usually occurs in people with an impaired immune system or generally poor health. GAE apparently results from either acanthamebic keratoconjunctivitis, via an uncommon phenomenon in which amoebae spread from the cornea to the CNS, or from the hematogenous spread of the ubiquitous organisms from primary inoculation sites in the lungs or skin to the CNS, where abscesses and focal granulomatous infections result. Characteristic granulomatous lesions in the CNS are a result of the host immune response and are most likely composed of CD4+ and CD8+ T cells, B cells, and infiltrating macrophages. The localization of immune cells in the brain suggests the involvement of multiple cyto/chemokines in protection as well as in pathophysiological complications. IFN-γ is one of the earliest cytokines to be involved and may play an important role in the activation of immune cells. IFN-γ, through the pro-inflammatory network, upregulates the release of specific cytokines, including TNF-α, IL-6, IL-β and IL-1α, which may initiate the immune response to the parasite in the brain (Benedetto et al. 2003). Other studies showed that microglial cells secrete IL-β, IL-α and TNF-α in response to the parasite (Marciano-Cabral et al. 2000). Interestingly, it has been observed that microglia primed with IFN-γ and TNF-α exhibit amoebicidal effects, but when

primed with IFN-γ and IL-6, exhibit amoebistatic effects (Benedetto and Auriault 2002a, b). **Cerebral amoebic abscess** caused by Entamoeba histolytica infection, is very rare and not related to immunodeficiency that causes cerebral abscess following haematogenous spread from liver (Stauffer and Ravdin 2003).

Visceral leishmaniasis (*Leishmania donovani*) is a relatively common infection and spreads haematogenously in the body but CNS involvement is extremely rare. Experimentally, *L. amazonensis* can cause encephalitis with parasites in the cerebral parenchyma (Abreu-Silva et al. 2003) and there is only one recorded case of CNS infection by *L. donovani* in human. A child with drug-refractory visceral leishmaniasis had meningitis associated with the presence of the parasites in the CSF (Prasad and Sen 1996).

4.6.2 CNS Inflammation Due to Metazoan (Helminthic) Infections

Neurocysticercosis is the most frequently encountered parasitic infestation of the CNS. Originally endemic in underdeveloped countries, predominantly Latin America, Africa, Asia, and some portions of Eastern Europe, it is becoming increasingly frequent in North America in immigrant populations. Humans become accidental hosts for the larval stage of Taenia Solium, (pork tapeworm), by ingesting contaminated material. The eggs hatch in the stomach and larvae burrow through the gut wall and become distributed by the circulatory system. There is a predilection for involvement of the brain. Patients most often present with seizures, elevated intracranial pressure, focal neurologic abnormalities, and altered mental status. Asymptomatic infections are also common. Four forms of neurocysticercosis are described: meningeal, parenchymal, ventricular and mixed. The cysticerci (hatched larva) are able to survive in the human brain by disarming host defenses. They secrete prostaglandins and other compounds (paramyosin, taeniastatin, sulfated polysaccharides) that inhibit or divert complement activation and cytokine production, resulting in only minimal host inflammation around the viable cysticercus. In addition, humoral antibodies do not kill the mature metacestode. Taeniastatin and other poorly defined factors may also interfere with lymphocyte proliferation and macrophage function, inhibiting normal cellular immune defenses (White et al. 1997; Terrazas 2008). The clinical manifestations commonly result when an inflammatory response develops around a degenerating cysticercus after it has died that can lead to an encephalitic syndrome in many patients, with temporary clinical deterioration (Garcia et al. 2003). In mouse model of neurocysticercosis, an upregulation of all known TLRs, except TLR5, has been reported (Mishra et al. 2006). In another model utilizing Taenia crassiceps, it was reported that specifically, TLR2-dependent signaling pathways are involved in the recognition of the parasite and in the subsequent activation of the innate immune system and production of inflammatory cytokines, which appear to be essential to limit infection during experimental cysticercosis (Reyes et al. 2011).

Echinococcosis is a parasitic disease caused by infection with tiny tapeworms of the genus Echinococcoccus. Infection with larvae of *E. granulosus* causes cystic echinocccosis and that of *E. multilocularis* causes alveolar hydatid disease. Both of the parasites are known to infect brain and form cysts or parasitic tumors. Cyst rupture is most frequently caused by trauma or during surgical procedures and may cause mild to severe anaphylactic reactions, even death, as a result of the release of cystic fluid. Not much is known about the immune reaction in CNS in response to these cysts; however, systemic reactions are known to happen in case of cyst rupture (Salunke et al. 2014).

Trichinosis is infection caused by the roundworm *Trichinella spiralis*. The parasite gains entry into the body by ingestion of contaminated meat of various animals. The parasite can infect several organs before it gains entry into the CNS where it results in encephalitis and meningitis. Trichinella larvae can migrate in CNS and cause diffuse lesions, obstruction of the blood vessels, and inflammatory infiltrate. Among the infiltrating cells, eosinophils stimulated by either chemotactic factor or cytokines, such as interleukin 5, have been reported to kill the larvae and cause vascular injuries (Bruschi et al. 2008) and TNF-alpha has been reported to regulate the eosinophil toxicity (Taratuto and Venturiello 1997).

Eosinophilic meningitis can be the result of noninfectious causes or infectious agents. Among the infectious agents, *Angiostrongylus cantonensis* (also known as the rat lungworm) and *Gnathostoma spinigerum* are the most common. The infection results in meningitis with a high percentage of eosinophils in the CSF (Graeff-Teixeira et al. 2009). Pathologically, eosinophilic meningitis is defined as the presence of ≥10 eosinophils/μL in CSF or at least 10 % eosinophils in the total CSF leukocyte count (Kuberski 1979, 1981). As eosinophils are not normally found in the CSF, their presence is suggestive of a number of different etiologies such as other infections, including Baylisascaris infection, toxocariasis, and neurocysticercosis; malignancies; medications; and the presence of intracranial foreign bodies (Lo Re and Gluckman 2003). Eosinophils are specialized in exocytotic degradation of large parasites, through the extrusion of cellular granules and contents. They are considered important effector cells of the adaptive immune response. Specifically, they are involved in the Th2-type response, which is mediated by a complex array of cytokines (interleukins 2, 4, 5, 10, 12, 13, 16, and 18 and transforming growth factor), chemokines (RANTES and eotaxins), and lipid mediators (platelet-activating factor and leukotriene C4) (Hogan 2007).

Larva migrans is a group of clinical syndromes that result from the movement of parasite larvae through host tissues. **Visceral larva migrans** occurs when parasitic larvae migrate through the internal organs of the host, CNS infections being the most serious form of the disease. *Toxocara canis* and *T. cati* are the most important causes of visceral larva migrans in humans, even though *Baylisascaris procyonis*, and *Ascaris suum* have also been reported to be involved (Beaver 1959). Infections with these parasites are manifested as acute eosinophilic meningoencephalitis (Othman 2012).

Strongyloidiasis is caused by the nematode Strongyloides stercoralis. Other Strongyloides include *S. fülleborni*, which infects chimpanzees and baboons and

may produce limited infections in humans. The larvae of the parasite may sometimes develop rapidly into the infective stage in the gut where they penetrate the intestinal lining instead of passing out of the body in the feces, as occurs normally. This modification of the life cycle, called internal autoinfection, explains persistent strongyloidiasis, which can last as long as 40 years in people who have moved to areas where the infection is not generally found (Pittella 2013). Autoinfection may produce heavy infections, dissemination to other organs and severe disease, especially in people with reduced immunity such as those receiving corticosteroids or other immunosuppressive therapy, or those with acquired immune deficiency due to retroviruses such as human T cell lymphotropic virus-1 (HTLV-1) (Takayanagui et al. 1995). CNS involvement includes parasitic meningitis (eosinophilic), brain abscess, and diffuse invasion of the brain (Dokmeci et al. 2013; Woll et al. 2013). *S. stercoralis* antigens activate eosinophils; induce the expression of MHC class II and T-cell co-stimulatory molecules. Activated eosinophils in turn stimulate the infiltrating T cells for antigen-specific immune responses (Padigel et al. 2006). Eosinophils are also believed to function as APCs for the induction of the primary and secondary Th2 immune responses to *S. stercoralis* (Padigel et al. 2007) indicating an essential role for eosinophils in the interface between innate and adaptive immune responses. Reports have shown that both eosinophils and neutrophils were found to be required in the protective innate immune response while only neutrophils were necessary for the protective adaptive immune response to larval *S. stercoralis* (Galioto et al. 2006).

Schistosomiasis is an acute and chronic parasitic disease caused by trematodes of the genus Schistosoma. There are two major forms of schistosomiasis—intestinal (caused by *Schistosoma mansoni, Schistosoma japonicum, Schistosoma mekongi, Schistosoma guineensis* and related *S. intercalatum*) and urogenital (caused by *Schistosoma haematobium*). Found predominantly in tropical and sub-tropical climates, schistosomiasis infects 240 million people in as many as 78 countries, with a vast majority of the burden occurring in Africa. Schistosomiasis ranks second only to malaria as the most common parasitic disease. **Neuroschistosomiasis** is an ectopic form of the disease that occurs when ova and or adult worms reach the CNS either when the ova are carried to the CNS through arterial or retrograde venous blood flow via the valveless perivertebral plexus of Batson, being deposited anywhere along the path of the blood flow, or the ova are deposited in situ after the anomalous migration of adult worms (Ferrari and Moreira 2011). Neuroschistosomiasis is mainly associated with *S. japonicum* infection. Involvement of the CNS in S. mansoni infection is neglected and underestimated. Neuroschistosomiasis can be classified into cerebral, spinal, and encephalomyelitic forms in the course of an acute or chronic infection (Vale et al. 2012). The most common site of clinically significant NS is the spinal cord where the pathological findings include a granulomatous intramedullary mass in the caudal spinal cord, radicular involvement with granulomatous changes surrounding the conus medullaris and nerve roots of the cauda equina, necrotic exudative granulomas and hemorrhage, and asymptomatic deposition of ova in the spinal cord (Scrimgeour and Gajdusek 1985; Carod Artal et al. 2004). The granulomatous reaction of the host to the presence of the ova is the

major factor in the pathogenesis of schistosomiais. Granulomas are strictly mediated by CD4+ T helper (Th) cells specific for egg antigens and can occur in environments dominated by either Th-1 or Th-2 type cytokines. The immune response to the antigens released from the ova is at a maximum intensity in the early stages of infection, leading to the formation of necrotic-exudative granulomas and the immune response declines over the course of the infection. It is followed by a spontaneous downregulation of the granulomatous response, characterized by delayed dermal reaction and production of cytokines and macrophage inhibitory factor. Even in presence of strong eosinophil stimulation promoter cytokines production declines concurrently with the waning of the granulomatous inflammation; this waxing and waning phase is followed by fibrosis which cumulative and mostly irreversible. Thus, the three stages of the granulomatous reaction (necrotic-exudative, productive and fibrotic) are strictly dependent on the evolution of the immunological response and the interaction between host and infection (Nascimento-Carvalho and Moreno-Carvalho 2005).

Paragonimiasis, is caused by infection with a number of species of trematodes belonging to the genus Paragonimus. The most common are *P. westermani, P. heterotremus, P. philippinensis, P. africanus, P. uterobilateralis, P. caliensis, P. kellicotti* and *P. mexicanus*. Ectopic paragonimiasis may result from erratic migration of the juvenile worms: the most frequent locations include the abdominal cavity and subcutaneous tissues and, most frequently, the brain: **cerebral paragonimiasis** is a severe condition that may be associated with headache, visual impairment, and epileptic seizures (Kohli et al. 2015). Not much is known about the detailed mechanism of cerebral inflammation caused by this parasite but it is believed to be mediated by eosinophils (Shin et al. 2005) as in the case of other trematodes. Another type of similar ectopic parasitic infection of the CNS is **cerebral fascioliasis** caused by two species of trematodes—*Fasciola hepatica* and *F. gigantic*. Eosinophilia (>500/mm^3) is observed in 96 % of cases of infection and with 2/3rd of the cases have general leukocytosis (>10,000/mm^3) (Mas-Coma et al. 2014).

4.7 Prion Infections in CNS

Prion diseases or transmissible spongiform encephalotheis (TSEs) represent a family of neurodegenerative disorders associated with the loss of brain cells and caused by proteins called prions (derived from ‘protein’ and ‘infection’). The diseases are found in both humans and animals, such as Creutzfeld–Jakob disease and mad cow disease, respectively. Although mostly harmless, prions can transform into infectious agents, which accumulate in the brain and destroy the nervous tissue. Prion diseases are caused by the templated misfolding of normal cellular prion protein (PrPC) into an abnormal form (PrPSc) that predominantly has a β-sheet structure and may show the biochemical properties of amyloid (Prusiner 1982).

Prion diseases have been hypothesized to be caused by misfolding of cellular prion protein that has been found to affect majorly immune-competent hosts. Although contradictory but several reports support the theory that disease-associated PrP gets deposited in lymphoid follicles and replicates in follicular dendritic cells (FDC) (Brown et al. 1999; Bruce et al. 2000; Bencsik et al. 2001; Lezmi et al. 2001). In addition FDCs have been hypothesized to act as the gateways to the CNS in case of peripheral infection of prion agents. Various studies conducted in transgenic mouse models suggest that in case of peripheral route of infection for prion pathogenesis the immune system plays a pivotal role in the propagation of the disease. Innate immune system is considered to be a protective system that is older in evolutionary terms than the adaptive immune system and forms the first line of defence to any invading pathogen. Pattern recognition receptors, such as TLRs, CLRs, NLRs and RLRs forms an integral part of innate immune system that helps to recognize the PAMP of the invading pathogens (Akira and Takeda 2004; Fritz et al. 2006; Geijtenbeek and Gringhuis 2009; Takeuchi and Akira 2009). In addition to these the innate immune system comprises of both cellular (inflammatory) and proteinaceous components that mobilizes rapidly in response to any alarming signals from the epithelia or resident innate immune cells. Hence in case of prion diseases, it is believed that the interaction between the innate immune system and prion agents in the earlier stages of infection is important for the disease progression. Epithelial and microfold cells form the first physical barrier to the infection. Since CNS happens to be the effecter organ of the prion diseases as discussed earlier; the distance between the sites of entry into the host (i.e., after escaping through the first physical barrier) to the FDC and subsequently the CNS becomes an important factor in prion pathogenesis. Thus, from the above discussion it is clear that rather than penetrating through the skin epithelial layers prion agents invades through oral and nasal routes more efficiently. The specialized microfold cells (M cells) localized to the follicle-associated epithelium (FAE) of intestinal Peyer's patches form the major gateway to the prion agents through the oral and nasal pathways. Albeit there have been reports on enterocyte derived extracellular vesicle and lumenal sampling by dendritic cells (DCs) to be other possible gateways (Rescigno et al. 2001; Kujala et al. 2011). Complement system provides the first active response to prion agents. It has been reported to opsonise the prion or TSE agents via the classical complement activation pathway involving complement components including C1q and C3 and hence aid in their targeting to the lymphoid follicles. C1qa, C2 or C3 deficient animal models have been observed to show a better peripheral system response to prion pathogenesis (Klein et al. 2001). The role of complement system in CNS is debatable.

Mast cells have been previously reported to have a higher expression level of prion proteins (PRPC) that make mast cells to be an interesting subject among the components of the innate immune system in the context of prion pathogenesis (Haddon et al. 2009). In CNS mast cells shed the PRPC via a proteolytic or lypolytic

cleavage mechanism by removing the glycosylphosphatidylinisotol (GPI) anchor from the prion protein. This makes the substrate available for the prion agents for further conversion into misfolded proteins (Haddon et al. 2009). The accumulation of misfolded proteins in brain is potent to amyloid formation in the brain even without clinical prion disease symptoms. While in peripheral system the activation of mast cells rely on C3a and CCL3 since there is little evidence of the induction of an adaptive immune response and specific anti-prion antibody generation during pathogenesis. Mononuclear phagocytes (MNPs) form an integral part of the innate immune system. Due to their diversity MNPs can be broadly categorized into following:

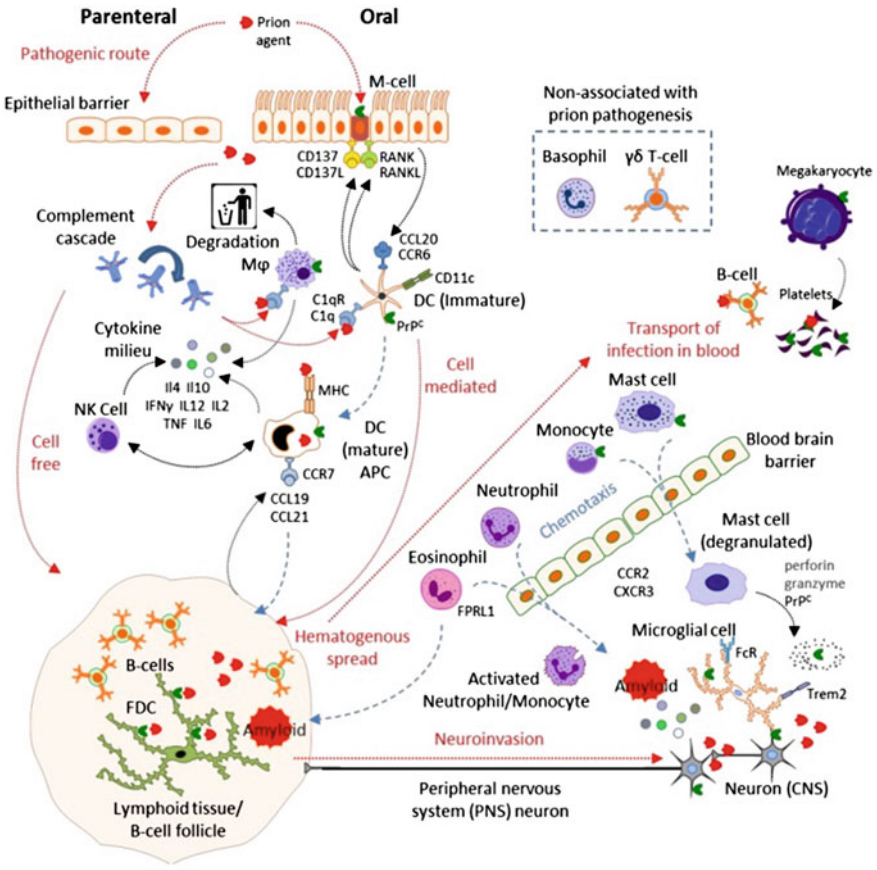

Fig. 4.4 Innate immunity and prion pathogenesis. Prions invade through the epithelial layers or enter through the oral route to enter the host system. Thereafter through various mechanisms like cell mediated or hematogonous spread prions migrate to the blood and enters the Central Nervous System (CNS). During the process the innate immune system both cellular and proteinaceous components play a major role in the prion pathogenesis. Adapted from Bradford and Mabbott (2012) (open access)

(i) Resident cells with degradative functions
(ii) Resident cells with antigen presenting functions
(iii) Systemic circulating cells responsive o inflammatory stimuli.

Due to the non-inflammatory nature of the disease the circulating MNPs are of less importance in the current context of the study. Macrophage has been evidenced to play a crucial role in degradative and prion clearance and of present is a critical therapeutic target in prion pathogenesis (Sassa et al. 2010). Antigen presenting cells (APCs) similar to complement system mediate the transportation of prion agents to lymphoid tissues. Depletion model experiments involving CD11c-expressing cells (a commonly used marker indicative of classical DC) have revealed the altered prion pathogenesis in cases of infection through oral and intraperitoneal routes. The CNS resident MNP, microglia however does not respond directly to the presence of misfolded prion protein but requires priming by other CNS cell types like neurons and astrocytes (Marella and Chabry 2004). Hence, MNPs are of considerable interest in developing therapeutic strategies against prion pathogenesis. Role of other components of innate immune system such as granulocytes, natural killer cells, γδT, megakaryocytes and platelet cells need further in-depth study to confirm their importance in the context of prion infection. Hence, from the above discussion and prior findings it becomes clear that the innate immune system function in disease pathogenesis operates via non- PRPC dependent mechanism(Loeuillet et al. 2010). Then again it gives rise to another question that what actually differentiates the components of the innate immune system in detecting the prions? There have been few reports that answer the question that stress upon the ability to detect prion protein gene (Prnp). Since the mature protein is labile or difficult to detect, their corresponding mRNA message might be helpful in answering the differential ability of the innate immune response (Ford et al. 2002). It has been observed that Prnp expression levels are highest among the macrophages, DC, microglia, Langerhans cells, and IFN-producing killer DCs as shown in Fig. 4.4.

Trangenic mouse models have been used to study the effect of various genes in the context of prion pathogenesis. Various genes have been proven to play active and few to play passive roles in the progression of prion infection. Knockout of Prnp also resulted in complete resistance to prion disease (Bueler et al. 1993). But majority of such knockdown studies have concluded that depletion model for a particular gene is not a solution to block prion infection. Hence, there is need of multiple gene targeting. Hence, a pathway analysis of innate immunity associated genes implicated in prion pathogenesis and determined major host upstream regulators of prion pathogenesis-associated gene expression helps in drafting an effective strategy against the disease (Bradford and Mabbott 2012).

In conclusion, innate immune system plays an important role in the initial stages of prion infection starting from its entry to getting transferred to the CNS. In the peripheral and CNS too the innate immune system actively responds to the prion

proteins and alters the progression of the disease. Among the various components of the innate immune system the MNPs hold critical importance. Hence, further in-depth studies along with therapeutic strategies involving multiple gene targeting would hold promising future in prion research.

References

Abeyama K, Stern DM, Ito Y, Kawahara K, Yoshimoto Y, Tanaka M, Uchimura T, Ida N, Yamazaki Y, Yamada S, Yamamoto Y, Yamamoto H, Iino S, Taniguchi N, Maruyama I (2005) The N-terminal domain of thrombomodulin sequesters high-mobility group-B1 protein, a novel antiinflammatory mechanism. J Clin Investig 115:1267–1274

Abreu-Silva AL, Calabrese KS, Tedesco RC, Mortara RA, Goncalves da Costa SC (2003) Central nervous system involvement in experimental infection with *Leishmania* (*Leishmania*) *amazonensis*. Am J Trop Med Hyg 68:661–665

Adams JH, Haller L, Boa FY, Doua F, Dago A, Konian K (1986) Human African trypanosomiasis (T.b. gambiense): a study of 16 fatal cases of sleeping sickness with some observations on acute reactive arsenical encephalopathy. Neuropathol Appl Neurobiol 12:81–94

Agarwal N, Lamichhane G, Gupta R, Nolan S, Bishai WR (2009) Cyclic AMP intoxication of macrophages by a *Mycobacterium tuberculosis* adenylate cyclase. Nature 460:98–102

Akira S, Takeda K (2004) Toll-like receptor signalling. Nat Rev Immunol 4:499–511

Almenoff PL, Johnson A, Lesser M, Mattman LH (1996) Growth of acid fast L forms from the blood of patients with sarcoidosis. Thorax 51:530–533

Al-Tawfiq JA, Boukhamseen A (2011) Cerebral phaeohyphomycosis due to Rhinocladiella mackenziei (formerly Ramichloridium mackenziei): case presentation and literature review. J Infect Pub Health 4:96–102

Anneken K, Fischera M, Evers S, Kloska S, Husstedt IW (2006) Recurrent vacuolar myelopathy in HIV infection. J Infect 52:e181–e183

Anstey NM, Weinberg JB, Hassanali MY, Mwaikambo ED, Manyenga D, Misukonis MA, Arnelle DR, Hollis D, McDonald MI, Granger DL (1996) Nitric oxide in Tanzanian children with malaria: inverse relationship between malaria severity and nitric oxide production/nitric oxide synthase type 2 expression. J Exp Med 184:557–567

Antinori S (2013) New insights into HIV/AIDS-associated cryptococcosis. ISRN AIDS 2013:471363

Arisoy ES, Arisoy AE, Dunne WM Jr (1994) Clinical significance of fungi isolated from cerebrospinal fluid in children. Pediatr Infect Dis J 13:128–133

Arlaud GJ, Gaboriaud C, Thielens NM, Budayova-Spano M, Rossi V, Fontecilla-Camps JC (2002) Structural biology of the C1 complex of complement unveils the mechanisms of its activation and proteolytic activity. Mol Immunol 39:383–394

Ashman RB, Bolitho EM, Fulurija A (1995) Cytokine mRNA in brain tissue from mice that show strain-dependent differences in the severity of lesions induced by systemic infection with Candida albicans yeast. J Infect Dis 172:823–830

Atmaca HT, Kul O, Karakus E, Terzi OS, Canpolat S, Anteplioglu T (2014) Astrocytes, microglia/macrophages, and neurons expressing Toll-like receptor 11 contribute to innate immunity against encephalitic Toxoplasma gondii infection. Neuroscience 269:184–191

Baddley JW, Salzman D, Pappas PG (2002) Fungal brain abscess in transplant recipients: epidemiologic, microbiologic, and clinical features. Clin Transplant 16:419–424

Bakardjiev A, Azimi PH, Ashouri N, Ascher DP, Janner D, Schuster FL, Visvesvara GS, Glaser C (2003) Amebic encephalitis caused by *Balamuthia mandrillaris*: report of four cases. Pediatr Infect Dis J 22:447–453

Baker DG, Woods TA, Butchi NB, Morgan TM, Taylor RT, Sunyakumthorn P, Mukherjee P, Lubick KJ, Best SM, Peterson KE (2013) Toll-like receptor 7 suppresses virus replication in

neurons but does not affect viral pathogenesis in a mouse model of Langat virus infection. J Gen Virol 94:336–347
Bale JF Jr (2014) Measles, mumps, rubella, and human parvovirus B19 infections and neurologic disease. Handbook of clinical neurology 121:1345–1353
Banuelos AF, Williams PL, Johnson RH, Bibi S, Fredricks DN, Gilroy SA, Bhatti SU, Aguet J, Stevens DA (1996) Central nervous system abscesses due to Coccidioides species. Clin Infect Dis 22:240–250
Baranova IN, Kurlander R, Bocharov AV, Vishnyakova TG, Chen Z, Remaley AT, Csako G, Patterson AP, Eggerman TL (2008) Role of human CD36 in bacterial recognition, phagocytosis, and pathogen-induced JNK-mediated signaling. J Immunol 181:7147–7156
Barker CF, Billingham RE (1977) Immunologically privileged sites. Adv Immunol 25:1–54
Beaver PC (1959) Visceral and cutaneous larva migrans. Public Health Rep 74:328–332
Bencsik A, Lezmi S, Hunsmann G, Baron T (2001) Close vicinity of PrP expressing cells (FDC) with noradrenergic fibers in healthy sheep spleen. Dev Immunol 8:235–241
Benedetto N, Auriault C (2002a) Complex network of cytokines activating murine microglial cell activity against *Acanthamoeba castellani*. Eur Cytokine Netw 13:351–357
Benedetto N, Auriault C (2002b) Prolactin-cytokine network in the defence against Acanthamoeba castellanii in murine microglia [corrected]. Eur Cytokine Netw 13:447–455
Benedetto N, Rossano F, Gorga F, Folgore A, Rao M, Romano Carratelli C (2003) Defense mechanisms of IFN-γ and LPS-primed murine microglia against Acanthamoeba castellanii infection. Int Immunopharmacol 3:825–834
Berger JR (1994) Tuberculous meningitis. Curr Opin Neurol 7:191–200
Bergmann CC, Lane TE, Stohlman SA (2006) Coronavirus infection of the central nervous system: host-virus stand-off. Nat Rev Microbiol 4:121–132
Bielefeldt-Ohmann H, Smirnova NP, Tolnay AE, Webb BT, Antoniazzi AQ, van Campen H, Hansen TR (2012) Neuro-invasion by a 'Trojan Horse' strategy and vasculopathy during intrauterine flavivirus infection. Int J Exp Pathol 93:24–33
Blasi E, Barluzzi R, Mazzolla R, Pitzurra L, Puliti M, Saleppico S, Bistoni F (1995) Biomolecular events involved in anticryptococcal resistance in the brain. Infect Immun 63:1218–1222
Blom AM, Hallstrom T, Riesbeck K (2009) Complement evasion strategies of pathogens-acquisition of inhibitors and beyond. Mol Immunol 46:2808–2817
Bobadilla K, Sada E, Jaime ME, Gonzalez Y, Ramachandra L, Rojas RE, Pedraza-Sanchez S, Michalak C, Gonzalez-Noriega A, Torres M (2013) Human phagosome processing of *Mycobacterium tuberculosis* antigens is modulated by interferon-γ and interleukin-10. Immunology 138:34–46
Bockenstedt LK, Wormser GP (2014) Review: unraveling Lyme disease. Arthritis Rheumatol (Hoboken, NJ) 66:2313–2323
Bonifati DM, Kishore U (2007) Role of complement in neurodegeneration and neuroinflammation. Mol Immunol 44:999–1010
Boothpur R, Brennan DC (2010) Human polyoma viruses and disease with emphasis on clinical BK and JC. J Clin Virol 47:306–312
Bradford BM, Mabbott NA (2012) Prion disease and the Innate immune system. Viruses 4 (12):3389–3419. doi:10.3390/v4123389
Braun JS, Sublett JE, Freyer D, Mitchell TJ, Cleveland JL, Tuomanen EI, Weber JR (2002) Pneumococcal pneumolysin and H_2O_2 mediate brain cell apoptosis during meningitis. J Clin Investig 109:19–27
Broderick C, Hoek RM, Forrester JV, Liversidge J, Sedgwick JD, Dick AD (2002) Constitutive retinal CD200 expression regulates resident microglia and activation state of inflammatory cells during experimental autoimmune uveoretinitis. Am J Pathol 161:1669–1677
Brown EJ, Frazier WA (2001) Integrin-associated protein (CD47) and its ligands. Trends Cell Biol 11:130–135

Brown KL, Stewart K, Ritchie DL, Mabbott NA, Williams A, Fraser H, Morrison WI, Bruce ME (1999) Scrapie replication in lymphoid tissues depends on prion protein-expressing follicular dendritic cells. Nat Med 5:1308–1312

Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC (2012) Hidden killers: human fungal infections. Science translational medicine 4:165rv113

Bruce ME, Brown KL, Mabbott NA, Farquhar CF, Jeffrey M (2000) Follicular dendritic cells in TSE pathogenesis. Immunol Today 21:442–446

Bruschi F, Korenaga M, Watanabe N (2008) Eosinophils and Trichinella infection: toxic for the parasite and the host? Trends Parasitol 24:462–467

Buchanan R, Bonthius DJ (2012) Measles virus and associated central nervous system sequelae. Semin Pediatr Neurol 19:107–114

Buchanan KL, Doyle HA (2000) Requirement for CD4(+) T lymphocytes in host resistance against *Cryptococcus neoformans* in the central nervous system of immunized mice. Infect Immun 68:456–462

Buchs S, Pfister P (1983) Candida meningitis. Course, prognosis and mortality before and after introduction of the new antimycotics. Mykosen 26:73–81

Bueler H, Aguzzi A, Sailer A, Greiner RA, Autenried P, Aguet M, Weissmann C (1993) Mice devoid of PrP are resistant to scrapie. Cell 73:1339–1347

Cai MS, Li ML, Zheng CF (2012) Herpesviral infection and Toll-like receptor 2. Protein Cell 3:590–601

Cantwell AR Jr (1982) Histologic observations of variably acid-fast pleomorphic bacteria in systemic sarcoidosis: a report of 3 cases. Growth 46:113–125

Cardoso AL, Guedes JR, Pereira de Almeida L, Pedroso de Lima MC (2012) miR-155 modulates microglia-mediated immune response by down-regulating SOCS-1 and promoting cytokine and nitric oxide production. Immunology 135:73–88

Carod Artal FJ, Vargas AP, Horan TA, Marinho PB, Coelho Costa PH (2004) *Schistosoma mansoni* myelopathy: clinical and pathologic findings. Neurology 63:388–391

Carruthers VB, Suzuki Y (2007) Effects of Toxoplasma gondii infection on the brain. Schizophr Bull 33:745–751

Casado JL, Quereda C, Oliva J, Navas E, Moreno A, Pintado V, Cobo J, Corral I (1997) Candidal meningitis in HIV-infected patients: analysis of 14 cases. Clin Infect Dis 25:673–676

Cermeno JR, Hernandez I, El Yasin H, Tinedo R, Sanchez R, Perez G, Gravano R, Ruiz A (2006) Meningoencephalitis by Naegleria fowleri: epidemiological study in Anzoategui state, Venezuela. Rev Soc Bras Med Trop 39:264–268

Chadwick DW, Hartley E, Mackinnon DM (1980) Meningitis caused by Candida tropicalis. Arch Neurol 37:175–176

Chai Q, He WQ, Zhou M, Lu H, Fu ZF (2014) Enhancement of blood-brain barrier permeability and reduction of tight junction protein expression are modulated by chemokines/cytokines induced by rabies virus infection. J Virol 88:4698–4710

Chai Q, She R, Huang Y, Fu ZF (2015) Expression of neuronal CXCL10 induced by rabies virus infection initiates infiltration of inflammatory cells, production of chemokines and cytokines, and enhancement of blood-brain barrier permeability. J Virol 89:870–876

Chao CC, Anderson WR, Hu S, Gekker G, Martella A, Peterson PK (1993) Activated microglia inhibit multiplication of Toxoplasma gondii via a nitric oxide mechanism. Clin Immunol Immunopathol 67:178–183

Chaudhuri JD (2000) Blood brain barrier and infection. Med Sci Monit 6:1213–1222

Chauhan VS, Sterka DG Jr, Furr SR, Young AB, Marriott I (2009) NOD2 plays an important role in the inflammatory responses of microglia and astrocytes to bacterial CNS pathogens. Glia 57:414–423

Cheeran MC, Hu S, Sheng WS, Rashid A, Peterson PK, Lokensgard JR (2005) Differential responses of human brain cells to West Nile virus infection. J Neurovirol 11:512–524

Chen CY, Lu PL, Lee KM, Chang TC, Lai CC, Chang K, Lin WR, Lin CY, Chen YH (2013) Acute meningitis caused by Cladosporium sphaerospermum. Am J Med Sci 346:523–525

Chen CJ, Ou YC, Li JR, Chang CY, Pan HC, Lai CY, Liao SL, Raung SL, Chang CJ (2014) Infection of pericytes in vitro by Japanese encephalitis virus disrupts the integrity of the endothelial barrier. J Virol 88:1150–1161

Cherian A, Thomas SV (2011) Central nervous system tuberculosis. Afr Health Sci 11:116–127

Cheung TW, Teich SA (1999) Cytomegalovirus infection in patients with HIV infection. Mt Sinai J Med 66:113–124

Chimelli L (2011) A morphological approach to the diagnosis of protozoal infections of the central nervous system. Pathol Res Int 2011:290853

Chin AC, Fournier B, Peatman EJ, Reaves TA, Lee WY, Parkos CA (2009) CD47 and TLR-2 cross-talk regulates neutrophil transmigration. J Immunol 183:5957–5963

Chowdhary A et al (2014) ESCMID and ECMM joint clinical guidelines for the diagnosis and management of systemic phaeohyphomycosis: diseases caused by black fungi. Clin Microbiol Infect 20(Suppl 3):47–75

Clark IA, Alleva LM (2009) Is human malarial coma caused, or merely deepened, by sequestration? Trends Parasitol 25:314–318

Cole JN, Barnett TC, Nizet V, Walker MJ (2011) Molecular insight into invasive group A streptococcal disease. Nat Rev Microbiol 9:724–736

Conant K, Lim ST, Randall B, Maguire-Zeiss KA (2012) Matrix metalloproteinase dependent cleavage of cell adhesion molecules in the pathogenesis of CNS dysfunction with HIV and methamphetamine. Curr HIV Res 10:384–391

Corcoran C, Rebe K, van der Plas H, Myer L, Hardie DR (2008) The predictive value of cerebrospinal fluid Epstein-Barr viral load as a marker of primary central nervous system lymphoma in HIV-infected persons. J Clin Virol 42:433–436

Couch JR, Romyg DA (1977) Histoplasma meningitis with common variable hypogammaglobulinemia. Neurologia, neurocirugia, psiquiatria 18:403–412

Cramer JP, Nussler AK, Ehrhardt S, Burkhardt J, Otchwemah RN, Zanger P, Dietz E, Gellert S, Bienzle U, Mockenhaupt FP (2005) Age-dependent effect of plasma nitric oxide on parasite density in Ghanaian children with severe malaria. Trop Med Int Health 10:672–680

Creagh EM, O'Neill LA (2006) TLRs, NLRs and RLRs: a trinity of pathogen sensors that co-operate in innate immunity. Trends Immunol 27:352–357

Cree BA (2014) Acute inflammatory myelopathies. Handbook of clinical neurology 122:613–667

Daffis S, Samuel MA, Suthar MS, Gale M Jr, Diamond MS (2008) Toll-like receptor 3 has a protective role against West Nile virus infection. J Virol 82:10349–10358

Dantas AM, Yamane R, Camara AG (1990) South American blastomycosis: ophthalmic and oculomotor nerve lesions. Am J Trop Med Hyg 43:386–388

Das Sarma J (2014) Microglia-mediated neuroinflammation is an amplifier of virus-induced neuropathology. J Neurovirol 20:122–136

Davis BK, Wen H, Ting JP (2011) The inflammasome NLRs in immunity, inflammation, and associated diseases. Annu Rev Immunol 29:707–735

de Almeida SM, Rebelatto CL, Queiroz-Telles F, Werneck LC (2005) Major histocompatibility complex and central nervous system involvement by paracoccidioidomycosis. J Infect 51: 140–143

de Vries HE, Hendriks JJ, Honing H, De Lavalette CR, van der Pol SM, Hooijberg E, Dijkstra CD, van den Berg TK (2002) Signal-regulatory protein α-CD47 interactions are required for the transmigration of monocytes across cerebral endothelium. J Immunol 168:5832–5839

del Pozo MM, Bermejo F, Molina JA, de la Fuente EC, Martinez-Martin P, Benito-Leon J (1998) Chronic neutrophilic meningitis caused by Candida albicans. Neurologia (Barcelona, Spain) 13:362–366

Delhaye S, Paul S, Blakqori G, Minet M, Weber F, Staeheli P, Michiels T (2006) Neurons produce type I interferon during viral encephalitis. Proc Natl Acad Sci USA 103:7835–7840

Dobbie M, Crawley J, Waruiru C, Marsh K, Surtees R (2000) Cerebrospinal fluid studies in children with cerebral malaria: an excitotoxic mechanism? Am J Trop Med Hyg 62:284–290

Dokmeci O, Forshay B, Scholand SJ (2013) Worms on the brain: fatal meningoencephalitis from disseminated strongyloides infection. Conn Med 77:31–33

du Bois RM, Goh N, McGrath D, Cullinan P (2003) Is there a role for microorganisms in the pathogenesis of sarcoidosis? J Intern Med 253:4–17

Ebner F, Brandt C, Thiele P, Richter D, Schliesser U, Siffrin V, Schueler J, Stubbe T, Ellinghaus A, Meisel C, Sawitzki B, Nitsch R (2013) Microglial activation milieu controls regulatory T cell responses. J Immunol 191:5594–5602

Eisen DP, Minchinton RM (2003) Impact of mannose-binding lectin on susceptibility to infectious diseases. Clin Infect Dis 37:1496–1505

Elsheikha HM, Khan NA (2010) Protozoa traversal of the blood-brain barrier to invade the central nervous system. FEMS Microbiol Rev 34:532–553

Elward K, Gasque P (2003) "Eat me" and "don't eat me" signals govern the innate immune response and tissue repair in the CNS: emphasis on the critical role of the complement system. Mol Immunol 40:85–94

Faix RG (1983) Candida parapsilosis meningitis in a premature infant. Pediatr Infect Dis 2: 462–464

Farina C, Aloisi F, Meinl E (2007) Astrocytes are active players in cerebral innate immunity. Trends Immunol 28:138–145

Ferrari TC, Moreira PR (2011) Neuroschistosomiasis: clinical symptoms and pathogenesis. The Lancet 10:853–864

Fischer HG, Nitzgen B, Reichmann G, Hadding U (1997a) Cytokine responses induced by *Toxoplasma gondii* in astrocytes and microglial cells. Eur J Immunol 27:1539–1548

Fischer HG, Nitzgen B, Reichmann G, Gross U, Hadding U (1997b) Host cells of *Toxoplasma gondii* encystation in infected primary culture from mouse brain. Parasitol Res 83:637–641

Fischer HG, Bonifas U, Reichmann G (2000) Phenotype and functions of brain dendritic cells emerging during chronic infection of mice with *Toxoplasma gondii*. J Immunol 164: 4826–4834

Flo TH, Halaas O, Lien E, Ryan L, Teti G, Golenbock DT, Sundan A, Espevik T (2000) Human toll-like receptor 2 mediates monocyte activation by Listeria monocytogenes, but not by group B streptococci or lipopolysaccharide. J Immunol 164:2064–2069

Ford MJ, Burton LJ, Li H, Graham CH, Frobert Y, Grassi J, Hall SM, Morris RJ (2002) A marked disparity between the expression of prion protein and its message by neurones of the CNS. Neuroscience 111:533–551

Forrellad MA, Klepp LA, Gioffré A, Sabio y García J, Morbidoni HR, de la Paz Santangelo M, Cataldi AA, Bigi F (2013) Virulence factors of the *Mycobacterium tuberculosis* complex. Virulence 4:3–66

Fritz JH, Ferrero RL, Philpott DJ, Girardin SE (2006) Nod-like proteins in immunity, inflammation and disease. Nat Immunol 7:1250–1257

Fu YR, Liu XJ, Li XJ, Shen ZZ, Yang B, Wu CC, Li JF, Miao LF, Ye HQ, Qiao GH, Rayner S, Chavanas S, Davrinche C, Britt WJ, Tang Q, McVoy M, Mocarski E, Luo MH (2015) MicroRNA miR-21 attenuates human cytomegalovirus replication in neural cells by targeting Cdc25a. J Virol 89:1070–1082

Galioto AM, Hess JA, Nolan TJ, Schad GA, Lee JJ, Abraham D (2006) Role of eosinophils and neutrophils in innate and adaptive protective immunity to larval strongyloides stercoralis in mice. Infect Immun 74:5730–5738

Garcia HH, Gonzalez AE, Evans CA, Gilman RH (2003) Taenia solium cysticercosis. Lancet 362:547–556

Garner JA, LaVail JH (1999) Differential anterograde transport of HSV type 1 viral strains in the murine optic pathway. J Neurovirol 5:140–150

Gasque P (2004) Complement: a unique innate immune sensor for danger signals. Mol Immunol 41:1089–1098

Gasque P, Singhrao SK, Neal JW, Wang P, Sayah S, Fontaine M, Morgan BP (1998) The receptor for complement anaphylatoxin C3a is expressed by myeloid cells and nonmyeloid cells in

inflamed human central nervous system: analysis in multiple sclerosis and bacterial meningitis. J Immunol 160:3543–3554
Geijtenbeek TB, Gringhuis SI (2009) Signalling through C-type lectin receptors: shaping immune responses. Nat Rev Immunol 9:465–479
Gerber J, Nau R (2010) Mechanisms of injury in bacterial meningitis. Curr Opin Neurol 23: 312–318
Ghoshal A, Das S, Ghosh S, Mishra MK, Sharma V, Koli P, Sen E, Basu A (2007) Proinflammatory mediators released by activated microglia induces neuronal death in Japanese encephalitis. Glia 55:483–496
Gilsdorf JR, Marrs CF, Foxman B (2004) Haemophilus influenzae: genetic variability and natural selection to identify virulence factors. Infect Immun 72:2457–2461
Girmenia C, Micozzi A, Venditti M, Meloni G, Iori AP, Bastianello S, Martino P (1991) Fluconazole treatment of *Blastoschizomyces capitatus* meningitis in an allogeneic bone marrow recipient. Eur J Clin Microbiol Infect Dis 10:752–756
Gluska S, Zahavi EE, Chein M, Gradus T, Bauer A, Finke S, Perlson E (2014) Rabies Virus Hijacks and accelerates the p75NTR retrograde axonal transport machinery. PLoS Pathog 10: e1004348
Gonyea EF (1978) The spectrum of primary blastomycotic meningitis: a review of central nervous system blastomycosis. Annals of neurology 3:26–39
Graeff-Teixeira C, da Silva AC, Yoshimura K (2009) Update on eosinophilic meningoencephalitis and its clinical relevance. Clin Microbiol Rev 22:322–348, Table of Contents
Gralinski LE, Ashley SL, Dixon SD, Spindler KR (2009) Mouse adenovirus type 1-induced breakdown of the blood-brain barrier. J Virol 83:9398–9410
Griffiths MR, Gasque P, Neal JW (2009) The multiple roles of the innate immune system in the regulation of apoptosis and inflammation in the brain. J Neuropathol Exp Neurol 68:217–226
Guedes J, Cardoso AL, Pedroso de Lima MC (2013) Involvement of microRNA in microglia-mediated immune response. Clin Dev Immunol 2013:186872
Guo L, Song Z, Li M, Wu Q, Wang D, Feng H, Bernard P, Daugherty A, Huang B, Li XA (2009) Scavenger receptor BI protects against septic death through its role in modulating inflammatory response. J Biol Chem 284:19826–19834
Haddon DJ, Hughes MR, Antignano F, Westaway D, Cashman NR, McNagny KM (2009) Prion protein expression and release by mast cells after activation. J Infect Dis 200:827–831
Halonen SK, Chiu F, Weiss LM (1998) Effect of cytokines on growth of *Toxoplasma gondii* in murine astrocytes. Infect Immun 66:4989–4993
Han YW, Choi JY, Uyangaa E, Kim SB, Kim JH, Kim BS, Kim K, Eo SK (2014) Distinct dictation of Japanese encephalitis virus-induced neuroinflammation and lethality via triggering TLR3 and TLR4 signal pathways. PLoS Pathog 10:e1004319
Hanke ML, Kielian T (2011) Toll-like receptors in health and disease in the brain: mechanisms and therapeutic potential. Clin Sci (Lond) 121:367–387
Harley WB, Lomis M, Haas DW (1994) Marked polymorphonuclear pleocytosis due to blastomycotic meningitis: case report and review. Clin Infect Dis 18:816–818
Hasbun R (2014) Cerebrospinal fluids in central nervous system infections, in Scheld WM, Whitley RJ, Marra, CM eds. Infections of the central nervous system 4th edition:11
Hause L, Al-Salleeh FM, Petro TM (2007) Expression of IL-27 p28 by Theiler's virus-infected macrophages depends on TLR3 and TLR7 activation of JNK-MAP-kinases. Antiviral Res 76:159–167
Hawley DA, Schaefer JF, Schulz DM, Muller J (1983) Cytomegalovirus encephalitis in acquired immunodeficiency syndrome. Am J Clin Pathol 80:874–877
Hayashi F, Smith KD, Ozinsky A, Hawn TR, Yi EC, Goodlett DR, Eng JK, Akira S, Underhill DM, Aderem A (2001) The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature 410:1099–1103
Heney C, Song E, Kellen A, Raal F, Miller SD, Davis V (1989) Cerebral phaeohyphomycosis caused by Xylohypha bantiana. Eur J Clin Microbiol Infect Dis 8:984–988

Herculano-Houzel S (2009) The human brain in numbers: a linearly scaled-up primate brain. Frontiers in human neuroscience 3:31

Hernandez JC, Stevenson M, Latz E, Urcuqui-Inchima S (2012) HIV type 1 infection up-regulates TLR2 and TLR4 expression and function in vivo and in vitro. AIDS Res Hum Retroviruses 28:1313–1328

Hernangomez M, Carrillo-Salinas FJ, Mecha M, Correa F, Mestre L, Loria F, Feliu A, Docagne F, Guaza C (2014) Brain innate immunity in the regulation of neuroinflammation: therapeutic strategies by modulating CD200-CD200R interaction involve the cannabinoid system. Curr Pharm Des 20:4707–4722

Hoarau JJ, Krejbich-Trotot P, Jaffar-Bandjee MC, Das T, Thon-Hon GV, Kumar S, Neal JW, Gasque P (2011) Activation and control of CNS innate immune responses in health and diseases: a balancing act finely tuned by neuroimmune regulators (NIReg). CNS Neurol Disord: Drug Targets 10:25–43

Hogan SP (2007) Recent advances in eosinophil biology. Int Arch Allergy Immunol 143(Suppl 1): 3–14

Hornbeek H, Ackerman BH, Reigart CL, Stair-Buchmann M, Guilday RE, Patton ML, Haith LR Jr (2012) Pseudallescheria boydii infection of the brain. Surgical infections 13:179–180

Hou YJ, Banerjee R, Thomas B, Nathan C, Garcia-Sastre A, Ding A, Uccellini MB (2013) SARM is required for neuronal injury and cytokine production in response to central nervous system viral infection. J Immunol 191:875–883

Huang SN, Harris LS (1963) Acute disseminated penicilliosis: report of a case and review of pertinent literature. Am J Clin Pathol 39:167–174

Hunt NH, Grau GE (2003) Cytokines: accelerators and brakes in the pathogenesis of cerebral malaria. Trends Immunol 24:491–499

Hunter CA, Gow JW, Kennedy PG, Jennings FW, Murray M (1991) Immunopathology of experimental African sleeping sickness: detection of cytokine mRNA in the brains of *Trypanosoma brucei* brucei-infected mice. Infect Immun 59:4636–4640

Hunter CA, Jennings FW, Kennedy PG, Murray M (1992) Astrocyte activation correlates with cytokine production in central nervous system of *Trypanosoma brucei* brucei-infected mice. Lab Invest J Tech Methods Pathol 67:635–642

Huttova M, Hartmanova I, Kralinsky K, Filka J, Uher J, Kurak J, Krizan S, Krcmery V Jr (1998a) Candida fungemia in neonates treated with fluconazole: report of forty cases, including eight with meningitis. Pediatr Infect Dis J 17:1012–1015

Huttova M, Kralinsky K, Horn J, Marinova I, Iligova K, Fric J, Spanik S, Filka J, Uher J, Kurak J, Krcmery V Jr (1998b) Prospective study of nosocomial fungal meningitis in children–report of 10 cases. Scand J Infect Dis 30:485–487

Ichiyama T, Isumi H, Yoshitomi T, Nishikawa M, Matsubara T, Furukawa S (2002) NF-κB activation in cerebrospinal fluid cells from patients with meningitis. Neurol Res 24:709–712

Idro R, Marsh K, John CC, Newton CR (2010) Cerebral malaria: mechanisms of brain injury and strategies for improved neurocognitive outcome. Pediatr Res 68:267–274

Jadhav VS, Krause KH, Singh SK (2014) HIV-1 Tat C modulates NOX2 and NOX4 expressions through miR-17 in a human microglial cell line. J Neurochem 131:803–815

Jain SK, Paul-Satyaseela M, Lamichhane G, Kim KS, Bishai WR (2006) *Mycobacterium tuberculosis* invasion and traversal across an in vitro human blood-brain barrier as a pathogenic mechanism for central nervous system tuberculosis. J Infect Dis 193:1287–1295

Jamjoom A, al-Abedeen Jamjoom Z, al-Hedaithy S, Jamali A, Naim Ur R, Malabarey T (1992) Ventriculitis and hydrocephalus caused by Candida albicans successfully treated by antimycotic therapy and cerebrospinal fluid shunting. Br J Neurosurg 6:501–504

Janot L, Secher T, Torres D, Maillet I, Pfeilschifter J, Quesniaux VF, Landmann R, Ryffel B, Erard F (2008) CD14 works with toll-like receptor 2 to contribute to recognition and control of Listeria monocytogenes infection. J Infect Dis 198:115–124

Jellinger KA, Setinek U, Drlicek M, Bohm G, Steurer A, Lintner F (2000) Neuropathology and general autopsy findings in AIDS during the last 15 years. Acta Neuropathol 100:213–220

John CC, Opika-Opoka R, Byarugaba J, Idro R, Boivin MJ (2006) Low levels of RANTES are associated with mortality in children with cerebral malaria. J Infect Dis 194:837–845

John CC, Panoskaltsis-Mortari A, Opoka RO, Park GS, Orchard PJ, Jurek AM, Idro R, Byarugaba J, Boivin MJ (2008) Cerebrospinal fluid cytokine levels and cognitive impairment in cerebral malaria. Am J Trop Med Hyg 78:198–205

Johnson HL, Jin F, Pirko I, Johnson AJ (2014) Theiler's murine encephalomyelitis virus as an experimental model system to study the mechanism of blood-brain barrier disruption. J Neurovirol 20:107–112

Johnston LJ, Halliday GM, King NJ (1996) Phenotypic changes in Langerhans' cells after infection with arboviruses: a role in the immune response to epidermally acquired viral infection? J Virol 70:4761–4766

Jones TC, Bienz KA, Erb P (1986) In vitro cultivation of *Toxoplasma gondii* cysts in astrocytes in the presence of gamma interferon. Infect Immun 51:147–156

Jonsson S, Musher DM, Chapman A, Goree A, Lawrence EC (1985) Phagocytosis and killing of common bacterial pathogens of the lung by human alveolar macrophages. J Infect Dis 152:4–13

Jung NY, Kim E (2014) Cerebral phaeohyphomycosis: a rare cause of brain abscess. J Korean Neurosurg Soc 56:444–447

Kanmogne GD, Primeaux C, Grammas P (2005) HIV-1 gp120 proteins alter tight junction protein expression and brain endothelial cell permeability: implications for the pathogenesis of HIV-associated dementia. J Neuropathol Exp Neurol 64:498–505

Kantarcioglu AS, Apaydin H, Yucel A, de Hoog GS, Samson RA, Vural M, Ozekmekci S (2004) Central nervous system infection due to *Penicillium chrysogenum*. Mycoses 47:242–248

Kastenbauer S, Koedel U, Weih F, Ziegler-Heitbrock L, Pfister HW (2004) Protective role of NF-κB1 (p50) in experimental pneumococcal meningitis. Eur J Pharmacol 498:315–318

Kaul M, Zheng J, Okamoto S, Gendelman HE, Lipton SA (2005) HIV-1 infection and AIDS: consequences for the central nervous system. Cell Death Differ 12(Suppl 1):878–892

Kaushik DK, Gupta M, Das S, Basu A (2010) Kruppel-like factor 4, a novel transcription factor regulates microglial activation and subsequent neuroinflammation. J Neuroinflamm 7:68

Kaushik DK, Mukhopadhyay R, Kumawat KL, Gupta M, Basu A (2012) Therapeutic targeting of Kruppel-like factor 4 abrogates microglial activation. J Neuroinflamm 9:57

Kaushik DK, Thounaojam MC, Kumawat KL, Gupta M, Basu A (2013) Interleukin-1β orchestrates underlying inflammatory responses in microglia via Kruppel-like factor 4. J Neurochem 127:233–244

Kawai T, Akira S (2011) Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. Immunity 34:637–650

Kennedy PG (2004) Human African trypanosomiasis of the CNS: current issues and challenges. J Clin Investig 113:496–504

Kershaw P, Freeman R, Templeton D, DeGirolami PC, DeGirolami U, Tarsy D, Hoffmann S, Eliopoulos G, Karchmer AW (1990) *Pseudallescheria boydii* infection of the central nervous system. Arch Neurol 47:468–472

Kethireddy S, Andes D (2007) CNS pharmacokinetics of antifungal agents. Expert Opin Drug Metabol Toxicol 3:573–581

Khan NA, Iqbal J, Siddiqui R (2012) Escherichia coli K1-induced cytopathogenicity of human brain microvascular endothelial cells. Microb Pathog 53:269–275

Khuth ST, Akaoka H, Pagenstecher A, Verlaeten O, Belin MF, Giraudon P, Bernard A (2001) Morbillivirus infection of the mouse central nervous system induces region-specific upregulation of MMPs and TIMPs correlated to inflammatory cytokine expression. J Virol 75:8268–8282

Kigerl KA, de Rivero Vaccari JP, Dietrich WD, Popovich PG, Keane RW (2014) Pattern recognition receptors and central nervous system repair. Exp Neurol 258:5–16

Kim KS (2008) Mechanisms of microbial traversal of the blood-brain barrier. Nat Rev Microbiol 6:625–634

Kim WK, Corey S, Alvarez X, Williams K (2003) Monocyte/macrophage traffic in HIV and SIV encephalitis. J Leukoc Biol 74:650–656

Kim Y, Zhou P, Qian L, Chuang JZ, Lee J, Li C, Iadecola C, Nathan C, Ding A (2007) MyD88-5 links mitochondria, microtubules, and JNK3 in neurons and regulates neuronal survival. J Exp Med 204:2063–2074

Klein J, Sato A (2000) The HLA system. Second of two parts. N Engl J Med 343:782–786

Klein MA, Kaeser PS, Schwarz P, Weyd H, Xenarios I, Zinkernagel RM, Carroll MC, Verbeek JS, Botto M, Walport MJ, Molina H, Kalinke U, Acha-Orbea H, Aguzzi A (2001) Complement facilitates early prion pathogenesis. Nat Med 7:488–492

Klein M, Koedel U, Pfister HW (2006) Oxidative stress in pneumococcal meningitis: a future target for adjunctive therapy? Prog Neurobiol 80:269–280

Knowles DM (2003) Etiology and pathogenesis of AIDS-related non-Hodgkin's lymphoma. Hematol Oncol Clin North Am 17:785–820

Koedel U, Bayerlein I, Paul R, Sporer B, Pfister HW (2000) Pharmacologic interference with NF-κB activation attenuates central nervous system complications in experimental Pneumococcal meningitis. J Infect Dis 182:1437–1445

Kohli S, Farooq O, Jani RB, Wolfe GI (2015) Cerebral paragonimiasis: an unusual manifestation of a rare parasitic infection. Pediatr Neurol 52:366–369

Koning N, Swaab DF, Hoek RM, Huitinga I (2009) Distribution of the immune inhibitory molecules CD200 and CD200R in the normal central nervous system and multiple sclerosis lesions suggests neuron-glia and glia-glia interactions. J Neuropathol Exp Neurol 68:159–167

Koyuncu OO, Hogue IB, Enquist LW (2013) Virus infections in the nervous system. Cell Host Microbe 13:379–393

Kozel TR (1993) Activation of the complement system by the capsule of *Cryptococcus neoformans*. Curr Top Med Mycol 5:1–26

Kravitz GR, Davies SF, Eckman MR, Sarosi GA (1981) Chronic blastomycotic meningitis. Am J Med 71:501–505

Kristensson K, Nygard M, Bertini G, Bentivoglio M (2010) African trypanosome infections of the nervous system: parasite entry and effects on sleep and synaptic functions. Prog Neurobiol 91:152–171

Kristensson K, Masocha W, Bentivoglio M (2013) Mechanisms of CNS invasion and damage by parasites. Handbook Clin Neurol 114:11–22

Kuberski T (1979) Eosinophils in the cerebrospinal fluid. Ann Intern Med 91:70–75

Kuberski T (1981) Eosinophils in cerebrospinal fluid: criteria for eosinophilic meningitis. Hawaii Med J 40:97–98

Kujala P, Raymond CR, Romeijn M, Godsave SF, van Kasteren SI, Wille H, Prusiner SB, Mabbott NA, Peters PJ (2011) Prion uptake in the gut: identification of the first uptake and replication sites. PLoS Pathog 7:e1002449

Lacroix S, Feinstein D, Rivest S (1998) The bacterial endotoxin lipopolysaccharide has the ability to target the brain in upregulating its membrane CD14 receptor within specific cellular populations. Brain Pathol (Zurich, Switzerland) 8:625–640

Lafay F, Coulon P, Astic L, Saucier D, Riche D, Holley A, Flamand A (1991) Spread of the CVS strain of rabies virus and of the avirulent mutant AvO1 along the olfactory pathways of the mouse after intranasal inoculation. Virology 183:320–330

Laflamme N, Soucy G, Rivest S (2001) Circulating cell wall components derived from gram-negative, not gram-positive, bacteria cause a profound induction of the gene-encoding Toll-like receptor 2 in the CNS. J Neurochem 79:648–657

Lamb TJ, Brown DE, Potocnik AJ, Langhorne J (2006) Insights into the immunopathogenesis of malaria using mouse models. Expert Rev Mol Med 8:1–22

Lambert C, Desbarats J, Arbour N, Hall JA, Olivier A, Bar-Or A, Antel JP (2008) Dendritic cell differentiation signals induce anti-inflammatory properties in human adult microglia. J Immunol 181:8288–8297

Lane TE, Liu MT, Chen BP, Asensio VC, Samawi RM, Paoletti AD, Campbell IL, Kunkel SL, Fox HS, Buchmeier MJ (2000) A central role for CD4(+) T cells and RANTES in virus-induced central nervous system inflammation and demyelination. J Virol 74:1415–1424

Le T, Huu Chi N, Kim Cuc NT, Manh Sieu TP, Shikuma CM, Farrar J, Day JN (2010) AIDS-associated *Penicillium marneffei* infection of the central nervous system. Clin Infect Dis 51:1458–1462

Lech M, Susanti HE, Rommele C, Grobmayr R, Gunthner R, Anders HJ (2012) Quantitative expression of C-type lectin receptors in humans and mice. Int J Mol Sci 13:10113–10131

Lee YR, Liu MT, Lei HY, Liu CC, Wu JM, Tung YC, Lin YS, Yeh TM, Chen SH, Liu HS (2006) MCP-1, a highly expressed chemokine in dengue haemorrhagic fever/dengue shock syndrome patients, may cause permeability change, possibly through reduced tight junctions of vascular endothelium cells. J Gen Virol 87:3623–3630

Leiguarda R, Roncoroni A, Taratuto AL, Jost L, Berthier M, Nogues M, Freilij H (1990) Acute CNS infection by *Trypanosoma cruzi* (Chagas' disease) in immunosuppressed patients. Neurology 40:850–851

Lejon V, Lardon J, Kenis G, Pinoges L, Legros D, Bisser S, N'Siesi X, Bosmans E, Buscher P (2002) Interleukin (IL)-6, IL-8 and IL-10 in serum and CSF of *Trypanosoma brucei* gambiense sleeping sickness patients before and after treatment. Trans R Soc Trop Med Hyg 96:329–333

Lewis SD, Butchi NB, Khaleduzzaman M, Morgan TW, Du M, Pourciau S, Baker DG, Akira S, Peterson KE (2008) Toll-like receptor 7 is not necessary for retroviral neuropathogenesis but does contribute to virus-induced neuroinflammation. J Neurovirol 14:492–502

Lezmi S, Bencsik A, Baron T (2001) CNA42 monoclonal antibody identifies FDC as PrPsc accumulating cells in the spleen of scrapie affected sheep. Vet Immunol Immunopathol 82:1–8

Li J, Faber M, Dietzschold B, Hooper DC (2011) The role of toll-like receptors in the induction of immune responses during rabies virus infection. Adv Virus Res 79:115–126

Li F, Wang Y, Yu L, Cao S, Wang K, Yuan J, Wang C, Cui M, Fu ZF (2015) Viral infection of the Central Nervous System and neuroinflammation precede Blood Brain Barrier disruption during Japanese Encephalitis. J Virol

Licinio J, Wong ML (1997) Pathways and mechanisms for cytokine signaling of the central nervous system. J Clin Investig 100:2941–2947

Lima MA (2013) Progressive multifocal leukoencephalopathy: new concepts. Arq Neuropsiquiatr 71:699–702

Liu Y, Mittal R, Solis NV, Prasadarao NV, Filler SG (2011) Mechanisms of Candida albicans trafficking to the brain. PLoS Pathog 7:e1002305

Lo Re V III, Gluckman SJ (2003) Eosinophilic meningitis. Am J Med 114:217–223

Loddick SA, Wong ML, Bongiorno PB, Gold PW, Licinio J, Rothwell NJ (1997) Endogenous interleukin-1 receptor antagonist is neuroprotective. Biochem Biophys Res Commun 234: 211–215

Loeuillet C, Lemaire-Vieille C, Naquet P, Cesbron-Delauw MF, Gagnon J, Cesbron JY (2010) Prion replication in the hematopoietic compartment is not required for neuroinvasion in scrapie mouse model. PLoS ONE 5

Lokensgard JR, Hu S, Sheng W, vanOijen M, Cox D, Cheeran MC, Peterson PK (2001) Robust expression of TNF-alpha, IL-1beta, RANTES, and IP-10 by human microglial cells during nonproductive infection with herpes simplex virus. J Neurovirol 7:208–219

Lopez-Castejon G, Corbett D, Goldrick M, Roberts IS, Brough D (2012) Inhibition of calpain blocks the phagosomal escape of Listeria monocytogenes. PLoS ONE 7:e35936

Lortholary O, Improvisi L, Rayhane N, Gray F, Fitting C, Cavaillon JM, Dromer F (1999) Cytokine profiles of AIDS patients are similar to those of mice with disseminated *Cryptococcus neoformans* infection. Infect Immun 67:6314–6320

Louboutin JP, Agrawal L, Reyes BA, Van Bockstaele EJ, Strayer DS (2010) HIV-1 gp120-induced injury to the blood-brain barrier: role of metalloproteinases 2 and 9 and relationship to oxidative stress. J Neuropathol Exp Neurol 69:801–816

Lyratzopoulos G, Ellis M, Nerringer R, Denning DW (2002) Invasive infection due to penicillium species other than P. marneffei. J Infect 45:184–195
Macauley MS, Crocker PR, Paulson JC (2014) Siglec-mediated regulation of immune cell function in disease. Nat Rev 14:653–666
MacLean L, Odiit M, Sternberg JM (2001) Nitric oxide and cytokine synthesis in human African trypanosomiasis. J Infect Dis 184:1086–1090
Maffei CM, Mirels LF, Sobel RA, Clemons KV, Stevens DA (2004) Cytokine and inducible nitric oxide synthase mRNA expression during experimental murine cryptococcal meningoencephalitis. Infect Immun 72:2338–2349
Mahajan VK (2014) Sporotrichosis: an overview and therapeutic options. Dermatol Res Pract 2014:272376
Marciano-Cabral F (2009) Free-living amoebae as agents of human infection. J Infect Dis 199:1104–1106
Marciano-Cabral F, Puffenbarger R, Cabral GA (2000) The increasing importance of Acanthamoeba infections. J Eukaryot Microbiol 47:29–36
Marella M, Chabry J (2004) Neurons and astrocytes respond to prion infection by inducing microglia recruitment. J Neurosci 24:620–627
Mas-Coma S, Agramunt VH, Valero MA (2014) Neurological and ocular fascioliasis in humans. Adv Parasitol 84:27–149
McKimmie CS, Johnson N, Fooks AR, Fazakerley JK (2005) Viruses selectively upregulate Toll-like receptors in the central nervous system. Biochem Biophys Res Commun 336: 925–933
Medana IM, Hien TT, Day NP, Phu NH, Mai NT, Chu'ong LV, Chau TT, Taylor A, Salahifar H, Stocker R, Smythe G, Turner GD, Farrar J, White NJ, Hunt NH (2002) The clinical significance of cerebrospinal fluid levels of kynurenine pathway metabolites and lactate in severe malaria. J Infect Dis 185:650–656
Meier UC, Giovannoni G, Tzartos JS, Khan G (2012) Translational Mini-Review Series on B cell subsets in disease. B cells in multiple sclerosis: drivers of disease pathogenesis and Trojan horse for Epstein-Barr virus entry to the central nervous system? Clin Exp Immunol 167:1–6
Mendel E, Milefchik EN, Amadi J, Gruen P (1994) Coccidioidomycosis brain abscess. Case report. J Neurosurg 81:614–616
Miller RG, Storey JR, Greco CM (1990) Ganciclovir in the treatment of progressive AIDS-related polyradiculopathy. Neurology 40:569–574
Miller F, Afonso PV, Gessain A, Ceccaldi PE (2012) Blood-brain barrier and retroviral infections. Virulence 3:222–229
Minghetti L, Ajmone-Cat MA, De Berardinis MA, De Simone R (2005) Microglial activation in chronic neurodegenerative diseases: roles of apoptotic neurons and chronic stimulation. Brain Res Brain Res Rev 48:251–256
Mischel PS, Vinters HV (1995) Coccidioidomycosis of the central nervous system: neuropathological and vasculopathic manifestations and clinical correlates. Clin Infect Dis 20:400–405
Mishra BB, Mishra PK, Teale JM (2006) Expression and distribution of Toll-like receptors in the brain during murine neurocysticercosis. J Neuroimmunol 181:46–56
Mishra MK, Dutta K, Saheb SK, Basu A (2009) Understanding the molecular mechanism of blood-brain barrier damage in an experimental model of Japanese encephalitis: correlation with minocycline administration as a therapeutic agent. Neurochem Int 55:717–723
Mitchell AM, Mitchell TJ (2010) Streptococcus pneumoniae: virulence factors and variation. Clin Microbiol Infect 16:411–418
Mitchell L, Smith SH, Braun JS, Herzog KH, Weber JR, Tuomanen EI (2004) Dual phases of apoptosis in pneumococcal meningitis. J Infect Dis 190:2039–2046
Mody CH, Tyler CL, Sitrin RG, Jackson C, Toews GB (1991) Interferon-γ activates rat alveolar macrophages for anticryptococcal activity. Am J Respir Cell Mol Biol 5:19–26
Mori T, Ebe T (1992) Analysis of cases of central nervous system fungal infections reported in Japan between January 1979 and June 1989. Intern Med (Tokyo, Japan) 31:174–179

Mori I, Nishiyama Y, Yokochi T, Kimura Y (2005) Olfactory transmission of neurotropic viruses. J Neurovirol 11:129–137

Mukherjee P, Woods TA, Moore RA, Peterson KE (2013) Activation of the innate signaling molecule MAVS by bunyavirus infection upregulates the adaptor protein SARM1, leading to neuronal death. Immunity 38:705–716

Mukhopadhyay S, Chen Y, Sankala M, Peiser L, Pikkarainen T, Kraal G, Tryggvason K, Gordon S (2006) MARCO, an innate activation marker of macrophages, is a class A scavenger receptor for Neisseria meningitidis. Eur J Immunol 36:940–949

Mulcahy G, O'Neill S, Fanning J, McCarthy E, Sekiya M (2005) Tissue migration by parasitic helminths—An immunoevasive strategy? Trends Parasitol 21:273–277

Munster VJ, Prescott JB, Bushmaker T, Long D, Rosenke R, Thomas T, Scott D, Fischer ER, Feldmann H, de Wit E (2012) Rapid Nipah virus entry into the central nervous system of hamsters via the olfactory route. Sci Rep 2:736

Nakamuta S, Endo H, Higashi Y, Kousaka A, Yamada H, Yano M, Kido H (2008) Human immunodeficiency virus type 1 gp120-mediated disruption of tight junction proteins by induction of proteasome-mediated degradation of zonula occludens-1 and -2 in human brain microvascular endothelial cells. J Neurovirol 14:186–195

Nascimento-Carvalho CM, Moreno-Carvalho OA (2005) Neuroschistosomiasis due to Schistosoma mansoni: a review of pathogenesis, clinical syndromes and diagnostic approaches. Rev Inst Med Trop Sao Paulo 47:179–184

Nazli A, Kafka JK, Ferreira VH, Anipindi V, Mueller K, Osborne BJ, Dizzell S, Chauvin S, Mian MF, Ouellet M, Tremblay MJ, Mossman KL, Ashkar AA, Kovacs C, Bowdish DM, Snider DP, Kaul R, Kaushic C (2013) HIV-1 gp120 induces TLR2- and TLR4-mediated innate immune activation in human female genital epithelium. J Immunol 191:4246–4258

Nazmi A, Dutta K, Basu A (2011) RIG-I mediates innate immune response in mouse neurons following Japanese encephalitis virus infection. PLoS ONE 6:e21761

Nazmi A, Mukhopadhyay R, Dutta K, Basu A (2012) STING mediates neuronal innate immune response following Japanese encephalitis virus infection. Sci Rep 2:347

Nazmi A, Mukherjee S, Kundu K, Dutta K, Mahadevan A, Shankar SK, Basu A (2014) TLR7 is a key regulator of innate immunity against Japanese encephalitis virus infection. Neurobiol Dis 69:235–247

Nicholas PK, Mauceri L, Slate Ciampa A, Corless IB, Raymond N, Barry DJ, Viamonte Ros A (2007) Distal sensory polyneuropathy in the context of HIV/AIDS. J Assoc Nurses AIDS Care 18:32–40

Noritomi DT, Bub GL, Beer I, da Silva AS, de Cleva R, Gama-Rodrigues JJ (2005) Multiple brain abscesses due to Penicillium spp infection. Rev Inst Med Trop Sao Paulo 47:167–170

Ohka S, Nihei C, Yamazaki M, Nomoto A (2012) Poliovirus trafficking toward central nervous system via human poliovirus receptor-dependent and -independent pathway. Front Microbiol 3:147

Okazawa H, Motegi S, Ohyama N, Ohnishi H, Tomizawa T, Kaneko Y, Oldenborg PA, Ishikawa O, Matozaki T (2005) Negative regulation of phagocytosis in macrophages by the CD47-SHPS-1 system. J Immunol 174:2004–2011

O'Neill LA, Bowie AG (2007) The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. Nat Rev 7:353–364

Osawa R, Williams KL, Singh N (2011) The inflammasome regulatory pathway and infections: role in pathophysiology and clinical implications. J Infect 62:119–129

Osiyemi OO, Dowdy LM, Mallon SM, Cleary T (2001) Cerebral phaeohyphomycosis due to a novel species: report of a case and review of the literature. Transplantation 71:1343–1346

Othman AA (2012) Therapeutic battle against larval toxocariasis: are we still far behind? Acta Trop 124:171–178

Padigel UM, Lee JJ, Nolan TJ, Schad GA, Abraham D (2006) Eosinophils can function as antigen-presenting cells to induce primary and secondary immune responses to Strongyloides stercoralis. Infect Immun 74:3232–3238

Padigel UM, Hess JA, Lee JJ, Lok JB, Nolan TJ, Schad GA, Abraham D (2007) Eosinophils act as antigen-presenting cells to induce immunity to Strongyloides stercoralis in mice. J Infect Dis 196:1844–1851

Palus M, Bily T, Elsterova J, Langhansova H, Salat J, Vancova M, Ruzek D (2014) Infection and injury of human astrocytes by tick-borne encephalitis virus. J Gen Virol 95:2411–2426

Pareek S, Roy S, Kumari B, Jain P, Banerjee A, Vrati S (2014) MiR-155 induction in microglial cells suppresses Japanese encephalitis virus replication and negatively modulates innate immune responses. J Neuroinflamm 11:97

Parker JC Jr, McCloskey JJ, Lee RS (1978) The emergence of candidosis. The dominant postmortem cerebral mycosis. Am J Clin Pathol 70:31–36

Pedersen M, Benfield TL, Skinhoej P, Jensen AG (2006) Haematogenous Staphylococcus aureus meningitis. A 10-year nationwide study of 96 consecutive cases. BMC Infect Dis 6:49

Peltier DC, Simms A, Farmer JR, Miller DJ (2010) Human neuronal cells possess functional cytoplasmic and TLR-mediated innate immune pathways influenced by phosphatidylinositol-3 kinase signaling. J Immunol 184:7010–7021

Pentreath VW (1989) Neurobiology of sleeping sickness. Parasitol Today 5:215–218

Pentreath VW (1995) Royal Society of Tropical Medicine and Hygiene Meeting at Manson House, London, 19 May 1994. Trypanosomiasis and the nervous system. Pathology and immunology. Trans R Soc Trop Med Hyg 89:9–15

Perry VH, Andersson PB (1992) The inflammatory response in the CNS. Neuropathol Appl Neurobiol 18:454–459

Pietila M, Lehtonen S, Tuovinen E, Lahteenmaki K, Laitinen S, Leskela HV, Natynki A, Pesala J, Nordstrom K, Lehenkari P (2012) CD200 positive human mesenchymal stem cells suppress TNF-alpha secretion from CD200 receptor positive macrophage-like cells. PLoS ONE 7:e31671

Pittella JE (2009) Central nervous system involvement in Chagas disease: a hundred-year-old history. Trans R Soc Trop Med Hyg 103:973–978

Pittella JE (2013) Pathology of CNS parasitic infections. Handb Clin Neurol 114:65–88

Prasad LS, Sen S (1996) Migration of Leishmania donovani amastigotes in the cerebrospinal fluid. Am J Trop Med Hyg 55:652–654

Prehaud C, Megret F, Lafage M, Lafon M (2005) Virus infection switches TLR-3-positive human neurons to become strong producers of beta interferon. J Virol 79:12893–12904

Preuss MA, Faber ML, Tan GS, Bette M, Dietzschold B, Weihe E, Schnell MJ (2009) Intravenous inoculation of a bat-associated rabies virus causes lethal encephalopathy in mice through invasion of the brain via neurosecretory hypothalamic fibers. PLoS Pathog 5:e1000485

Price RW (1986) Neurobiology of human herpesvirus infections. CRC Crit Rev Clin Neurobiol 2:61–123

Prusiner SB (1982) Novel proteinaceous infectious particles cause scrapie. Science (New York, NY) 216:136–144

Qin H, Yeh WI, De Sarno P, Holdbrooks AT, Liu Y, Muldowney MT, Reynolds SL, Yanagisawa LL, Fox TH 3rd, Park K, Harrington LE, Raman C, Benveniste EN (2012) Signal transducer and activator of transcription-3/suppressor of cytokine signaling-3 (STAT3/SOCS3) axis in myeloid cells regulates neuroinflammation. Proc Natl Acad Sci USA 109:5004–5009

Racaniello VR (2006) One hundred years of poliovirus pathogenesis. Virology 344:9–16

Raman Sharma R (2010) Fungal infections of the nervous system: current perspective and controversies in management. Int J Surg (London, England) 8:591–601

Rambach G, Maier H, Vago G, Mohsenipour I, Lass-Florl C, Defant A, Wurzner R, Dierich MP, Speth C (2008) Complement induction and complement evasion in patients with cerebral aspergillosis. Microbes Infect/Institut Pasteur 10:1567–1576

Randall RE, Goodbourn S (2008) Interferons and viruses: an interplay between induction, signalling, antiviral responses and virus countermeasures. J Gen Virol 89:1–47

Randall PJ, Hsu NJ, Lang D, Cooper S, Sebesho B, Allie N, Keeton R, Francisco NM, Salie S, Labuschagné A, Quesniaux V, Ryffel B, Kellaway L, Jacobs M (2014) Neurons are host cells for *Mycobacterium tuberculosis*. Infect Immun 82:1880–1890

Ransohoff RM, Kivisakk P, Kidd G (2003) Three or more routes for leukocyte migration into the central nervous system. Nat Rev 3:569–581

Raymond J (2012) Neisseria meningitidis: characterisation and epidemiology. Arch Pediatr 19 (Suppl 2):S55–S60

Reinert LS, Harder L, Holm CK, Iversen MB, Horan KA, Dagnaes-Hansen F, Ulhoi BP, Holm TH, Mogensen TH, Owens T, Nyengaard JR, Thomsen AR, Paludan SR (2012) TLR3 deficiency renders astrocytes permissive to herpes simplex virus infection and facilitates establishment of CNS infection in mice. J Clin Investig 122:1368–1376

Rescigno M, Urbano M, Valzasina B, Francolini M, Rotta G, Bonasio R, Granucci F, Kraehenbuhl JP, Ricciardi-Castagnoli P (2001) Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. Nat Immunol 2:361–367

Reuter D, Schneider-Schaulies J (2010) Measles virus infection of the CNS: human disease, animal models, and approaches to therapy. Med Microbiol Immunol 199:261–271

Reyes JL, Gonzalez MI, Ledesma-Soto Y, Satoskar AR, Terrazas LI (2011) TLR2 mediates immunity to experimental cysticercosis. Int J Bio Sci 7:1323–1333

Rhoades RE, Tabor-Godwin JM, Tsueng G, Feuer R (2011) Enterovirus infections of the central nervous system. Virology 411:288–305

Rich AR, McCordock HA (1933) Pathogenesis of tubercular meningitis. Bull John Hopkins Hosp 52:5–13

Rivest S (2009) Regulation of innate immune responses in the brain. Nat Rev 9:429–439

Roberts TK, Eugenin EA, Lopez L, Romero IA, Weksler BB, Couraud PO, Berman JW (2012) CCL2 disrupts the adherens junction: implications for neuroinflammation. Lab Invest J Tech Methods Pathol 92:1213–1233

Rock RB, Hu S, Gekker G, Sheng WS, May B, Kapur V, Peterson PK (2005) Mycobacterium tuberculosis-induced cytokine and chemokine expression by human microglia and astrocytes: effects of dexamethasone. J Infect Dis 192:2054–2058

Rom WN, Garay SM (2004) Tuberculosis, 2nd edn. Lippincott Williams & Wilkins, Philadelphia, PA

Romani L (2011) Immunity to fungal infections. Nat Rev 11:275–288

Romero JR, Newland JG (2003) Viral meningitis and encephalitis: traditional and emerging viral agents. Semin Pediatr Infect Dis 14:72–82

Roos A, Xu W, Castellano G, Nauta AJ, Garred P, Daha MR, van Kooten C (2004) Mini-review: a pivotal role for innate immunity in the clearance of apoptotic cells. Eur J Immunol 34:921–929

Saidha S, Sotirchos ES, Eckstein C (2012) Etiology of sarcoidosis: does infection play a role? Yale J Biol Med 85:133–141

Sakaguchi M, Okano H (2012) Neural stem cells, adult neurogenesis, and galectin-1: from bench to bedside. Dev Neurobiol 72:1059–1067

Salunke P, Patra DP, Mukherjee KK (2014) Delayed cerebral vasospasm and systemic inflammatory response syndrome following intraoperative rupture of cerebral hydatid cyst. Acta Neurochir (Wien) 156:613–614

Sarma PS, Durairaj P, Padhye AA (1993) Candida lusitaniae causing fatal meningitis. Postgrad Med J 69:878–880

Sassa Y, Inoshima Y, Ishiguro N (2010) Bovine macrophage degradation of scrapie and BSE PrPSc. Vet Immunol Immunopathol 133:33–39

Savarin C, Bergmann CC (2008) Neuroimmunology of central nervous system viral infections: the cells, molecules and mechanisms involved. Curr Opin Pharmacol 8:472–479

Savarin C, Bergmann CC, Hinton DR, Stohlman SA (2013) MMP-independent role of TIMP-1 at the blood brain barrier during viral encephalomyelitis. ASN Neuro 5:e00127

Scheld WM, Koedel U, Nathan B, Pfister HW (2002) Pathophysiology of bacterial meningitis: mechanism(s) of neuronal injury. J Infect Dis 186(Suppl 2):S225–S233

Schluter D, Hein A, Dorries R, Deckert-Schluter M (1995) Different subsets of T cells in conjunction with natural killer cells, macrophages, and activated microglia participate in the intracerebral immune response to *Toxoplasma gondii* in athymic nude and immunocompetent rats. Am J Pathol 146:999–1007

Schluter D, Kaefer N, Hof H, Wiestler OD, Deckert-Schluter M (1997) Expression pattern and cellular origin of cytokines in the normal and *Toxoplasma gondii*-infected murine brain. Am J Pathol 150:1021–1035

Schluter D, Bertsch D, Frei K, Hubers SB, Wiestler OD, Hof H, Fontana A, Deckert-Schluter M (1998) Interferon-γ antagonizes transforming growth factor-β2-mediated immunosuppression in murine *Toxoplasma encephalitis*. J Neuroimmunol 81:38–48

Schluter D, Deckert M, Hof H, Frei K (2001) *Toxoplasma gondii* infection of neurons induces neuronal cytokine and chemokine production, but gamma interferon- and tumor necrosis factor-stimulated neurons fail to inhibit the invasion and growth of *T. gondii*. Infect Immun 69:7889–7893

Scott EN, Kaufman L, Brown AC, Muchmore HG (1987) Serologic studies in the diagnosis and management of meningitis due to Sporothrix schenckii. N Engl J Med 317:935–940

Scrimgeour EM, Gajdusek DC (1985) Involvement of the central nervous system in Schistosoma mansoni and *S. haematobium* infection. A review. Brain 108(Pt 4):1023–1038

Sellner J, Tauber MG, Leib SL (2010) Pathogenesis and pathophysiology of bacterial CNS infections. Handb Clin Neurol 96:1–16

Sharafeldin A, Eltayeb R, Pashenkov M, Bakhiet M (2000) Chemokines are produced in the brain early during the course of experimental African trypanosomiasis. J Neuroimmunol 103: 165–170

Sharma N, Verma R, Kumawat KL, Basu A, Singh SK (2015) miR-146a suppresses cellular immune response during Japanese encephalitis virus JaOArS982 strain infection in human microglial cells. J Neuroinflamm 12:30

Sheen TR, Ebrahimi CM, Hiemstra IH, Barlow SB, Peschel A, Doran KS (2010) Penetration of the blood-brain barrier by *Staphylococcus aureus*: contribution of membrane-anchored lipoteichoic acid. J Mol Med (Berlin, Germany) 88:633–639

Shi Z, Cai Z, Sanchez A, Zhang T, Wen S, Wang J, Yang J, Fu S, Zhang D (2011) A novel Toll-like receptor that recognizes vesicular stomatitis virus. J Biol Chem 286:4517–4524

Shih AY, Fernandes HB, Choi FY, Kozoriz MG, Liu Y, Li P, Cowan CM, Klegeris A (2006) Policing the police: astrocytes modulate microglial activation. J Neurosci 26:3887–3888

Shin T (2013) The pleiotropic effects of galectin-3 in neuroinflammation: a review. Acta Histochem 115:407–411

Shin MH, Chung YB, Kita H (2005) Degranulation of human eosinophils induced by *Paragonimus westermani*-secreted protease. Korean J Parasitol 43:33–37

Silva AA, Roffe E, Marino AP, dos Santos PV, Quirico-Santos T, Paiva CN, Lannes-Vieira J (1999) Chagas' disease encephalitis: intense CD8+ lymphocytic infiltrate is restricted to the acute phase, but is not related to the presence of *Trypanosoma cruzi* antigens. Clin Immunol (Orlando Fla) 92:56–66

Singhrao SK, Neal JW, Rushmere NK, Morgan BP, Gasque P (1999) Differential expression of individual complement regulators in the brain and choroid plexus. Lab Invest J Tech Methods Pathol 79:1247–1259

Sips GJ, Wilschut J, Smit JM (2012) Neuroinvasive flavivirus infections. Rev Med Virol 22:69–87

Sisk JM, Witwer KW, Tarwater PM, Clements JE (2013) SIV replication is directly downregulated by four antiviral miRNAs. Retrovirology 10:95

Skattum L, van Deuren M, van der Poll T, Truedsson L (2011) Complement deficiency states and associated infections. Mol Immunol 48:1643–1655

Skiada A, Vrana L, Polychronopoulou H, Prodromou P, Chantzis A, Tofas P, Daikos GL (2009) Disseminated zygomycosis with involvement of the central nervous system. Clin Microbiol Infect 15(Suppl 5):46–49

Solomon T, Lewthwaite P, Perera D, Cardosa MJ, McMinn P, Ooi MH (2010) Virology, epidemiology, pathogenesis, and control of enterovirus 71. Lancet Infect Dis 10:778–790

Sood S, Vaid VK, Sharma M, Bhartiya H (2014) Cerebral phaeohyphomycosis by *Exophiala dermatitidis*. Indian J Med Microbiol 32:188–190

Spanos JP, Hsu N-J, Jacobs M (2015) Microglia are crucial regulators of neuro-immunity during central nervous system tuberculosis. Front Cell Neurosci 9:182

Speth C, Rambach G, Wurzner R, Lass-Florl C (2008) Complement and fungal pathogens: an update. Mycoses 51:477–496

Stauffer W, Ravdin JI (2003) Entamoeba histolytica: an update. Curr Opin Infect Dis 16:479–485

Stavros K, Simpson DM (2014) Understanding the etiology and management of HIV-associated peripheral neuropathy. Curr HIV/AIDS Rep 11:195–201

Strazielle N, Ghersi-Egea JF (2000) Choroid plexus in the central nervous system: biology and physiopathology. J Neuropathol Exp Neurol 59:561–574

Strazza M, Pirrone V, Wigdahl B, Nonnemacher MR (2011) Breaking down the barrier: the effects of HIV-1 on the blood-brain barrier. Brain Res 1399:96–115

Sturdevant CB, Joseph SB, Schnell G, Price RW, Swanstrom R, Spudich S (2015) Compartmentalized replication of R5 T Cell-Tropic HIV-1 in the central nervous system early in the course of infection. PLoS Pathog 11:e1004720

Suen WW, Prow NA, Hall RA, Bielefeldt-Ohmann H (2014) Mechanism of West Nile virus neuroinvasion: a critical appraisal. Viruses 6:2796–2825

Suh HS, Zhao ML, Choi N, Belbin TJ, Brosnan CF, Lee SC (2009) TLR3 and TLR4 are innate antiviral immune receptors in human microglia: role of IRF3 in modulating antiviral and inflammatory response in the CNS. Virology 392:246–259

Suri P, Chhina DK, Kaushal V, Kaushal RK, Singh J (2014) Cerebral phaeohyphomycosis due to cladophialophora bantiana—A case report and review of literature from India. J Clin Diagn Res 8:DD01–DD05

Surmont I, Vergauwen B, Marcelis L, Verbist L, Verhoef G, Boogaerts M (1990) First report of chronic meningitis caused by *Trichosporon beigelii*. Eur J Clin Microbiol Infect Dis 9:226–229

Suzuki Y, Claflin J, Wang X, Lengi A, Kikuchi T (2005) Microglia and macrophages as innate producers of interferon-gamma in the brain following infection with *Toxoplasma gondii*. Int J Parasitol 35:83–90

Swanson PA 2nd, McGavern DB (2015) Viral diseases of the central nervous system. Curr Opin Virol 11C:44–54

Tabor-Godwin JM, Ruller CM, Bagalso N, An N, Pagarigan RR, Harkins S, Gilbert PE, Kiosses WB, Gude NA, Cornell CT, Doran KS, Sussman MA, Whitton JL, Feuer R (2010) A novel population of myeloid cells responding to coxsackievirus infection assists in the dissemination of virus within the neonatal CNS. J Neurosci 30:8676–8691

Tailleux L, Schwartz O, Herrmann JL, Pivert E, Jackson M, Amara A, Legres L, Dreher D, Nicod LP, Gluckman JC, Lagrange PH, Gicquel B, Neyrolles O (2003) DC-SIGN is the major *Mycobacterium tuberculosis* receptor on human dendritic cells. J Exp Med 197:121–127

Takayanagui OM, Lofrano MM, Araugo MB, Chimelli L (1995) Detection of Strongyloides stercoralis in the cerebrospinal fluid of a patient with acquired immunodeficiency syndrome. Neurology 45:193–194

Takeuchi O, Akira S (2009) Innate immunity to virus infection. Immunol Rev 227:75–86

Taratuto AL, Venturiello SM (1997) Trichinosis. Brain pathology (Zurich, Switzerland) 7: 663–672

Tato CM, Hunter CA (2002) Host-pathogen interactions: subversion and utilization of the NF-κB pathway during infection. Infect Immun 70:3311–3317

Terrazas LI (2008) The complex role of pro- and anti-inflammatory cytokines in cysticercosis: immunological lessons from experimental and natural hosts. Curr Top Med Chem 8:383–392

Thompson KA, Cherry CL, Bell JE, McLean CA (2011) Brain cell reservoirs of latent virus in presymptomatic HIV-infected individuals. Am J Pathol 179:1623–1629

Thornton CR (2009) Tracking the emerging human pathogen Pseudallescheria boydii by using highly specific monoclonal antibodies. Clin Vaccine Immunol 16:756–764
Thounaojam MC, Kundu K, Kaushik DK, Swaroop S, Mahadevan A, Shankar SK, Basu A (2014) MicroRNA 155 regulates Japanese encephalitis virus-induced inflammatory response by targeting Src homology 2-containing inositol phosphatase 1. J Virol 88:4798–4810
Tien RD, Chu PK, Hesselink JR, Duberg A, Wiley C (1991) Intracranial cryptococcosis in immunocompromised patients: CT and MR findings in 29 cases. Ajnr 12:283–289
Vale TC, de Sousa-Pereira SR, Ribas JG, Lambertucci JR (2012) Neuroschistosomiasis mansoni: literature review and guidelines. Neurologist 18:333–342
van de Veerdonk FL, Kullberg BJ, van der Meer JW, Gow NA, Netea MG (2008) Host-microbe interactions: innate pattern recognition of fungal pathogens. Curr Opin Microbiol 11:305–312
van Den Pol AN, Mocarski E, Saederup N, Vieira J, Meier TJ (1999) Cytomegalovirus cell tropism, replication, and gene transfer in brain. J Neurosci 19:10948–10965
van Marle G, Antony J, Ostermann H, Dunham C, Hunt T, Halliday W, Maingat F, Urbanowski MD, Hobman T, Peeling J, Power C (2007) West Nile virus-induced neuroinflammation: glial infection and capsid protein-mediated neurovirulence. J Virol 81:10933–10949
van Riel D, Verdijk R, Kuiken T (2015) The olfactory nerve: a shortcut for influenza and other viral diseases into the central nervous system. J Pathol 235:277–287
Vanittanakom N, Cooper CR Jr, Fisher MC, Sirisanthana T (2006) Penicillium marneffei infection and recent advances in the epidemiology and molecular biology aspects. Clin Microbiol Rev 19:95–110
Verma S, Lo Y, Chapagain M, Lum S, Kumar M, Gurjav U, Luo H, Nakatsuka A, Nerurkar VR (2009) West Nile virus infection modulates human brain microvascular endothelial cells tight junction proteins and cell adhesion molecules: transmigration across the in vitro blood-brain barrier. Virology 385:425–433
Villalba M, Hott M, Martin C, Aguila B, Valdivia S, Quezada C, Zambrano A, Concha MI, Otth C (2012) Herpes simplex virus type 1 induces simultaneous activation of Toll-like receptors 2 and 4 and expression of the endogenous ligand serum amyloid A in astrocytes. Med Microbiol Immunol 201:371–379
Walsh TJ, Anaissie EJ, Denning DW, Herbrecht R, Kontoyiannis DP, Marr KA, Morrison VA, Segal BH, Steinbach WJ, Stevens DA, van Burik JA, Wingard JR, Patterson TF (2008) Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. Clin Infect Dis 46:327–360
Wang T, Town T, Alexopoulou L, Anderson JF, Fikrig E, Flavell RA (2004) Toll-like receptor 3 mediates West Nile virus entry into the brain causing lethal encephalitis. Nat Med 10: 1366–1373
Watanabe M, Suyama K, Hashimoto K, Sato M, Ohara S, Abe Y, Kawasaki Y, Yamaguchi S, Saijo M, Hosoya M (2013) Mumps virus-associated acute encephalopathy: case report and review of the literature. J Child Neurol 28:243–245
White AC Jr, Robinson P, Kuhn R (1997) Taenia solium cysticercosis: host-parasite interactions and the immune response. Chem Immunol 66:209–230
Williams DW, Calderon TM, Lopez L, Carvallo-Torres L, Gaskill PJ, Eugenin EA, Morgello S, Berman JW (2013) Mechanisms of HIV entry into the CNS: increased sensitivity of HIV infected CD14+CD16+ monocytes to CCL2 and key roles of CCR2, JAM-A, and ALCAM in diapedesis. PLoS ONE 8:e69270
Woldeamanuel YW, Girma B (2014) A 43-year systematic review and meta-analysis: case-fatality and risk of death among adults with tuberculous meningitis in Africa. J Neurol 261:851–865
Wolinsky JS, Baringer JR, Margolis G, Kilham L (1974) Ultrastructure of mumps virus replication in newborn hamster central nervous system. Lab Invest J Tech Methods Pathol 31:403–412
Woll F, Gotuzzo E, Montes M (2013) Strongyloides stercoralis infection complicating the central nervous system. Handb Clin Neurol 114:229–234

Wu HS, Kolonoski P, Chang YY, Bermudez LE (2000a) Invasion of the brain and chronic central nervous system infection after systemic Mycobacterium avium complex infection in mice. Infect Immun 68:2979–2984

Wu SJ, Grouard-Vogel G, Sun W, Mascola JR, Brachtel E, Putvatana R, Louder MK, Filgueira L, Marovich MA, Wong HK, Blauvelt A, Murphy GS, Robb ML, Innes BL, Birx DL, Hayes CG, Frankel SS (2000b) Human skin Langerhans cells are targets of dengue virus infection. Nat Med 6:816–820

Wuerfel E, Infante-Duarte C, Glumm R, Wuerfel JT (2010) Gadofluorine M-enhanced MRI shows involvement of circumventricular organs in neuroinflammation. J Neuroinflamm 7:70

Xie G, Welte T, Wang J, Whiteman MC, Wicker JA, Saxena V, Cong Y, Barrett AD, Wang T (2013) A West Nile virus NS4B-P38G mutant strain induces adaptive immunity via TLR7-MyD88-dependent and independent signaling pathways. Vaccine 31:4143–4151

Yong VW, Power C, Forsyth P, Edwards DR (2001) Metalloproteinases in biology and pathology of the nervous system. Nat Rev Neurosci 2:502–511

Yu M, Wang H, Ding A, Golenbock DT, Latz E, Czura CJ, Fenton MJ, Tracey KJ, Yang H (2006) HMGB1 signals through toll-like receptor (TLR) 4 and TLR2. Shock (Augusta. Ga 26: 174–179

Zanetta JP (1998) Structure and functions of lectins in the central and peripheral nervous system. Acta Anat 161:180–195

Zhao P, Zhao L, Zhang K, Feng H, Wang H, Wang T, Xu T, Feng N, Wang C, Gao Y, Huang G, Qin C, Yang S, Xia X (2012) Infection with street strain rabies virus induces modulation of the microRNA profile of the mouse brain. Virol J 9:159

Demyelinating Diseases and Neuroinflammation

5

Manmeet Singh and Jayasri Das Sarma

Abstract

Demyelination is a neuropathological condition of the nervous system, where the myelin sheath of neurons is damaged. This damage impairs the conduction of signals in the affected neurons. Several central nervous system (CNS) demyelinating disorders have been described in humans including, multiple sclerosis (MS), neuromyelitis optica (Devic's disease), acute disseminated encephalomyelitis, and osmotic demyelination (central pontine myelinolysis, extrapontine myelinolysis). The primary cellular target in demyelination pathology is believed to be myelin itself or the myelin-forming cells, oligodendrocytes in the CNS and Schwann cells in the peripheral nervous system (PNS). The mechanisms of demyelinating diseases are essentially unknown. It has been apparent in several current studies that demyelination/axonal loss occurs mainly *by inflammation composed predominantly of lymphocytes and monocytes/macrophages.* However, evidences suggest that demyelination/axonal loss may not be entirely immune mediated and could be due to direct virus or toxin-induced damage. Microglia, the major resident immune cells in the CNS, are considered as the key cellular mediators of neuroinflammatory demyelinating processes. Chronic/remitting neurological disease such as MS has long been considered an inflammatory autoimmune disease with the infiltration of peripheral myelin-specific T cells into the CNS. With the rapid advancement in the field of microglia and astrocytic neurobiology, the term neuroinflammation progressively started to denote chronic CNS cell-specific inflammation in MS. The direct glial responses

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in MS are different from conventional peripheral immune responses. The presence of activated microglia in the chronic active inflammatory demyelinating lesions is the foundation of neuroinflammatory pathology of demyelination.

Abbreviations

ADEM	Acute disseminated encephalomyelitis
BBB	Blood–brain barrier
CNS	Central nervous system
EAE	Experimental autoimmune encephalomyelitis
MBP	Myelin basic protein
MHC	Major histocompatibility complex
MRI	Magnetic resonance imaging
MRS	Magnetic Resonance spectrometry
MS	Multiple Sclerosis
NAWM	Normal appearing white matter
NMO	Neuromyelitis optica
PAI-1	Plasmin activator inhibitor
PLP	Proteolipid protein
PML	Progressive multifocal leukoencephalopathy
PNS	Peripheral nervous system
PP-MS	Primary progressive multiple sclerosis
RORγt	Retinoic acid receptor-related orphan receptor γt
RR-MS	Relapsing-remitting multiple sclerosis
SP-MS	Secondary progressive multiple sclerosis
TCR	T cell receptor
TLR	Toll-like receptor

5.1 Introduction

The myelin sheath is a greatly extended and modified plasma membrane that insulates axons in a spiral fashion and is essential for rapid propagation of neuronal action potentials (Fig. 5.1). In comparison to most biological membranes it has a high lipid to protein ratio with cerebroside (galactosyl ceramide) as the most typical lipid, and myelin basic protein (MBP) and proteolipid protein (PLP) as main protein components. Intertwining hydrocarbon chains of sphingomyelin serve to strengthen the myelin sheath (Fig. 5.2). Oligodendrocytes create myelin for the central nervous system (CNS), while Schwann cells myelinate the axons of the peripheral nervous system (PNS) (Fig. 5.3). In myelinated nerve fibers, action potentials propagate

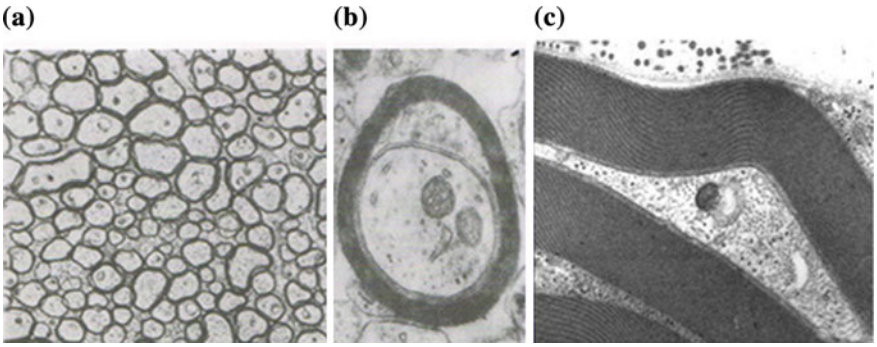

Fig. 5.1 Electron microscopic appurtenances of normal white matter: **a** section of white matter, **b** single neuronal axon wreathed by myelin sheath, **c** higher magnification picture of myelin (Unpublished data)

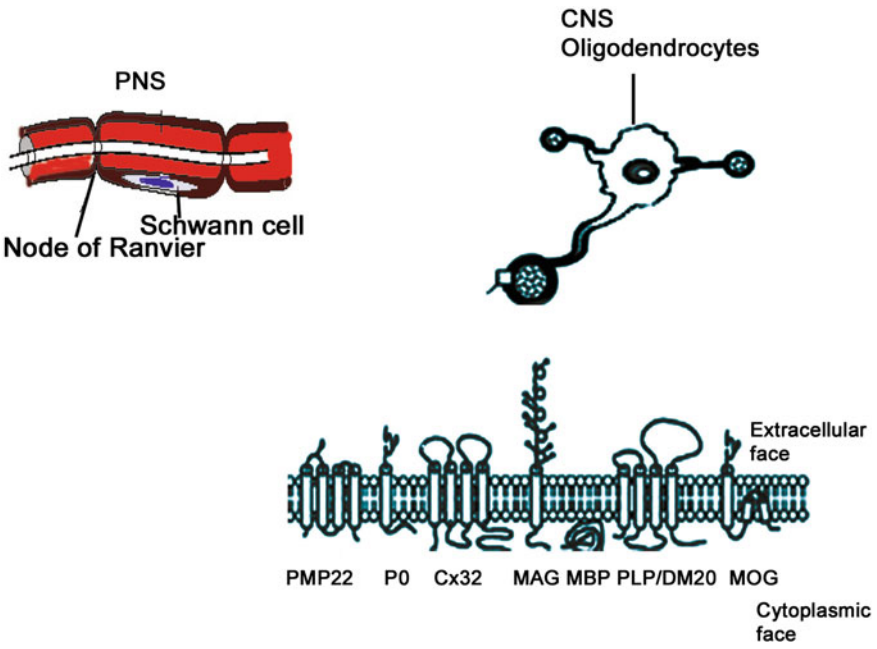

Fig. 5.2 Constituents of myelin

from one neuron to another by saltation and result in faster propagation than unmyelinated nerve fibers. Neural cells in coordination with glial cells (oligodendrocytes, microglia, astrocytes, and other glia cells) maintain homeostasis within themselves and also with the peripheral immune cells across the blood–brain barrier (BBB) (Fig. 5.4). Cytokines are major factors that play a role in response to any physical or physiological injury resulting in perturbation of the resident state of the

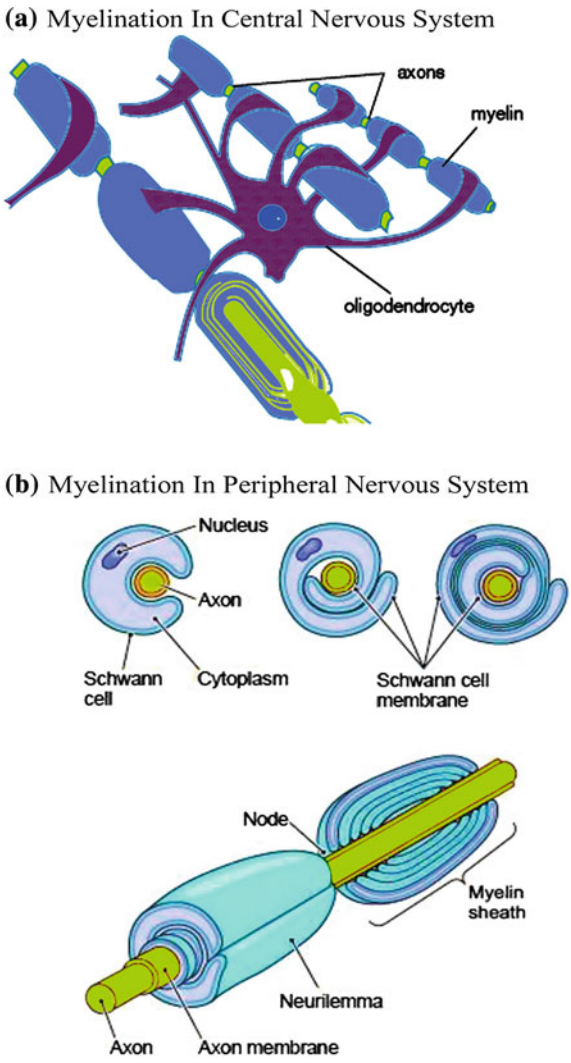

Fig. 5.3 Showing oligodendrocytes involved in myelination of axons in the central nervous system and Schwann cell in the peripheral nervous system
a oligodendrocyte is encircling multiple neurons by their projections.
b Showing Schwann cells encircling the axon of PNS

CNS. Demyelination is an acquired disorder resulting from pathological conditions that could be due to immunological, genetic, environmental, or infectious factors, in which normal myelin degenerates, exposing axons to the extracellular environment and leading to reduction in function of normal neuron-to-neuron communication. When the myelin sheath is damaged, nerve impulses slow or even stop, causing neurological problems. Among the demyelinating diseases, multiple sclerosis (MS) is the most common demyelinating disease of the CNS. As opposed to the destruction of normal myelin that is seen in demyelination, dysmyelination refers to malformed and defective myelin sheath. Dysmyelination often arises from hereditary mutations that affect the synthesis and formation of myelin.

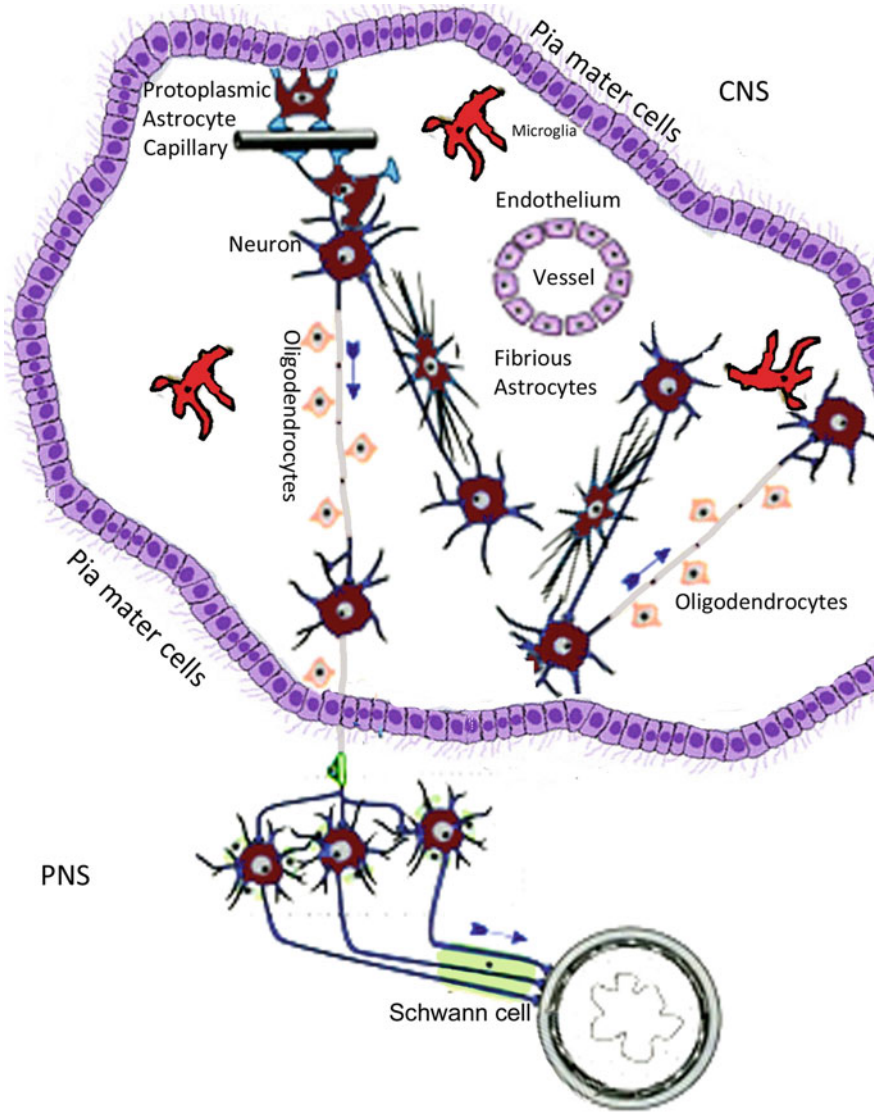

Fig. 5.4 Showing different cell types in the nervous system, endothelial cells, vessels, microglia cell, oligodendrocytes, neural cells communicating with each other

The pathological hallmark of MS is the presence of focal demyelinated plaques with partial axonal preservation and reactive glial scar formation in the white and gray matter of the CNS. In addition, there is diffuse damage throughout the normal appearing white and gray matter. With disease progression, these alterations are associated with increasing global brain atrophy. While MS is the most common neurological diseases in the CNS there are also other types of demyelinating disease, including the following:

- Idiopathic optic neuritis—inflammation of the optic nerve in one or both eyes
- Neuromyelitis optica (NMO; Devic's disease)—inflammation and demyelination of the central nervous system, especially of the optic nerve and spinal cord
- Transverse myelitis—inflammation of the spinal cord
- Acute disseminated encephalomyelitis—inflammation of the brain and spinal cord
- Adrenoleukodystrophy and adrenomyeloneuropathy—rare, inherited metabolic disorders.

MS and other demyelinating diseases most commonly result in vision loss, muscle weakness, muscle stiffness, and spasms, loss of coordination, loss of sensation, pain, and changes in bladder and bowel function. A major challenge in the field of pathology in MS came from recent developments in magnetic resonance imaging (MRI) and spectroscopic (MRS) techniques. MRI and MRS permitted the study of the dynamic evolution of brain and spinal cord damage during the course of the disease.

Different types of actively demyelinating lesions are found in MS brains in the early stage of the disease, which apparently reflect different immunopathologic mechanisms of their formation (Lucchinetti et al. 2000). All these pathologies develop on the background of an inflammatory reaction and there is no major quantitative or qualitative difference in the extent of inflammation or the composition of inflammatory infiltrates (Lucchinetti et al. 2000). Inflammation in demyelinating lesions is mediated most often by a T cell-driven process, which leads to profound activation of macrophages and microglia. However, it seems that these basic lesions are modified by differences in the fine tuning of the inflammatory reaction, by recruitment of additional effectors mechanisms, as well as by the reaction of the target tissue in response to the inflammatory reaction. It was originally described that the respective patterns of tissue destruction are common in all active lesions of an individual patient, but differ between patients or patient subgroups (Lucchinetti et al. 2000). This suggests a genuine interindividual heterogeneity between patients, possibly determined by the genetic background (Lassmann et al. 2001). The patterns of demyelination clearly segregate in the extreme variants of inflammatory demyelinating diseases, Devic's NMO being associated with antibody and complement-mediated tissue damage (Lucchinetti et al. 2002) and Balo's concentric sclerosis with hypoxia-like tissue injury (Stadelmann et al. 2005).

5.2 Neuro-Immunopathogenesis of Multiple Sclerosis (MS)

MS is a heterogeneous and complex disease that is characterized by inflammation, demyelination, and axon degeneration in the CNS. This pathology most likely results from a primary defect in the immune system that targets components of the myelin sheath, resulting in secondary effects on neurons.

MS lesions are characterized by infiltration of lymphocytes and antibody-producing plasma cells into the perivascular region of the brain and spinal cord white matter, an increase in microglia and astrocytes, and demyelination (Frohman et al. 2006). The deposition of antibodies and complement around demyelinated lesions (Frohman et al. 2006) and axonal degeneration in the progression phase of MS have also been observed (Trapp and Nave 2008). When damage and the ensuing inflammatory response are transient, remyelination of nerves can take place as part of normal repair. However, in the presence of chronic inflammation, such as in MS, remyelination is severely impaired and leads to axon degeneration and the eventual demise of the neuron.

MS pathology and pathogenesis are apparently much more complex than originally anticipated. Pathology favors the concept that all neurodegenerative events in MS are driven by the inflammatory component, although this view is still controversial. From this perspective, extensive research efforts have been devoted to understand the nature of the inflammatory process and to ameliorating tissue injury by therapies directed against the immune response. Focal white matter lesions in MS, the classical plaques, are defined by the triad of inflammation, primary demyelination, and reactive astrocytic scar formation. Anti-inflammatory, immunomodulatory, or immunosuppressive treatments prevent, at least in part, the development of new plaques (Noseworthy et al. 2000).

5.2.1 Inflammation, Neuroinflammation and Neurodegeneration

While some chronic/remitting neurological demyelinating diseases, such as MS, have long been recognized as inflammatory, the term neuroinflammation has come to denote chronic, CNS-specific, inflammation-like glial responses that do not reproduce the classical characteristics of inflammation in the periphery but that may engender neurodegenerative events. New understanding of neuroinflammation has come from rapid advances in the field of microglial and astrocytic neurobiology over the past decade. These advances have led to the recognition that glia, primarily microglia, respond to tissue insult with a complex array of inflammatory cytokines secretion. Microglia are now recognized as the prime components of an intrinsic brain immune system (Stadelmann et al. 2005), and as such they have become a main focus in cellular neuroimmunology and therefore in neuroinflammation. This is not the inflammation of the adaptive mammalian immune response, with its array of specialized T cells and the made-to-order antibodies produced through complex gene rearrangements. This is, instead, the innate immune system, upon which adaptive immunity is built (Frohman et al. 2006).

Recent insights gave rise to the concept that in MS neurodegeneration may occur independently of the peripheral inflammatory process. This view was further supported by the poor efficacy of current anti-inflammatory, immunomodulatory, or immunosuppressive treatments to stop clinical deterioration when patients have entered the progressive phase of the disease (Noseworthy et al. 2000).

In contrast to classic neurodegenerative diseases, all lesions in MS, regardless of the stage and type of disease, are associated with inflammation (Frischer et al. 2009). Progressive MS rather reflects a compartmentalization of the inflammatory response in the CNS than absence of inflammation. **Based on these observations it was suggested that brain damage in MS is mediated by two possibly independent events**—(1) an inflammatory reaction, which drives the formation of focal white matter lesions, and, (2) neurodegeneration, which is responsible for diffuse and progressive brain damage (Burt et al. 2003; Coles et al. 2006). This view was further boosted by a study on very early MS lesions in a patient who died a few hours after clinical disease onset due to a lesion located in the brainstem (Barnett and Prineas 2004). In this case, oligodendrocyte apoptosis and initial demyelination were associated with microglia activation, but no infiltration of the lesion parenchyma by T cells was found. From these studies, the possibility arises that **MS is triggered by degeneration of oligodendrocytes and myelin, which is followed by an inflammatory reaction, which potentiates tissue injury**.

Inflammation is the primary event in MS, which drives subsequent tissue injury. However, preexisting neurodegeneration may amplify tissue injury in a proinflammatory environment (Perry et al. 2010). Neurodegeneration always leads to microglia activation, which primarily is involved in removal of tissue debris and provides neuroprotection. Such preactivated microglia are, however, more easily converted into highly pathogenic effector cells, when exposed to proinflammatory cytokines in an inflammatory environment (Perry et al. 2010). Thus, preexistent neurodegeneration may render the nervous system more susceptible to inflammation-mediated tissue injury. Innate immune response in the brain is considered to be a potentially pathogenic factor in a number of CNS diseases that lack the prominent leukocytic infiltrates of adaptive immune responses, but that do have activated microglia and astrocytes, i.e., **neuroinflammation**. Thus, the term neuroinflammation can be described as lesions where limited neuronal insults trigger glial cell activation without breakdown of the blood-brain barrier and without concomitant leukocyte/blood monocyte infiltration.

5.3 Neuroimmunopathology of Demyelination and Axonal Loss in MS

Both the innate and acquired immune systems are involved in MS pathology. Several lines of evidence demonstrate that immune cells outside of the CNS such as dendritic cells are key players in MS pathogenesis (Bailey et al. 2007). Although autoreactive T and B cells play major roles in MS pathology, it is the **innate immune system** that initiates the disease. For example, naive T cells recognize the myelin-specific antigen MBP when this autoantigen is presented in the context of Major Histocompatibility Complec (MHC) by antigen-presenting cells such as dendritic cells, macrophages, and microglia. Antigen-presenting cells not only present the antigen to T lymphocytes but also provide costimulation and produce

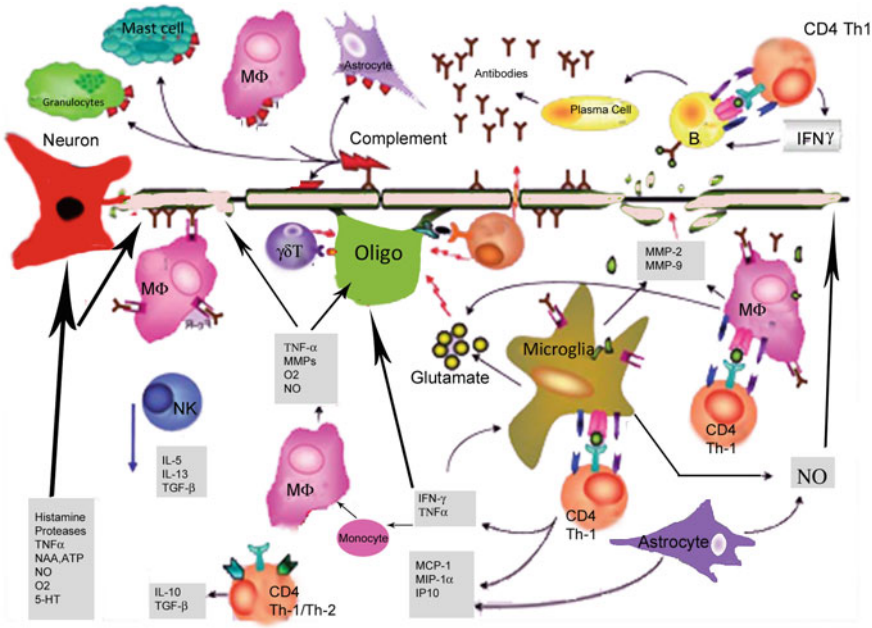

Fig. 5.5 Schematic diagram depicting the pathogenetic steps and contributing factors that lead to tissue damage in MS

the cytokines required for T cells to differentiate into effector cells. Figure 5.5 shows a simplified view of MS pathogenesis within the CNS.

5.3.1 Activation of T Helper Cells

Innate immune cells influence differentiation of distinct subsets of T helper cells in demyelination (Marta et al. 2009). Recently, phagocytosis of infected apoptotic cells was reported to be a physiological signal that induces activation of effector T cells in vivo (Torchinsky et al. 2009). Autoreactive T and B lymphocytes play roles as amplifiers and effectors in MS. Th1 helper T cells were initially thought to play a crucial role in MS pathogenesis. However, characterization of specific functions of IL-12, IL-23, and other IL-12 family members has uncovered essential roles for a subset of T helper cells called Th17 cells in the pathogenesis of MS (Cua et al. 2003). These Th17 cells secrete members of the IL-17 proinflammatory cytokine family, especially IL-17A and IL-17F (Korn et al. 2009), and play a key role in infection by pathogens and in gut immunity. The differentiation and activation of Th17 cells requires signaling though the T cell receptor (TCR) as well as a mixture of cytokines produced by antigen-presenting cells. These include IL-1β for human and IL-6 for mice, and TGF-b as well as IL-23 and other cytokines. Th17 cells also secrete IL-21, which induces activation of Th17 cells in an autocrine manner

(Korn et al. 2009). The retinoic acid receptor-related orphan receptor γt (RORγt) plays a key role in Th17 cell differentiation. Treg cells, which are anti-inflammatory helper T cells, play an opposing role by inhibiting the activity of Th17 cells. Differentiation of this cell type requires the Foxp3 transcription factor (Littman and Rudensky 2010).

5.3.2 Autoreactive T Cells

It is widely believed that MS is driven by autoimmunity. This view is highly influenced by experimental data on autoimmune encephalomyelitis, a disease which leads to a pathologic process in the CNS featuring many aspects of MS (Storch et al. 1998). This view is further supported by immunologic studies, which demonstrate the existence of autoreactive T cells (Pette et al. 1990) and autoantibodies in MS patients (O'Connor et al. 2001). Anti-MOG antibodies, which are potentially demyelinating, can be extracted from MS lesions (O'Connor et al. 2005). Furthermore, MHC class II complexes with MBP peptides have been detected on antigen-presenting cells in MS lesions (Krogsgaard et al. 2000). Unfortunately, however, a single MS-specific autoimmune reaction has not been identified, and autoreactive T cells and autoantibodies are also part of the normal immune repertoire of healthy individuals (Pette et al. 1990). Taken together, these data suggest that autoimmune reactions play a role in the pathogenesis of the disease: whether they are the primary cause of the disease or provide a secondary mechanism, amplifying the formation of the lesions, is yet unresolved. Another important aspect regarding autoimmunity in MS is that until recently only class II restricted CD4+ T lymphocytes have been regarded as effector cells mediating CNS autoimmunity. More recently, however, new experimental models were developed which clearly show that class I MHC restricted CD8+ T cells, too, can induce brain inflammation and organ-specific autoimmunity (Huseby et al. 2001; Sun et al. 2001). CD8+ cells dominate the T cell infiltrates in all MS lesions, regardless of the clinical subtype of the disease or the stage of the lesion, and these cells show dominant clonal expansion in the lesions. Furthermore, such CD8+ T cell clones may remain stable in the patient's immune system for several years (Skulina et al. 2004) and autoreactive class I restricted T cells can be found in the circulation of MS patients (Friese and Fugger 2005). It is possible, but so far undetermined, that autoimmunity, mediated by such T cells, may be more specific for MS than that mediated by class II restricted CD4+ T cells. Alternatively, the clonally expanded T cell response may also be directed against a foreign (infectious) antigen.

Autoreactive T cells, both of CD4+ and CD8+ T cell phenotype, can be isolated from peripheral blood of MS patients (Pette et al. 1990; Kerlero de Rosbo et al. 1997), and macrophages or microglia cells within MS lesions may express antigenic peptides from CNS proteins on their surface (Krogsgaard et al. 2000). Furthermore, clonal expansion of T cells within the lesions suggests their antigen-driven proliferation (Babbe et al. 2000; Skulina et al. 2004). Despite these advances, major questions are still unresolved. It is not known whether autoreactive T cells in MS

lesions are the exception or the rule. Another problem in the interpretation of the role of T cells in lesion pathogenesis in MS is that leukocytes may not necessarily be harmful. Moreover, T cells, B cells, and monocytes can produce neurotrophins (Kerschensteiner et al. 1999; Moalem et al. 2000), and brain-derived neurotrophic factor, in active MS plaques (Kerschensteiner et al. 1999; Stadelmann et al. 2002).

Cytotoxic T cells are able to destroy oligodendrocytes as well as axons through specific recognition of their cognate antigen, presented by MHC class I molecules (Evans et al. 1996; Medana et al. 2001). In experimental models this may lead to the formation of large confluent demyelinating plaques, closely similar to those seen in MS (Saxena et al. 2008). In the brains of MS patients MHC class I molecules are widely expressed in all tissue components (Hoftberger et al. 2004) and CD8+ T cells, which express granzyme B as a marker for cytotoxic activation, are sometimes found in close contact to oligodendrocytes in acute MS lesions (Neumann et al. 2002). T cells can also destroy neurons by antigen-independent mechanisms (Nitsch et al. 2004), possibly involving death receptors of the tumor necrosis factor family (Aktas et al. 2005). Such death receptors are also expressed in actively demyelinating MS lesions (Dowling et al. 1996; D'Souza et al. 1996; Bonetti and Raine 1997) although their specific cellular expression is unclear.

5.3.3 Microglia and Macrophages

Besides T lymphocytes, macrophages and activated microglia cells are abundant in active MS lesions. These cells can form close contacts with myelin sheaths as well as dystrophic axons (Ferguson et al. 1997; Trapp et al. 1998) and they are engaged in the removal and digestion of tissue debris (Bruck et al. 1995). These phagocytes are derived both from hematogenous monocytes as well as from the pool of local microglia (Li et al. 1996). In early MS lesions, macrophages which express the chemokine receptors CCR1 and CCR5 are abundant in the perivascular space. This chemokine receptor profile is a typical feature of circulating monocytes, which can pass the BBB. With maturation of the lesions, the number of CCR1-positive phagocytes decreases dramatically, while CCR5 appears on the majority of cells, which are engaged in tissue removal (Trebst et al. 2001). The dynamics of CCR1 versus CCR5 expression in the lesions suggests that the majority of phagocytes within active MS lesions come from the microglia pool and this is supported by the observation of an activation gradient of microglia from the periplaque white matter into the active plaques (Lassmann 2011).

Phagocytes in MS lesions can express a large number of molecules, which are engaged in migration, phagocytosis, antigen presentation, and tissue injury. They include adhesion molecules (Cannella and Raine 1995), chemokine receptors (Huang et al. 2000), scavenger receptors (Fabriek et al. 2005; Marik et al. 2007), Fc receptors (Ulvestad et al. 1994), MHC molecules (Traugott 1987), costimulatory molecules (Windhagen et al. 1995; Gerritse et al. 1996), proteases (Cuzner and

Opdenakker 1999; Lindberg et al. 2001), Toll-like receptors (TLRs) (Bsibsi et al. 2002), and inducible nitric oxide synthase (Liu et al. 2001). Most of these molecules are dominantly upregulated appears to depend upon the type of tissue damage, the stage of the individual lesion, and the disease stage. Recently, different lesion types have been identified in patients with active disease, involving T cells and macrophages alone, pathogenic autoantibodies, or a hypoxia-like tissue injury, most likely mediated through the action of reactive oxygen and nitrogen intermediates (Lucchinetti et al. 2000; Aboul-Enein et al. 2003). When comparing these lesions with each other, major differences in the patterns of phagocyte activation can be seen. In particular, the plaques with signs of hypoxia-like tissue injury reveal only mild to moderate expression of immune-driven macrophage activation antigens and a very low expression of the chemokine receptor CCR5 (Trebst et al. 2001), while scavenger receptors and enzymes involved in radical production are prominently expressed (Stadelmann et al. 2005; Fischer et al. 2012). On the contrary, macrophages which have ingested tissue debris in nervous system lesions may obtain a deactivated phenotype (Boven et al. 2006). Therefore, it seems that the local microenvironment determines the pattern of macrophage and microglia activation in MS lesions. Activated macrophages can induce demyelination and axonal injury through a variety of toxic effector mechanisms, including tumor necrosis factor-a (Probert et al. 2000), proteases (Anthony et al. 1998), reactive oxygen or nitric oxide species (Smith and Lassmann 2002), or excitotoxins (Lipton 1998). Recently, phagocytosis of infected apoptotic cells (which trigger TLR signaling) was reported to be a physiological signal that induces activation of effector T cells in vivo (Torchinsky et al. 2009).

5.3.4 B Lymphocytes and Plasma Cells

B lymphocytes and plasma cells are another important component of the inflammatory response in MS lesions (Corcione et al. 2005). They are rare in early stages of acute and relapsing MS (Frischer et al. 2009). However, their relative contribution increases with chronicity of the disease (Ozawa et al. 1994) and they are particularly prominent in inflammatory aggregates in the meninges, which show features of lymphatic B-cell follicles (Serafini et al. 2004; Franciotta et al. 2008). B cells and plasma cells produce immunoglobulin within the lesions, including IgG, IgM, and IgA (Tavolato 1975; Mussini et al. 1977; Esiri 1980). This is reflected in MS patients by intrathecal immunoglobulin synthesis, which, when detected in the cerebrospinal fluid, contributes to differential diagnosis. B cells, however, may also produce a variety of cytokines and chemokines and it is possible that these factors also contribute to the proinflammatory environment in MS lesions. Interestingly, systemic elimination of B cells with an antibody directed against CD20 is an effective therapy for MS patients in early stages of the disease (Hauser et al. 2008).

5.4 Antibody-Mediated Demyelination and Tissue Destruction

Specific binding of antibodies to myelin or oligodendrocytes is difficult to detect unequivocally in MS lesions because much of the CNS tissue in this disease is covered by immunoglobulins, either entering the brain through the leaky BBB or being locally produced by infiltrating plasma cells (Tavolato 1975). However, in some lesions a profound accentuation of IgG or IgM staining at sites of active demyelination can be found, associated with macrophages, present in close apposition to myelin sheaths (Prineas and Graham 1981). In a recent study binding of immunoglobulin IgG and IgM to oligodendrocytes and axons has been observed in the majority of acute and chronic active MS lesions (Sadaba et al. 2012).

5.5 Other Factors May Contribute in Demyelination Pathology

Activated macrophages and microglia in MS lesions express Fc and complement receptors, which enable them to bind and interact with antibody-opsonized myelin (Ulvestad et al. 1994). Experimental studies have shown that impaired fibrinolysis within brain tissue may augment inflammation, demyelination, and axonal injury (Akassoglou et al. 2004; East et al. 2005). Neuropathologic studies suggest that in MS lesions, too, fibrinolysis may be impaired (Gveric et al. 2003). Fibrin deposits were found by immunocytochemistry on macrophages and demyelinated axons, and these deposits were associated with a decreased fibrinolytic activity within the plaques. Urokinase and tissue plasmin activator were expressed in macrophages in the lesions, and plasmin activator inhibitor (PAI-1) was also upregulated (Gveric et al. 2001, 2003). PAI-1 was bound to tissue plasmin activator, suggesting inhibition of the respective proteolytic activity. An impairment of this cascade may directly be deleterious to axons and neurons (Gveric et al. 2005) or lead to activation of microglia through integrin receptors or TLRs (Smiley et al. 2001; Flick et al. 2004).

5.6 Mechanism of Migration of Inflammatory Cells

The basic mechanisms of T lymphocyte migration through the BBB in the course of immune surveillance and brain inflammation are well understood. Preactivation of cells in the peripheral immune system seems to be necessary for them to pass the barrier (Hickey et al. 1991). Much less is currently known about how B lymphocytes and monocytes enter the CNS. Migration of leukocytes through the wall of cerebral vessels requires the expression and activation of adhesion molecules (Springer 1994) and the interaction of chemokines with their specific receptors (Luster 1998). Depending upon the type of leukocyte and the nature of the

inflammatory process, the cells migrate by a paracellular route through tight junctions between adjacent endothelial cells or by a transcellular route through transendothelial cytoplasmic channels (Engelhardt and Wolburg 2004). Leukocyte migration through cerebral endothelia is not necessarily associated with disturbance of the BBB for proteins. Impermeable junctions can temporarily be formed between endothelial cells and transmigrating leukocytes. Besides, the binding of leukocytes to endothelial cells and specific cell/cell signaling through adhesion molecules and chemokine receptors, proteases, secreted by leukocytes, are required for paracellular migration and for the dissolution of the vessel's basement membrane. All three components of this migration process, the adhesion molecules (Sobel et al. 1990; Cannella and Raine 1995), the chemokine system (Huang et al. 2000), and the proteases (Cuzner and Opdenakker 1999), are highly redundant and it is therefore not surprising that a large number of these molecules have been identified in active MS lesions (Engelhardt and Ransohoff 2005; Greenwood et al. 2011).

5.7 Axonal Injury and Destruction

The presence of plaques with primary demyelination is the hallmark of the pathologic diagnosis of MS. This means that myelin sheaths are completely destroyed and lost, while axons remain at least partially preserved. Nevertheless, it was common knowledge, dating from the earliest pathologic studies of MS, that axons are also affected by the disease process (Kornek and Lassmann 1999). Acutely injured axons have been seen in close contact with macrophages or activated microglia cells, suggesting their role in axonal destruction and even some attempts of axonal sprouting have been depicted within lesions (Schirmer et al. 2013). Extensive axonal destruction and loss within plaques were associated with poor functional recovery (Charcot, 1880). Despite all this knowledge, the importance of axonal injury in MS was largely ignored until the past two decades. Two different mechanisms lead to axonal destruction in MS. Fulminant axonal injury in actively demyelinating lesions is most likely mediated by cells or mediators of the inflammatory reaction. In addition, however, there seems to be a slowly progressive axonal loss that occurs in inactive plaques (possibly due to the lack of proper trophic support), which is largely absent once substantial remyelination takes place. Axonal loss is profound in chronic MS plaques. Close apposition of activated macrophages or microglia to dystrophic axons is regularly found (Ferguson et al. 1997; Trapp et al. 1998; Kornek et al. 2000). A variety of proteases (Cuzner and Opdenakker 1999) and inducible nitric oxide synthase (Liu et al. 2001) are expressed in macrophages in active lesions. Alterations in the expression of enzymes, involved in glutamate biosynthesis and degradation, indicate the involvement of excitotoxic mechanisms (Werner et al. 2001). And, finally, a variety of sodium (Craner et al. 2004) and voltage-gated calcium channels (Kornek et al. 2001) are aberrantly expressed within axons in MS lesions, which indicate their involvement in functional disturbances and structural damage.

Interestingly, not all axons in MS lesions are similarly vulnerable. Axonal loss mainly affects thin fibers, while thick fibers are much more resistant (Evangelou et al. 2001). This pattern of affecting thinner fibers is also reflected in the degeneration of neurons whose axons project into demyelinated plaques.

5.8 Clinical Subtypes of MS

Based on clinical observations it has been postulated that the pathogenesis of the disease in the progressive stage of MS is different from that seen in patients with relapsing-remitting disease.

Based on the clinical observation there are three major forms of MS (Fig. 5.6). In the early stage of the disease, many MS patients exhibit a ***relapsing-remitting (RR)-MS*** type of disease. RR-MS is the most frequent (85–90 %) and affects women about twice as often as men. However, with time, the recovery of these RR patients is impaired and eventually leads to irreversible progression, that is, ***secondary progressive MS (SP)-MS***. The majority of RR-MS patients develop secondary progressive (SP)-MS. In contrast, about 10–15 % of MS patients do not show any remission (Goverman 2009; Sospedra and Martin 2005) and these patients present with insidious disease onset and steady progression, termed ***primary progressive (PP)-MS***.

Active demyelination and tissue injury in the progressive stage of MS are associated with inflammation. There are, however, differences in the nature of the inflammatory process between early and late stages of the disease. Pathologically, all the typical features of MS are present in patients with primary progressive disease, including inflammation and focal plaques of demyelination with reactive astrocytic scarring (Lassmann 2012; Lassmann et al. 2007), but inflammation within focal white matter plaques is less severe compared to that in patients with SP-MS (Revesz et al. 1994). Systematic analysis of cortical demyelination and

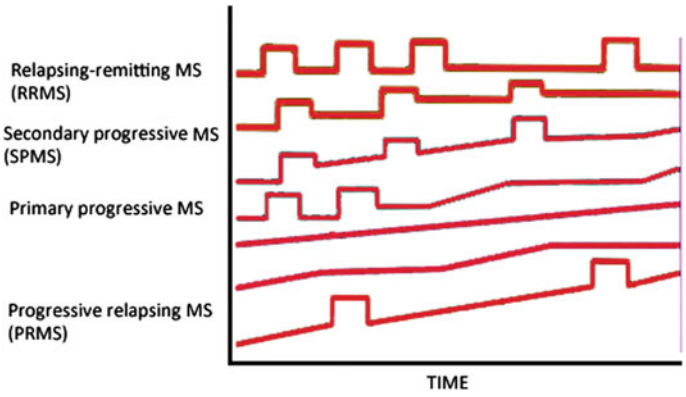

Fig. 5.6 Clinical patterns of MS

diffuse white matter injury revealed that both of these pathologies are present in PP-MS and SP-MS in about the same extent and severity (Kutzelnigg et al. 2005).

According to the structure and shape, several types of active lesions can be distinguished (Lassmann et al. 1998). ***Acute plaques*** show synchronous myelin destruction throughout the entire lesion and macrophages within the lesions all contain myelin degradation products at the same (early) stage of myelin digestion. ***Chronic active plaques*** have an inactive lesion center, which is surrounded by a rim of macrophages with early myelin degradation products. ***Slowly expanding active plaques*** (Kutzelnigg et al. 2005) also consist of an inactive lesion center and are surrounded by a rim of macrophages and activated microglia. While acute or chronic active lesions are most frequent in early MS and become rare in the progressive stage, slowly expanding lesions show the opposite distribution in relation to MS course (Frischer et al. 2009).

Based on clinical observations, it has been postulated that the pathogenesis of the disease in the progressive stage of MS is different from that seen in patients with RR disease. While new lesions in the cortex and white matter in early MS are induced by new waves of leukocytes, entering the CNS from the circulation and being associated with profound BBB leakage, inflammation at least in part becomes trapped behind a closed or repaired BBB in patients with progressive disease (Hochmeister et al. 2006).

On MRI, focal white matter lesions are present, although fewer numbers and smaller lesion areas are seen compared to those found in relapsing or secondary progressive disease (Thompson et al. 1991; Fu et al. 1998; Pelletier et al. 2003; Rocca et al. 2003). In contrast, diffuse damage of the NAWM (normal appearing white matter) and brain atrophy are pronounced. Furthermore, as in SP-MS (Howell et al. 2011) active tissue injury in the cortex in PP-MS is associated with meningeal inflammation (Choi et al. 2012).

Thus, all pathological features typical for MS are present in patients with PP-MS and SP-MS. The relative contribution to the disease process, however, seems to be different from that seen in RR-MS (Lassmann 2012). New focal white matter plaques, although present, are rare in progressive MS compared to RR-MS and are associated with less inflammation. In contrast, slow expansion of preexisting lesions, cortical demyelination as well as diffuse inflammation, microglia activation, and axonal injury in the NAWM are extensive in patients with PP-MS and SP-MS.

Oxidative injury seems to be a prime mechanism of tissue injury in RR-MS as well as progressive MS. Activation of microglia due to accumulating neurodegeneration may further amplify tissue injury in the chronic inflammatory environment in the brain of patients with progressive MS (Lassmann 2012). Thus, in the progressive stage the disease process is in part compartmentalized within the brain and spinal cord and this may explain the failure of current treatments, which predominantly target the peripheral immune response.

5.9 Other Primary Demyelinating Diseases

5.9.1 Optic Neuritis

Acute optic neuritis is the most common optic neuropathy affecting young adults. In its typical form, optic neuritis presents as an inflammatory demyelinating disorder of the optic nerve. A typical form of optic neuritis can occur, either in association with other inflammatory disorders or in isolation. The pathophysiology of optic neuritis in MS has been studied in human beings and in animal models. The optic nerve lesion is pathologically very similar to MS brain lesions. In the acute phase, inflammatory demyelination occurs resulting in varying degrees of conduction block and visual loss. Predominant T cell activation occurs in the acute phase, with release of proinflammatory cytokines although there could also be B-cell involvement and microglial activation. Resolution of inflammation and visual recovery occurs over the next few weeks. Remyelination occurs, although it is usually incomplete and sodium channels are redistributed over demyelinated segments. This redistribution improves conduction but can make surviving axons vulnerable to damage. Visual recovery can be incomplete, probably because of the effects of persistent demyelination and axonal loss. Advances in optical coherence tomography, visual evoked potentials, and MRI have provided insight into the pathophysiological processes and clinical correlations for optic neuritis.

The patterns of demyelination clearly segregate in the extreme variants of inflammatory demyelinating diseases, Devic's NMO being always associated with antibody and complement-mediated tissue damage (Lucchinetti et al. 2002) and Balo's concentric sclerosis with hypoxia-like tissue injury (Stadelmann et al. 2005). Barnett and Prineas, 2004 suggest that MS lesions can also start with pattern III (hypoxia-like) changes and, when they further actively expand, develop into a pattern of complement-mediated demyelination (pattern II) (Barnett and Prineas 2004).

5.9.2 Devic's Neuromyelitis Optica: A Distinct Disease Entity

NMO is apparently an autoimmune disease directed against an astrocytic antigen, which is reflected by primary astrocytic damage followed by demyelination and neurodegeneration. Lennon et al. (2004, 2005) identified a novel autoantibody response in patients with Devic's type of NMO. These autoantibodies stain astrocytic processes in the perivascular and superficial glia limiting membrane and have subsequently been found to react with the water channel protein aquaporin 4. The distribution of this antigen explains well the massive perivascular antibody and complement deposition in the spinal cord of patients with Devic's disease (Lucchinetti et al. 2002). Lesions in patients with aquaporin 4 antibodies are mainly located within the optic nerves and the spinal cord; they are destructive, not only

affecting astrocytes, but also resulting in pronounced myelin, oligodendrocyte, or axonal injury and loss. In rare cases, however, extensive destructive lesions are also seen in the brain. In the spinal cord, they mainly affect the central gray matter and extend over several spinal cord segments. This characteristic appearance is also well reflected in MRI. T cell-mediated autoimmune reactions against aquaporin 4 have recently been detected also in NMO patients (Varrin-Doyer et al. 2012). However, aquaporin 4 antibodies are so far only found in about 70–90 % of patients with NMO (Lennon et al. 2004; Takahashi et al. 2007) and the pathogenesis of disease in these aquaporin 4 antibody-negative patients is currently unclear. Recent studies indicate that some aquaporin 4 antibody-negative NMO patients may have circulating antibodies against an epitope of MOG, which is expressed on the surface of oligodendrocytes and myelin (Kitley et al. 2012; Rostasy et al. 2013).

5.9.3 Balo's Disease is Part of the MS Spectrum, But Involves Specific Additional Mechanisms of Tissue Injury

Typical Balo's concentric sclerosis is an acute or subacute inflammatory demyelinating disease, which leads to very large white matter lesions with concentric layering of myelinated and demyelinated tissue (Courville and Cooper 1970) (Balo, 1928) (Fig. 5.7). Typical Balo's concentric sclerosis is very rare, but some layers of concentric demyelination are frequently found in actively demyelinating plaques of patients with acute or early relapsing MS (Barnett and Prineas 2004; Stadelmann et al. 2005). Thus, Balo's disease is not a disease entity distinct from MS, but it reflects an extreme variant of tissue alterations, which to a lower extent are also present in other MS patients. Concentric demyelination was exclusively found in lesions which showed the structural features of hypoxia-like tissue injury, reflected by the loss of MOG and by oligodendrocyte apoptosis. When these lesions were in the active stage of demyelination, a profound expression of

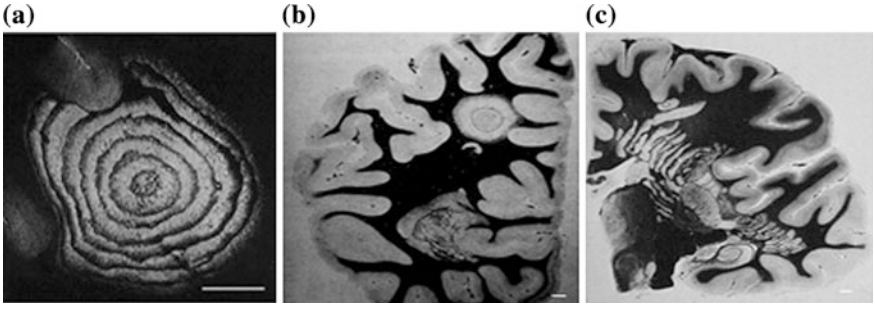

Fig. 5.7 **a** Original case of Balo: several anastomoses are located in lower half of lesion, **b** lesion, **c** progress of the pathological process from a center located in a constrained area, showing formation of bands

proteins, which are involved in tissue preconditioning was expressed in a small concentric zone outside the outermost actively demyelinating rim as well as in one to two most peripherally located layers of preserved myelin. In contrast, at sites of active demyelination and tissue destruction the tissue was heavily infiltrated by macrophages. Apoptotic-like cell death is frequently encountered in areas adjacent to ongoing myelin destruction (Yao et al. 1994). Axonal injury and loss are profound within the demyelinated rims, but only minor in myelinated areas (Courville and Cooper 1970).

5.9.4 Acute Disseminated Encephalomyelitis (ADEM)

ADEM is a monophasic demyelinating illness that can present with clinical, imaging, and laboratory manifestations indistinguishable from an acute MS attack (Griffin 1990). ADEM is defined as an acute monophasic, sometimes relapsing disease with clinical features, distinct from MS, with rare incidence of oligoclonal bands in the cerebrospinal fluid and with a high incidence of gray-matter involvement (de Seze et al. 2007). In pathology, ADEM lesions are characterized by profound inflammation, the inflammatory infiltrates being mainly composed of T cells and activated macrophages. This is associated with perivenous demyelination. In contrast to acute and chronic MS, no confluent demyelinated plaques are seen. Furthermore, profound inflammation and microglia activation are also present in the gray matter, including the cerebral cortex, but this inflammation is not associated with primary demyelination (Young et al. 2010). It has recently been shown that a fraction of patients with childhood ADEM have high circulating antibody titers against an epitope of MOG, which is recognized by demyelinating antibodies (O'Connor et al. 2007). Typical ADEM is seen in pediatric populations and has more of an explosive course associated with alterations in mental status, and a post-viral or postvaccination history is often elicited. This disease is associated with significant responses to myelin proteins, indicated both by T cell and antibody measurements.

5.9.5 Other Demyelinating Conditions

In several other primary demyelinating diseases, myelinoclastic (myelin destructive) pathogenic cellular mechanisms of injury are also known to occur. Examples of these are selective infection of oligodendrocytes by *papovavirus* in progressive multifocal leukoencephalopathy (PML) (Khoury et al. 2014) and toxic injury to oligodendrocytes in cyanide and hexachlorophene poisoning. Central pontine myelitis is a focal damage to brain myelin that occurs following metabolic disturbances and rapid correction of hyponatrema.

5.10 Dysmyelinating Disease

Leukodystrophies (also called dysmyelinating diseases) are genetic diseases that result in primary demyelination. However, the myelin being removed is inherently abnormal. Most of these disorders are due to defects in genes affecting myelin structural proteins or enzymes affecting myelin structural proteins or enzymes affecting myelin turnover. The result is an abnormal or unstable myelin. Although many leukodystrophies are evident in infancy or childhood, onset of the diseases in adults can result from degeneration of myelin that was apparently normal and functional at earlier stages. In some of the leukodystrophies with early onset and massive demyelination, there is nearly a complete lack of normal myelin production.

Adrenoleukodystrophy is due to a genetic defect of a peroxisomal transporter. In humans, there is also one example, in which a genetically determined defect of lipid metabolism is in a subset of patients associated with an inflammatory demyelinating disease, which in some aspects resembles MS. It is reflected in different clinical manifestation, including cerebral adrenoleukodystrophy, which is an inflammatory disease of the CNS, resulting in extensive demyelination of the brain white matter (Moser 1997). Other patients with the same genetic defect suffer from adrenomyeloneuropathy, a noninflammatory degenerative disease, mainly affecting the spinal cord. Although in adrenoleukodystrophy inflammation is clearly triggered as a secondary reaction to the metabolic defect, it is not clear so far which immunological events are responsible. One possible mechanism is that altered fatty acid chains may trigger autoimmune reactions against glycolipids in genetically susceptible individuals. Such a scenario is supported by the dominant infiltration of early lesions by CD8+ T cells and the expression of CD1, a molecule which is involved in lipid antigen presentation (Ito et al. 2001). Furthermore, in contrast to adrenomyeloneuropathy, there is no variant of MS where neurodegeneration is seen in the absence of inflammation.

5.11 Experimental Animal Model to Study the Pathogenesis of MS

Over the past several decades, a number of animal models have been developed in order to understand a variety of aspects of human MS. The main driving force for animal studies stems from the following limitations of human studies: overall limited access to human MS tissue, biopsies are rarely performed and autopsy samples are usually biased towards a chronic disease state; experimental circumstances cannot easily be modified in clinical trials and mechanistic studies addressing disease pathomechanism(s) cannot readily be performed in patients. Similarly, Genetic complexity, together with variability in the pathology, symptoms, and clinical course of MS, suggest the possibility of multiple disease-initiating pathways. Disease heterogeneity may account for the difficulty in

identifying triggers of MS. One key to better understand the heterogeneity of pathogenesis of MS depends on the appropriate choice of experimental animal models. The most commonly studied animal models of MS are the purely experimental autoimmune/allergic encephalomyelitis models.

5.11.1 Experimental Autoimmune Encephalomyelitis (EAE)

Experimental autoimmune encephalomyelitis (EAE) is a model in which animals are immunized with a myelin derived antigen and adjuvant. By varying the genetic background and immunization protocol, EAE can reproduce the symptoms of the major forms of human MS (Goverman and Brabb 1996; Mix et al. 2008; Miller et al. 1997), providing a reasonable strategy for reproducing distinct features of CNS pathology mediated by similar immunogenetic mechanisms. EAE is primarily used as an animal model of autoimmune inflammatory diseases of the CNS, and it resembles MS, the prototypical such disease, in many respects (Wang et al. 2006; Steinman and Zamvil 2005, 2006; Farooqi et al. 2010). EAE was first described as inflammatory model of MS in 1933 (Rivers et al. 1933; Rivers and Schwentker 1935) and is still a popular and widely used model. EAE is a complex condition in which the interaction between a variety of immunopathological and neuropathological mechanisms leads to an approximation of the key pathological features of MS: inflammation, demyelination, axonal loss, and gliosis. EAE is very heterogeneous in terms of induction methods, clinical and pathological features, and amenability to treatment, all of which add to its complexity. EAE is induced by the MBP–PLP fusion protein MP4, MOG peptide 35–55, or PLP peptide 178–191 in mice, which, respectively, display distinct features of CNS pathology (Kuerten et al. 2011). Major differences between the three models reside in the region- / tract-specificity and disseminated nature of spinal cord degeneration, the extent and kinetics of demyelination, and the involvement of motor neurons in the disease. The counter-regulatory mechanisms of resolution of inflammation and remyelination also occur in EAE, which, therefore can also serve as a model for these processes. Some models are more similar to other, less common inflammatory CNS disorders, such as the monophasic acute disseminated encephalomyelitis (ADEM) or neuromyelitis optica (NMO, Devic's disease). Increasingly, the use of EAE has expanded considerably beyond the laboratory study of MS and the development of MS therapeutics. EAE has also become a very well characterized model for organ-specific autoimmune disease in general. Indeed, several recent first reports of key novel functions of immunologically important molecules, or of a novel knockout mouse were published with EAE data as the in vivo validation model. Examples include the discovery of ROR-γ (RORC) as a master transcription factor for Th17 cell development (Ivanov and Linden 2007), the identification of the aryl hydrocarbon receptor (AHR) as an essential component in the development of both Treg and Th17 responses (Veldhoen et al. 2008) and the differential role of the related molecules IL-12 and IL-23 in the susceptibility to autoimmune demyelination (Becher et al. 2002; Gran et al. 2002; Cua et al. 2003). EAE has a complex

neuropharmacology, and many of the drugs that are in current or imminent use in MS have been developed, tested, or validated on the basis of EAE studies. From the pathogenesis point of view, also EAE is a good model for studying MS mechanisms, even more so than for testing or developing drugs (Farooqi et al. 2010). On a more positive note, however, diversity within the field of EAE has its advantages. Each model may accurately mimic one particular facet of MS. In terms of providing clues to the MS pathogenesis and allowing development of treatments, a most exciting and rewarding approach was that of the bidirectional translational studies (Lock et al. 2002; Robinson et al. 2003; Steinman and Zamvil 2003; Han et al. 2008). This involved gene expression profiling in MS brain, identification of a number of plausible novel targets and then testing and validating these targets in EAE. Several such targets have been identified in this fashion, some supported by small previous studies in EAE, and these targets have a potential for being translated into MS treatment soon. Such targets include osteopontin, platelet activating factor receptor, histamine receptors, and alpha-B crystallin (Lock et al. 2002; Han et al. 2008; Steinman 2009). This makes EAE a very versatile system to use in translational neuro- and immunopharmacology. But the model needs to be tailored to the scientific question being asked as like all animal models, EAE has limitations. A major difference between MS and EAE is that the latter requires an external immunization step to develop, whereas in humans, the sensitization to autoantigens is obviously not artificially induced (Gran et al. 2002). Sensitization to myelin antigens in EAE typically occurs through the use of adjuvant, usually containing bacterial components highly capable of activating the innate immune system via pattern recognition receptors (Libbey et al. 2010). In EAE, the inducing antigens are known, whereas in MS, there is no unique identified antigen. Thus, important differences between these conditions may be due to how autoreactive T cells are primed and activated. Taken together, we must conclude that despite numerous drawbacks, EAE has been an extremely valuable model in investigating pathogenesis and developing new medications to help those suffering from MS.

Similarly, a few animal models exist in which viral infection triggers CNS autoimmunity. Infection with Theiler's Murine Encephalomyelitis Virus induces CNS autoimmune disease in susceptible mouse strains via bystander activation of myelin antigen–specific CD4+ T cells (Vanderlugt and Vanderlugt and Miller 2002). Bystander activation is facilitated by myelin damage that occurs during the initial clearance of virus by CD8+ T cells, which results in presentation of myelin epitopes by antigen-presenting cells to CD4+ myelin antigen-specific T cells that were nonspecifically recruited to the CNS. This phenomenon of epitope spreading from viral antigen-specific CD8+ T cells to self-reactive, myelin-specific CD4+ T cells results in a chronic disease that resembles MS (Miller et al. 1997). Correspondingly, infection with the highly neurovirulent murine hepatitis virus strain, MHV-JHM induces a chronic demyelinating disease that depends only on the activity of virus-specific T cells rather than on the emergence of myelin-specific T cells during the course of infection (Hosking and Lane 2009; Wu et al. 2000). In contrast, MHV-A59, another neurotropic strain of MHV, induced demyelination in adult immune compromised mice lacking B and T cells, and depletion of CD4+ or

CD8+ T cells after the acute stage of infection does not reduce demyelination (Matthews et al. 2002). Indeed, one of our recent studies shows MHV-A59 or the demyelination induced by its isogenic spike protein (host-attachment protein) recombinant strain, RSA59, exhibit inflammatory CNS demyelination consisting of a mixed population of inflammatory cells, predominantly macrophages/microglia and a smaller population of T lymphocytes, but not conventional CD4+ or CD8+ T cells (Shindler et al. 2008; Das Sarma et al. 2009). Thus, MHV-A59 or its recombinant strain RSA59 (Das Sarma et al. 2002, 2008, 2009) may induce demyelination via unique mechanisms, and it is likely that in the absence of an adaptive immune response, MHV-A59 or RSA59 infection in the CNS is responsible for the onset of demyelination, possibly secondary to axonal and/or neuronal injury.

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References

Aboul-Enein F, Rauschka H, Kornek B, Stadelmann C, Stefferl A, Bruck W, Lucchinetti C, Schmidbauer M, Jellinger K, Lassmann H (2003) Preferential loss of myelin-associated glycoprotein reflects hypoxia-like white matter damage in stroke and inflammatory brain diseases. J Neuropathol Exp Neurol 62(1):25–33

Akassoglou K, Adams RA, Bauer J, Mercado P, Tseveleki V, Lassmann H, Probert L, Strickland S (2004) Fibrin depletion decreases inflammation and delays the onset of demyelination in a tumor necrosis factor transgenic mouse model for multiple sclerosis. Proc Natl Acad Sci USA 101(17):6698–6703. doi:10.1073/pnas.0303859101

Aktas O, Smorodchenko A, Brocke S, Infante-Duarte C, Schulze Topphoff U, Vogt J, Prozorovski T, Meier S, Osmanova V, Pohl E, Bechmann I, Nitsch R, Zipp F (2005) Neuronal damage in autoimmune neuroinflammation mediated by the death ligand TRAIL. Neuron 46(3):421–432. doi:10.1016/j.neuron.2005.03.018

Anthony DC, Miller KM, Fearn S, Townsend MJ, Opdenakker G, Wells GM, Clements JM, Chandler S, Gearing AJ, Perry VH (1998) Matrix metalloproteinase expression in an experimentally-induced DTH model of multiple sclerosis in the rat CNS. J Neuroimmunol 87 (1–2):62–72

Babbe H, Roers A, Waisman A, Lassmann H, Goebels N, Hohlfeld R, Friese M, Schroder R, Deckert M, Schmidt S, Ravid R, Rajewsky K (2000) Clonal expansions of CD8(+) T cells dominate the T cell infiltrate in active multiple sclerosis lesions as shown by micromanipulation and single cell polymerase chain reaction. J Exp Med 192(3):393–404

Bailey SL, Schreiner B, McMahon EJ, Miller SD (2007) CNS myeloid DCs presenting endogenous myelin peptides 'preferentially' polarize CD4+ T(H)-17 cells in relapsing EAE. Nat Immunol 8(2):172–180. doi:10.1038/ni1430

Barnett MH, Prineas JW (2004) Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. Annals of neurology 55(4):458–468. doi:10.1002/ana.20016

Becher B, Durell BG, Noelle RJ (2002) Experimental autoimmune encephalitis and inflammation in the absence of interleukin-12. J Clin Investig 110(4):493–497. doi:10.1172/JCI15751

Bonetti B, Raine CS (1997) Multiple sclerosis: oligodendrocytes display cell death-related molecules in situ but do not undergo apoptosis. Ann Neurol 42(1):74–84. doi:10.1002/ana.410420113

Boven LA, Van Meurs M, Van Zwam M, Wierenga-Wolf A, Hintzen RQ, Boot RG, Aerts JM, Amor S, Nieuwenhuis EE, Laman JD (2006) Myelin-laden macrophages are anti-inflammatory, consistent with foam cells in multiple sclerosis. Brain J Neurol 129(Pt 2): 517–526. doi:10.1093/brain/awh707

Bruck W, Porada P, Poser S, Rieckmann P, Hanefeld F, Kretzschmar HA, Lassmann H (1995) Monocyte/macrophage differentiation in early multiple sclerosis lesions. Ann Neurol 38 (5):788–796. doi:10.1002/ana.410380514

Bsibsi M, Ravid R, Gveric D, van Noort JM (2002) Broad expression of Toll-like receptors in the human central nervous system. J Neuropathol Exp Neurol 61(11):1013–1021

Burt RK, Cohen BA, Russell E, Spero K, Joshi A, Oyama Y, Karpus WJ, Luo K, Jovanovic B, Traynor A, Karlin K, Stefoski D, Burns WH (2003) Hematopoietic stem cell transplantation for progressive multiple sclerosis: failure of a total body irradiation-based conditioning regimen to prevent disease progression in patients with high disability scores. Blood 102(7):2373–2378. doi:10.1182/blood-2003-03-0877

Cannella B, Raine CS (1995) The adhesion molecule and cytokine profile of multiple sclerosis lesions. Ann Neurol 37(4):424–435. doi:10.1002/ana.410370404

Choi SR, Howell OW, Carassiti D, Magliozzi R, Gveric D, Muraro PA, Nicholas R, Roncaroli F, Reynolds R (2012) Meningeal inflammation plays a role in the pathology of primary progressive multiple sclerosis. Brain J Neurol 135(Pt 10):2925–2937. doi:10.1093/brain/aws189

Coles AJ, Cox A, Le Page E, Jones J, Trip SA, Deans J, Seaman S, Miller DH, Hale G, Waldmann H, Compston DA (2006) The window of therapeutic opportunity in multiple sclerosis: evidence from monoclonal antibody therapy. J Neurol 253(1):98–108. doi:10.1007/s00415-005-0934-5

Corcione A, Aloisi F, Serafini B, Capello E, Mancardi GL, Pistoia V, Uccelli A (2005) B-cell differentiation in the CNS of patients with multiple sclerosis. Autoimmun Rev 4(8):549–554. doi:10.1016/j.autrev.2005.04.012

Courville J, Cooper CW (1970) The cerebellar nuclei of Macaca mulatta: a morphological study. J Comp Neurol 140(2):241–254. doi:10.1002/cne.901400207

Craner MJ, Newcombe J, Black JA, Hartle C, Cuzner ML, Waxman SG (2004) Molecular changes in neurons in multiple sclerosis: altered axonal expression of Nav1.2 and Nav1.6 sodium channels and Na+/Ca2+ exchanger. Proc Natl Acad Sci USA 101(21):8168–8173. doi:10.1073/pnas.0402765101

Cua DJ, Sherlock J, Chen Y, Murphy CA, Joyce B, Seymour B, Lucian L, To W, Kwan S, Churakova T, Zurawski S, Wiekowski M, Lira SA, Gorman D, Kastelein RA, Sedgwick JD (2003) Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. Nature 421(6924):744–748. doi:10.1038/nature01355

Cuzner ML, Opdenakker G (1999) Plasminogen activators and matrix metalloproteases, mediators of extracellular proteolysis in inflammatory demyelination of the central nervous system. J Neuroimmunol 94(1–2):1–14

D'Souza SD, Bonetti B, Balasingam V, Cashman NR, Barker PA, Troutt AB, Raine CS, Antel JP (1996) Multiple sclerosis: Fas signaling in oligodendrocyte cell death. J Exp Med 184 (6):2361–2370

Das Sarma J, Scheen E, Seo SH, Koval M, Weiss SR (2002) Enhanced green fluorescent protein expression may be used to monitor murine coronavirus spread in vitro and in the mouse central nervous system. J Neurovirology 8(5):381–391. doi:10.1080/13550280290100815

Das Sarma J, Iacono K, Gard L, Marek R, Kenyon LC, Koval M, Weiss SR (2008) Demyelinating and nondemyelinating strains of mouse hepatitis virus differ in their neural cell tropism. J Virol 82(11):5519–5526. doi:10.1128/JVI.01488-07

Das Sarma J, Kenyon LC, Hingley ST, Shindler KS (2009) Mechanisms of primary axonal damage in a viral model of multiple sclerosis. J Neurosci Off J Soc Neurosci 29(33):10272–10280. doi:10.1523/JNEUROSCI.1975-09.2009

de Seze J, Debouverie M, Zephir H, Lebrun C, Blanc F, Bourg V, Wiertlewski S, Pittion S, Laplaud D, Le Page E, Deschamps R, Cabre P, Pelletier J, Malikova I, Clavelou P, Jaillon V, Defer G, Labauge P, Gout O, Boulay C, Edan G, Vermersch P (2007) Acute fulminant demyelinating disease: a descriptive study of 60 patients. Arch Neurol 64(10):1426–1432. doi:10.1001/archneur.64.10.1426

Dowling P, Shang G, Raval S, Menonna J, Cook S, Husar W (1996) Involvement of the CD95 (APO-1/Fas) receptor/ligand system in multiple sclerosis brain. J Exp Med 184(4):1513–1518

East E, Baker D, Pryce G, Lijnen HR, Cuzner ML, Gveric D (2005) A role for the plasminogen activator system in inflammation and neurodegeneration in the central nervous system during experimental allergic encephalomyelitis. Am J Pathol 167(2):545–554. doi:10.1016/S0002-9440(10)62996-3

Engelhardt B, Wolburg H (2004) Mini-review: transendothelial migration of leukocytes: through the front door or around the side of the house? Eur J Immunol 34(11):2955–2963. doi:10.1002/eji.200425327

Engelhardt B, Ransohoff RM (2005) The ins and outs of T-lymphocyte trafficking to the CNS: anatomical sites and molecular mechanisms. Trends Immunol 26(9):485–495. doi:10.1016/j.it.2005.07.004

Esiri MM (1980) Multiple sclerosis: a quantitative and qualitative study of immunoglobulin-containing cells in the central nervous system. Neuropathol Appl Neurobiol 6(1):9–21

Evangelou N, Konz D, Esiri MM, Smith S, Palace J, Matthews PM (2001) Size-selective neuronal changes in the anterior optic pathways suggest a differential susceptibility to injury in multiple sclerosis. Brain J Neurol 124(Pt 9):1813–1820

Evans CF, Horwitz MS, Hobbs MV, Oldstone MB (1996) Viral infection of transgenic mice expressing a viral protein in oligodendrocytes leads to chronic central nervous system autoimmune disease. J Exp Med 184(6):2371–2384

Fabriek BO, Van Haastert ES, Galea I, Polfliet MM, Dopp ED, Van Den Heuvel MM, Van Den Berg TK, De Groot CJ, Van Der Valk P, Dijkstra CD (2005) CD163-positive perivascular macrophages in the human CNS express molecules for antigen recognition and presentation. Glia 51(4):297–305. doi:10.1002/glia.20208

Farooqi N, Gran B, Constantinescu CS (2010) Are current disease-modifying therapeutics in multiple sclerosis justified on the basis of studies in experimental autoimmune encephalomyelitis? J Neurochem 115(4):829–844. doi:10.1111/j.1471-4159.2010.06982.x

Ferguson B, Matyszak MK, Esiri MM, Perry VH (1997) Axonal damage in acute multiple sclerosis lesions. Brain J Neurol 120(Pt 3):393–399

Fischer MT, Sharma R, Lim JL, Haider L, Frischer JM, Drexhage J, Mahad D, Bradl M, van Horssen J, Lassmann H (2012) NADPH oxidase expression in active multiple sclerosis lesions in relation to oxidative tissue damage and mitochondrial injury. Brain J Neurol 135(Pt 3):886–899. doi:10.1093/brain/aws012

Flick MJ, Du X, Witte DP, Jirouskova M, Soloviev DA, Busuttil SJ, Plow EF, Degen JL (2004) Leukocyte engagement of fibrin(ogen) via the integrin receptor alphaMbeta2/Mac-1 is critical for host inflammatory response in vivo. J Clin Investig 113(11):1596–1606. doi:10.1172/JCI20741

Franciotta D, Salvetti M, Lolli F, Serafini B, Aloisi F (2008) B cells and multiple sclerosis. Lancet Neurol 7(9):852–858. doi:10.1016/S1474-4422(08)70192-3

Friese MA, Fugger L (2005) Autoreactive CD8+ T cells in multiple sclerosis: a new target for therapy? Brain J Neurol 128(Pt 8):1747–1763. doi:10.1093/brain/awh578

Frischer JM, Bramow S, Dal-Bianco A, Lucchinetti CF, Rauschka H, Schmidbauer M, Laursen H, Sorensen PS, Lassmann H (2009) The relation between inflammation and neurodegeneration in multiple sclerosis brains. Brain J Neurol 132(Pt 5):1175–1189. doi:10.1093/brain/awp070

Frohman EM, Racke MK, Raine CS (2006) Multiple sclerosis—the plaque and its pathogenesis. N Engl J Med 354(9):942–955. doi:10.1056/NEJMra052130

Fu L, Matthews PM, De Stefano N, Worsley KJ, Narayanan S, Francis GS, Antel JP, Wolfson C, Arnold DL (1998) Imaging axonal damage of normal-appearing white matter in multiple sclerosis. Brain J Neurol 121(Pt 1):103–113

Gerritse K, Laman JD, Noelle RJ, Aruffo A, Ledbetter JA, Boersma WJ, Claassen E (1996) CD40-CD40 ligand interactions in experimental allergic encephalomyelitis and multiple sclerosis. Proc Natl Acad Sci USA 93(6):2499–2504

Goverman J (2009) Autoimmune T cell responses in the central nervous system. Nat Rev Immunol 9(6):393–407. doi:10.1038/nri2550

Goverman J, Brabb T (1996) Rodent models of experimental allergic encephalomyelitis applied to the study of multiple sclerosis. Lab Anim Sci 46(5):482–492

Gran B, Zhang GX, Yu S, Li J, Chen XH, Ventura ES, Kamoun M, Rostami A (2002) IL-12p35-deficient mice are susceptible to experimental autoimmune encephalomyelitis: evidence for redundancy in the IL-12 system in the induction of central nervous system autoimmune demyelination. J Immunol 169(12):7104–7110

Greenwood J, Heasman SJ, Alvarez JI, Prat A, Lyck R, Engelhardt B (2011) Review: leucocyte-endothelial cell crosstalk at the blood-brain barrier: a prerequisite for successful immune cell entry to the brain. Neuropathol Appl Neurobiol 37(1):24–39. doi:10.1111/j.1365-2990.2010.01140.x

Griffin DE (1990) Monophasic autoimmune inflammatory diseases of the CNS and PNS. Res Publ Assoc Res Nerv Ment Dis 68:91–104

Gveric D, Hanemaaijer R, Newcombe J, van Lent NA, Sier CF, Cuzner ML (2001) Plasminogen activators in multiple sclerosis lesions: implications for the inflammatory response and axonal damage. Brain J Neurol 124(Pt 10):1978–1988

Gveric D, Herrera B, Petzold A, Lawrence DA, Cuzner ML (2003) Impaired fibrinolysis in multiple sclerosis: a role for tissue plasminogen activator inhibitors. Brain J Neurol 126 (Pt 7):1590–1598. doi:10.1093/brain/awg167

Gveric D, Herrera BM, Cuzner ML (2005) tPA receptors and the fibrinolytic response in multiple sclerosis lesions. Am J Pathol 166(4):1143–1151. doi:10.1016/S0002-9440(10)62334-6

Han MH, Hwang SI, Roy DB, Lundgren DH, Price JV, Ousman SS, Fernald GH, Gerlitz B, Robinson WH, Baranzini SE, Grinnell BW, Raine CS, Sobel RA, Han DK, Steinman L (2008) Proteomic analysis of active multiple sclerosis lesions reveals therapeutic targets. Nature 451 (7182):1076–1081. doi:10.1038/nature06559

Hauser SL, Waubant E, Arnold DL, Vollmer T, Antel J, Fox RJ, Bar-Or A, Panzara M, Sarkar N, Agarwal S, Langer-Gould A, Smith CH, Group HT (2008) B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. N Engl J Med 358(7):676–688. doi:10.1056/NEJMoa0706383

Hickey WF, Hsu BL, Kimura H (1991) T-lymphocyte entry into the central nervous system. J Neurosci Res 28(2):254–260. doi:10.1002/jnr.490280213

Hochmeister S, Grundtner R, Bauer J, Engelhardt B, Lyck R, Gordon G, Korosec T, Kutzelnigg A, Berger JJ, Bradl M, Bittner RE, Lassmann H (2006) Dysferlin is a new marker for leaky brain blood vessels in multiple sclerosis. J Neuropathol Exp Neurol 65(9):855–865. doi:10.1097/01.jnen.0000235119.52311.16

Hoftberger R, Aboul-Enein F, Brueck W, Lucchinetti C, Rodriguez M, Schmidbauer M, Jellinger K, Lassmann H (2004) Expression of major histocompatibility complex class I molecules on the different cell types in multiple sclerosis lesions. Brain Pathol 14(1):43–50

Hosking MP, Lane TE (2009) The biology of persistent infection: inflammation and demyelination following murine coronavirus infection of the central nervous system. Curr Immunol Rev 5 (4):267–276. doi:10.2174/157339509789504005

Howell OW, Reeves CA, Nicholas R, Carassiti D, Radotra B, Gentleman SM, Serafini B, Aloisi F, Roncaroli F, Magliozzi R, Reynolds R (2011) Meningeal inflammation is widespread and linked to cortical pathology in multiple sclerosis. Brain J Neurol 134(Pt 9):2755–2771. doi:10.1093/brain/awr182

Huang D, Han Y, Rani MR, Glabinski A, Trebst C, Sorensen T, Tani M, Wang J, Chien P, O'Bryan S, Bielecki B, Zhou ZL, Majumder S, Ransohoff RM (2000) Chemokines and chemokine receptors in inflammation of the nervous system: manifold roles and exquisite regulation. Immunol Rev 177:52–67

Huseby ES, Liggitt D, Brabb T, Schnabel B, Ohlen C, Goverman J (2001) A pathogenic role for myelin-specific CD8(+) T cells in a model for multiple sclerosis. J Exp Med 194(5):669–676

Ito R, Melhem ER, Mori S, Eichler FS, Raymond GV, Moser HW (2001) Diffusion tensor brain MR imaging in X-linked cerebral adrenoleukodystrophy. Neurology 56(4):544–547

Ivanov S, Linden A (2007) Th-17 cells in the lungs? Expert Rev Respir Med 1(2):279–293. doi:10.1586/17476348.1.2.279

Kerlero de Rosbo N, Hoffman M, Mendel I, Yust I, Kaye J, Bakimer R, Flechter S, Abramsky O, Milo R, Karni A, Ben-Nun A (1997) Predominance of the autoimmune response to myelin oligodendrocyte glycoprotein (MOG) in multiple sclerosis: reactivity to the extracellular domain of MOG is directed against three main regions. Eur J Immunol 27(11):3059–3069. doi:10.1002/eji.1830271144

Kerschensteiner M, Gallmeier E, Behrens L, Leal VV, Misgeld T, Klinkert WE, Kolbeck R, Hoppe E, Oropeza-Wekerle RL, Bartke I, Stadelmann C, Lassmann H, Wekerle H, Hohlfeld R (1999) Activated human T cells, B cells, and monocytes produce brain-derived neurotrophic factor in vitro and in inflammatory brain lesions: a neuroprotective role of inflammation? J Exp Med 189(5):865–870

Khoury MN, Alsop DC, Agnihotri SP, Pfannl R, Wuthrich C, Ho ML, Hackney D, Ngo L, Anderson MP, Koralnik IJ (2014) Hyperintense cortical signal on magnetic resonance imaging reflects focal leukocortical encephalitis and seizure risk in progressive multifocal leukoencephalopathy. Ann Neurol 75(5):659–669. doi:10.1002/ana.24144

Kitley J, Woodhall M, Waters P, Leite MI, Devenney E, Craig J, Palace J, Vincent A (2012) Myelin-oligodendrocyte glycoprotein antibodies in adults with a neuromyelitis optica phenotype. Neurology 79(12):1273–1277. doi:10.1212/WNL.0b013e31826aac4e

Korn T, Bettelli E, Oukka M, Kuchroo VK (2009) IL-17 and Th17 Cells. Annu Rev Immunol 27:485–517. doi:10.1146/annurev.immunol.021908.132710

Kornek B, Lassmann H (1999) Axonal pathology in multiple sclerosis. A historical note. Brain Pathol 9(4):651–656

Kornek B, Storch MK, Weissert R, Wallstroem E, Stefferl A, Olsson T, Linington C, Schmidbauer M, Lassmann H (2000) Multiple sclerosis and chronic autoimmune encephalomyelitis: a comparative quantitative study of axonal injury in active, inactive, and remyelinated lesions. Am J Pathol 157(1):267–276. doi:10.1016/S0002-9440(10)64537-3

Kornek B, Storch MK, Bauer J, Djamshidian A, Weissert R, Wallstroem E, Stefferl A, Zimprich F, Olsson T, Linington C, Schmidbauer M, Lassmann H (2001) Distribution of a calcium channel subunit in dystrophic axons in multiple sclerosis and experimental autoimmune encephalomyelitis. Brain J Neurol 124(Pt 6):1114–1124

Krogsgaard M, Wucherpfennig KW, Cannella B, Hansen BE, Svejgaard A, Pyrdol J, Ditzel H, Raine C, Engberg J, Fugger L (2000) Visualization of myelin basic protein (MBP) T cell epitopes in multiple sclerosis lesions using a monoclonal antibody specific for the human histocompatibility leukocyte antigen (HLA)-DR2-MBP 85-99 complex. J Exp Med 191(8):1395–1412

Kuerten S, Gruppe TL, Laurentius LM, Kirch C, Tary-Lehmann M, Lehmann PV, Addicks K (2011) Differential patterns of spinal cord pathology induced by MP4, MOG peptide 35-55, and PLP peptide 178-191 in C57BL/6 mice. APMIS 119(6):336–346. doi:10.1111/j.1600-0463.2011.02744.x

Kutzelnigg A, Lucchinetti CF, Stadelmann C, Bruck W, Rauschka H, Bergmann M, Schmidbauer M, Parisi JE, Lassmann H (2005) Cortical demyelination and diffuse white matter injury in multiple sclerosis. Brain J Neurol 128(Pt 11):2705–2712. doi:10.1093/brain/awh641

Lassmann H (2011) Review: the architecture of inflammatory demyelinating lesions: implications for studies on pathogenesis. Neuropathol Appl Neurobiol 37(7):698–710. doi:10.1111/j.1365-2990.2011.01189.x

Lassmann H (2012) Cortical lesions in multiple sclerosis: inflammation versus neurodegeneration. Brain J Neurol 135(Pt 10):2904–2905. doi:10.1093/brain/aws260

Lassmann H, Raine CS, Antel J, Prineas JW (1998) Immunopathology of multiple sclerosis: report on an international meeting held at the Institute of Neurology of the University of Vienna. J Neuroimmunol 86(2):213–217

Lassmann H, Bruck W, Lucchinetti C (2001) Heterogeneity of multiple sclerosis pathogenesis: implications for diagnosis and therapy. Trends Mol Med 7(3):115–121

Lassmann H, Bruck W, Lucchinetti CF (2007) The immunopathology of multiple sclerosis: an overview. Brain Pathol 17(2):210–218. doi:10.1111/j.1750-3639.2007.00064.x

Lennon VA, Wingerchuk DM, Kryzer TJ, Pittock SJ, Lucchinetti CF, Fujihara K, Nakashima I, Weinshenker BG (2004) A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. Lancet 364(9451):2106–2112. doi:10.1016/S0140-6736(04)17551-X

Lennon VA, Kryzer TJ, Pittock SJ, Verkman AS, Hinson SR (2005) IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. J Exp Med 202(4):473–477. doi:10.1084/jem.20050304

Li H, Cuzner ML, Newcombe J (1996) Microglia-derived macrophages in early multiple sclerosis plaques. Neuropathol Appl Neurobiol 22(3):207–215

Libbey JE, Tsunoda I, Fujinami RS (2010) Studies in the modulation of experimental autoimmune encephalomyelitis. J Neuroimmune Pharmacol Off J Soc Neuroimmune Pharmacol 5(2):168–175. doi:10.1007/s11481-010-9215-x

Lindberg RL, De Groot CJ, Montagne L, Freitag P, van der Valk P, Kappos L, Leppert D (2001) The expression profile of matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) in lesions and normal appearing white matter of multiple sclerosis. Brain J Neurol 124 (Pt 9):1743–1753

Lipton SA (1998) Neuronal injury associated with HIV-1: approaches to treatment. Annu Rev Pharmacol Toxicol 38:159–177. doi:10.1146/annurev.pharmtox.38.1.159

Littman DR, Rudensky AY (2010) Th17 and regulatory T cells in mediating and restraining inflammation. Cell 140(6):845–858. doi:10.1016/j.cell.2010.02.021

Liu JS, Zhao ML, Brosnan CF, Lee SC (2001) Expression of inducible nitric oxide synthase and nitrotyrosine in multiple sclerosis lesions. Am J Pathol 158(6):2057–2066. doi:10.1016/S0002-9440(10)64677-9

Lock C, Hermans G, Pedotti R, Brendolan A, Schadt E, Garren H, Langer-Gould A, Strober S, Cannella B, Allard J, Klonowski P, Austin A, Lad N, Kaminski N, Galli SJ, Oksenberg JR, Raine CS, Heller R, Steinman L (2002) Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. Nat Med 8(5):500–508. doi:10.1038/nm0502-500

Lucchinetti C, Bruck W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H (2000) Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. Ann Neurol 47(6):707–717

Lucchinetti CF, Mandler RN, McGavern D, Bruck W, Gleich G, Ransohoff RM, Trebst C, Weinshenker B, Wingerchuk D, Parisi JE, Lassmann H (2002) A role for humoral mechanisms in the pathogenesis of Devic's neuromyelitis optica. Brain J Neurol 125(Pt 7):1450–1461

Luster AD (1998) Chemokines–chemotactic cytokines that mediate inflammation. N Engl J Med 338(7):436–445. doi:10.1056/NEJM199802123380706

Marik C, Felts PA, Bauer J, Lassmann H, Smith KJ (2007) Lesion genesis in a subset of patients with multiple sclerosis: a role for innate immunity? Brain J Neurol 130(Pt 11):2800–2815. doi:10.1093/brain/awm236

Marta M, Meier UC, Lobell A (2009) Regulation of autoimmune encephalomyelitis by toll-like receptors. Autoimmun Rev 8(6):506–509. doi:10.1016/j.autrev.2009.01.006

Matthews AE, Lavi E, Weiss SR, Paterson Y (2002) Neither B cells nor T cells are required for CNS demyelination in mice persistently infected with MHV-A59. J Neurovirology 8(3):257–264. doi:10.1080/13550280290049697

Medana I, Martinic MA, Wekerle H, Neumann H (2001) Transection of major histocompatibility complex class I-induced neurites by cytotoxic T lymphocytes. Am J Pathol 159(3):809–815. doi:10.1016/S0002-9440(10)61755-5

Miller SD, Vanderlugt CL, Begolka WS, Pao W, Yauch RL, Neville KL, Katz-Levy Y, Carrizosa A, Kim BS (1997) Persistent infection with Theiler's virus leads to CNS autoimmunity via epitope spreading. Nat Med 3(10):1133–1136

Mix E, Meyer-Rienecker H, Zettl UK (2008) Animal models of multiple sclerosis for the development and validation of novel therapies—potential and limitations. J Neurol 255(Suppl 6):7–14. doi:10.1007/s00415-008-6003-0

Moalem G, Gdalyahu A, Shani Y, Otten U, Lazarovici P, Cohen IR, Schwartz M (2000) Production of neurotrophins by activated T cells: implications for neuroprotective autoimmunity. J Autoimmun 15(3):331–345. doi:10.1006/jaut.2000.0441

Moser HW (1997) Adrenoleukodystrophy: phenotype, genetics, pathogenesis and therapy. Brain J Neurol 120(Pt 8):1485–1508

Mussini JM, Hauw JJ, Escourolle R (1977) Immunofluorescence studies of intra cytoplasmic immunoglobulin binding lymphoid cells (CILC) in the central nervous system. Report of 32 cases including 19 multiple sclerosis. Acta Neuropathol 40(3):227–232

Neumann H, Medana IM, Bauer J, Lassmann H (2002) Cytotoxic T lymphocytes in autoimmune and degenerative CNS diseases. Trends Neurosci 25(6):313–319

Nitsch R, Pohl EE, Smorodchenko A, Infante-Duarte C, Aktas O, Zipp F (2004) Direct impact of T cells on neurons revealed by two-photon microscopy in living brain tissue. J Neurosci off J Soc Neurosci 24(10):2458–2464. doi:10.1523/JNEUROSCI.4703-03.2004

Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG (2000) Multiple sclerosis. N Engl J Med 343(13):938–952. doi:10.1056/NEJM200009283431307

O'Connor KC, Bar-Or A, Hafler DA (2001) The neuroimmunology of multiple sclerosis: possible roles of T and B lymphocytes in immunopathogenesis. J Clin Immunol 21(2):81–92

O'Connor KC, Appel H, Bregoli L, Call ME, Catz I, Chan JA, Moore NH, Warren KG, Wong SJ, Hafler DA, Wucherpfennig KW (2005) Antibodies from inflamed central nervous system tissue recognize myelin oligodendrocyte glycoprotein. J Immunol 175(3):1974–1982

O'Connor KC, McLaughlin KA, De Jager PL, Chitnis T, Bettelli E, Xu C, Robinson WH, Cherry SV, Bar-Or A, Banwell B, Fukaura H, Fukazawa T, Tenembaum S, Wong SJ, Tavakoli NP, Idrissova Z, Viglietta V, Rostasy K, Pohl D, Dale RC, Freedman M, Steinman L, Buckle GJ, Kuchroo VK, Hafler DA, Wucherpfennig KW (2007) Self-antigen tetramers discriminate between myelin autoantibodies to native or denatured protein. Nat Med 13(2):211–217. doi:10.1038/nm1488

Ozawa K, Suchanek G, Breitschopf H, Bruck W, Budka H, Jellinger K, Lassmann H (1994) Patterns of oligodendroglia pathology in multiple sclerosis. Brain J Neurol 117(Pt 6):1311–1322

Pelletier D, Nelson SJ, Oh J, Antel JP, Kita M, Zamvil SS, Goodkin DE (2003) MRI lesion volume heterogeneity in primary progressive MS in relation with axonal damage and brain atrophy. J Neurol Neurosurg Psychiatry 74(7):950–952

Perry VH, Nicoll JA, Holmes C (2010) Microglia in neurodegenerative disease. Nat Rev Neurol 6(4):193–201. doi:10.1038/nrneurol.2010.17

Pette M, Fujita K, Wilkinson D, Altmann DM, Trowsdale J, Giegerich G, Hinkkanen A, Epplen JT, Kappos L, Wekerle H (1990) Myelin autoreactivity in multiple sclerosis: recognition of myelin basic protein in the context of HLA-DR2 products by T lymphocytes of multiple-sclerosis patients and healthy donors. Proc Natl Acad Sci USA 87(20):7968–7972

Prineas JW, Graham JS (1981) Multiple sclerosis: capping of surface immunoglobulin G on macrophages engaged in myelin breakdown. Ann Neurol 10(2):149–158. doi:10.1002/ana.410100205

Probert L, Eugster HP, Akassoglou K, Bauer J, Frei K, Lassmann H, Fontana A (2000) TNFR1 signalling is critical for the development of demyelination and the limitation of T-cell responses during immune-mediated CNS disease. Brain J Neurol 123(Pt 10):2005–2019

Revesz T, Kidd D, Thompson AJ, Barnard RO, McDonald WI (1994) A comparison of the pathology of primary and secondary progressive multiple sclerosis. Brain J Neurol 117(Pt 4):759–765

Rivers TM, Schwentker FF (1935) Encephalomyelitis accompanied by myelin destruction experimentally produced in monkeys. J Exp Med 61(5):689–702

Rivers TM, Sprunt DH, Berry GP (1933) Observations on attempts to produce acute disseminated encephalomyelitis in monkeys. J Exp Med 58(1):39–53

Robinson WH, Utz PJ, Steinman L (2003) Genomic and proteomic analysis of multiple sclerosis opinion. Cur Opin Immunol 15(6):660–667

Rocca MA, Iannucci G, Rovaris M, Comi G, Filippi M (2003) Occult tissue damage in patients with primary progressive multiple sclerosis is independent of T2-visible lesions—a diffusion tensor MR study. J Neurol 250(4):456–460. doi:10.1007/s00415-003-1024-1

Rostasy K, Mader S, Hennes EM, Schanda K, Gredler V, Guenther A, Blaschek A, Korenke C, Pritsch M, Pohl D, Maier O, Kuchukhidze G, Brunner-Krainz M, Berger T, Reindl M (2013) Persisting myelin oligodendrocyte glycoprotein antibodies in aquaporin-4 antibody negative pediatric neuromyelitis optica. Multiple Scler 19(8):1052–1059. doi:10.1177/1352458512470310

Sadaba MC, Tzartos J, Paino C, Garcia-Villanueva M, Alvarez-Cermeno JC, Villar LM, Esiri MM (2012) Axonal and oligodendrocyte-localized IgM and IgG deposits in MS lesions. J Neuroimmunol 247(1–2):86–94. doi:10.1016/j.jneuroim.2012.03.020

Saxena A, Bauer J, Scheikl T, Zappulla J, Audebert M, Desbois S, Waisman A, Lassmann H, Liblau RS, Mars LT (2008) Cutting edge: multiple sclerosis-like lesions induced by effector CD8 T cells recognizing a sequestered antigen on oligodendrocytes. J Immunol 181(3):1617–1621

Schirmer L, Merkler D, Konig FB, Bruck W, Stadelmann C (2013) Neuroaxonal regeneration is more pronounced in early multiple sclerosis than in traumatic brain injury lesions. Brain Pathol 23(1):2–12. doi:10.1111/j.1750-3639.2012.00608.x

Serafini B, Rosicarelli B, Magliozzi R, Stigliano E, Aloisi F (2004) Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis. Brain Pathol 14(2):164–174

Shindler KS, Kenyon LC, Dutt M, Hingley ST, Das Sarma J (2008) Experimental optic neuritis induced by a demyelinating strain of mouse hepatitis virus. J Virol 82(17):8882–8886. doi:10.1128/JVI.00920-08

Skulina C, Schmidt S, Dornmair K, Babbe H, Roers A, Rajewsky K, Wekerle H, Hohlfeld R, Goebels N (2004) Multiple sclerosis: brain-infiltrating CD8+ T cells persist as clonal expansions in the cerebrospinal fluid and blood. Proc Natl Acad Sci USA 101(8):2428–2433

Smiley ST, King JA, Hancock WW (2001) Fibrinogen stimulates macrophage chemokine secretion through toll-like receptor 4. J Immunol 167(5):2887–2894

Smith KJ, Lassmann H (2002) The role of nitric oxide in multiple sclerosis. Lancet Neurol 1(4):232–241

Sobel RA, Mitchell ME, Fondren G (1990) Intercellular adhesion molecule-1 (ICAM-1) in cellular immune reactions in the human central nervous system. Am J Pathol 136(6):1309–1316

Sospedra M, Martin R (2005) Immunology of multiple sclerosis. Annu Rev Immunol 23:683–747. doi:10.1146/annurev.immunol.23.021704.115707

Springer TA (1994) Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell 76(2):301–314

Stadelmann C, Kerschensteiner M, Misgeld T, Bruck W, Hohlfeld R, Lassmann H (2002) BDNF and gp145trkB in multiple sclerosis brain lesions: neuroprotective interactions between immune and neuronal cells? Brain J Neurol 125(Pt 1):75–85

Stadelmann C, Ludwin S, Tabira T, Guseo A, Lucchinetti CF, Leel-Ossy L, Ordinario AT, Bruck W, Lassmann H (2005) Tissue preconditioning may explain concentric lesions in Balo's type of multiple sclerosis. Brain J Neurol 128(Pt 5):979–987. doi:10.1093/brain/awh457

Steinman L (2009) A molecular trio in relapse and remission in multiple sclerosis. Nat Rev Immunol 9(6):440–447. doi:10.1038/nri2548

Steinman L, Zamvil S (2003) Transcriptional analysis of targets in multiple sclerosis. Nat Rev Immunol 3(6):483–492. doi:10.1038/nri1108

Steinman L, Zamvil SS (2005) Virtues and pitfalls of EAE for the development of therapies for multiple sclerosis. Trends Immunol 26(11):565–571. doi:10.1016/j.it.2005.08.014

Steinman L, Zamvil SS (2006) How to successfully apply animal studies in experimental allergic encephalomyelitis to research on multiple sclerosis. Ann Neurol 60(1):12–21. doi:10.1002/ana.20913

Storch MK, Stefferl A, Brehm U, Weissert R, Wallstrom E, Kerschensteiner M, Olsson T, Linington C, Lassmann H (1998) Autoimmunity to myelin oligodendrocyte glycoprotein in rats mimics the spectrum of multiple sclerosis pathology. Brain Pathol 8(4):681–694

Sun D, Whitaker JN, Huang Z, Liu D, Coleclough C, Wekerle H, Raine CS (2001) Myelin antigen-specific CD8+ T cells are encephalitogenic and produce severe disease in C57BL/6 mice. J Immunol 166(12):7579–7587

Takahashi T, Fujihara K, Nakashima I, Misu T, Miyazawa I, Nakamura M, Watanabe S, Shiga Y, Kanaoka C, Fujimori J, Sato S, Itoyama Y (2007) Anti-aquaporin-4 antibody is involved in the pathogenesis of NMO: a study on antibody titre. Brain J Neurol 130(Pt 5):1235–1243. doi:10.1093/brain/awm062

Tavolato BF (1975) Immunoglobulin G distribution in multiple sclerosis brain. An immunofluorescence study. J Neurol Sci 24(1):1–11

Thompson AJ, Kermode AG, Wicks D, MacManus DG, Kendall BE, Kingsley DP, McDonald WI (1991) Major differences in the dynamics of primary and secondary progressive multiple sclerosis. Ann Neurol 29(1):53–62. doi:10.1002/ana.410290111

Torchinsky MB, Garaude J, Martin AP, Blander JM (2009) Innate immune recognition of infected apoptotic cells directs T(H)17 cell differentiation. Nature 458(7234):78–82. doi:10.1038/nature07781

Trapp BD, Nave KA (2008) Multiple sclerosis: an immune or neurodegenerative disorder? Annu Rev Neurosci 31:247–269. doi:10.1146/annurev.neuro.30.051606.094313

Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mork S, Bo L (1998) Axonal transection in the lesions of multiple sclerosis. N Engl J Med 338(5):278–285. doi:10.1056/NEJM199801293380502

Traugott U (1987) Multiple sclerosis: relevance of class I and class II MHC-expressing cells to lesion development. J Neuroimmunol 16(2):283–302

Trebst C, Sorensen TL, Kivisakk P, Cathcart MK, Hesselgesser J, Horuk R, Sellebjerg F, Lassmann H, Ransohoff RM (2001) CCR1+/CCR5+ mononuclear phagocytes accumulate in the central nervous system of patients with multiple sclerosis. Am J Pathol 159(5):1701–1710

Ulvestad E, Williams K, Vedeler C, Antel J, Nyland H, Mork S, Matre R (1994) Reactive microglia in multiple sclerosis lesions have an increased expression of receptors for the Fc part of IgG. J Neurol Sci 121(2):125–131

Vanderlugt CL, Miller SD (2002) Epitope spreading in immune-mediated diseases: implications for immunotherapy. Nat Rev Immunol 2(2):85–95. doi:10.1038/nri724

Varrin-Doyer M, Spencer CM, Schulze-Topphoff U, Nelson PA, Stroud RM, Cree BA, Zamvil SS (2012) Aquaporin 4-specific T cells in neuromyelitis optica exhibit a Th17 bias and recognize Clostridium ABC transporter. Ann Neurol 72(1):53–64. doi:10.1002/ana.23651

Veldhoen M, Hirota K, Westendorf AM, Buer J, Dumoutier L, Renauld JC, Stockinger B (2008) The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. Nature 453(7191):106–109. doi:10.1038/nature06881

Wang C, Gold BG, Kaler LJ, Yu X, Afentoulis ME, Burrows GG, Vandenbark AA, Bourdette DN, Offner H (2006) Antigen-specific therapy promotes repair of myelin and axonal damage in established EAE. J Neurochem 98(6):1817–1827. doi:10.1111/j.1471-4159.2006.04081.x

Werner P, Pitt D, Raine CS (2001) Multiple sclerosis: altered glutamate homeostasis in lesions correlates with oligodendrocyte and axonal damage. Ann Neurol 50(2):169–180

Windhagen A, Newcombe J, Dangond F, Strand C, Woodroofe MN, Cuzner ML, Hafler DA (1995) Expression of costimulatory molecules B7-1 (CD80), B7-2 (CD86), and interleukin 12 cytokine in multiple sclerosis lesions. J Exp Med 182(6):1985–1996

Wu GF, Dandekar AA, Pewe L, Perlman S (2000) CD4 and CD8 T cells have redundant but not identical roles in virus-induced demyelination. J Immunol 165(4):2278–2286

Yao DL, Webster HD, Hudson LD, Brenner M, Liu DS, Escobar AI, Komoly S (1994) Concentric sclerosis (Balo): morphometric and in situ hybridization study of lesions in six patients. Ann Neurol 35(1):18–30. doi:10.1002/ana.410350105

Young NP, Weinshenker BG, Parisi JE, Scheithauer B, Giannini C, Roemer SF, Thomsen KM, Mandrekar JN, Erickson BJ, Lucchinetti CF (2010) Perivenous demyelination: association with clinically defined acute disseminated encephalomyelitis and comparison with pathologically confirmed multiple sclerosis. Brain J Neurol 133(Pt 2):333–348. doi:10.1093/brain/awp321

Neuroinflammation in Huntington's & Related Neurodegenerative Disorders

6

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Abstract

One of the common pathological features of most age-related neurodegenerative disorders is the accumulation of abnormal protein deposits as inclusion bodies. It could be neuronal intranuclear inclusions in case of Huntington's disease (HD), extracellular amyloid plaques, and intracellular neurofibrillary tangles in case of Alzheimer's disease (AD), Lewy bodies in case of Parkinson's disease (PD), and cytoplasmic inclusions in case of amyotrophic lateral sclerosis (ALS). Multiple mechanisms have been proposed to understand how these abnormal disease proteins induces neuronal dysfunction and neurodegeneration. However, neuroinflammation and oxidative stress are considered one of the most common phenomena that can be seen across all neurodegenerative disorders. Microglial cells play a key role in neuroinflammation. Continued activation of microglia and constant secretion of inflammatory molecules sets in the vicious cycle of inflammatory reactions in many of these neurodegenerative disorders. In this review we have focussed on the role of neuroinflammation in the pathogenesis of HD and other related neurodegenerative disorders.

Abbreviations

HD	Huntington's disease
AD	Alzheimer's disease, PD, Parkinson's disease
ALS	Amyotrophic lateral sclerosis
CSF	Cerebrospinal fluid
GFAP	Glial fibrillary acidic protein
BBB	Blood brain barrier
TNF-α	Tumour necrosis factor-alpha

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IL	Interleukin
CD	Cluster of differentiation
TGF	Transforming growth factor
IFN	Interferon
PPAR	Peroxisome proliferator-activated receptor
APP	Amyloid precursor protein
IKK	IkappaB kinase
NF-κB	Nuclear factor kappa B
COX	Cyclooxigenase
MCP-1	Monocyte chemoattractant protein-1
IP-10	IFN inducing protein-10
MIP	Macrophage inflammatory protein
Aβ	Amyloid-beta
LPS	Lipopolysaccharide
PET	Positron emission tomography
SOD	Superoxide dismutase
TLR	Toll-like receptor
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NSAIDs	Non-steroidal anti-inflammatory drugs

6.1 Introduction

Our brain is very efficient to stimulate immune and inflammatory responses to a number of insults and stresses (Rivest 2009). Many types of infections, stresses, chronic and acute neurodegenerative conditions are proficient to stimulate the innate immune system in the brain that results in the activation of the local immune cells, i.e., microglia and penetrating monocytes (Crutcher et al. 2006; Popovich and Longbrake 2008). Monocytes are the main constituent of the innate immune system and they eliminate dead cells through phagocytosis. In contrast, activated microglia secrete various inflammatory molecules, including TNF-α, IL-6, and nitric oxide (Hanisch 2002). Microglial cells are activated by the constituent released from damaged or injured cells inside the brain (Hanisch and Kettenmann 2007). Microglial activation is also regulated by astrocytes and neurons through various cytokines, chemokines and neurotransmitters, indicating a complex interplay among microglia, neurons and astrocytes. This complex process is usually termed as "neuroinflammation." Generally, acute neuroinflammatory reaction is beneficial to the brain, because it helps to reduce further injury and promotes the repair mechanism. On the other hand, chronic neuroinflammation is a longstanding and often self-propagating neuroinflammatory reaction that stays for long time after an initial damage. In addition to continued activation of microglia and continual secretion of inflammatory mediators, chronic inflammation also causes increased

oxidative and nitrosative stress (Tansey et al. 2007). The continuous release of inflammatory molecules activates additional microglia, stimulating their production and thereby promoting further release of inflammatory factors and thus generates a vicious inflammatory cycle. Chronic inflammation also leads to weakening of BBB permeability that could allow infiltration of peripheral macrophages into the brain parenchyma leading to further stimulation of inflammatory cycle (Rivest 2009).

Chronic neuroinflammation and elevated levels of several cytokines and chemokines are associated with many neurodegenerative disorders, including HD, AD, PD and ALS (Block and Hong 2005; McGeer and McGeer 2007a, b). Various reports have indicated that neuroinflammatory reactions can be observed much before the significant loss of neuronal populations in the progression of these diseases. This indicates inflammation plays a very prominent role in the progression of these diseases. In this article, we have reviewed the possible role of neuroinflammation, mainly mediated by microglial activation, in the pathophysiology of several neurodegenerative diseases.

6.2 Microglial Activation: A Central Event of Neuroinflammation

Microglia are the local tissue macrophages in the brain and are the key mediators of neuroinflammation. In the inactive state, microglia show a small cell soma and abundant processes (a ramified morphology). In normal brain tissue, these processes are dynamic structures that extend and retract for sampling and monitoring their microenvironment (Nimmerjahn et al. 2005). During the resting state, multiple cell surface receptors like CD-45, CD-14, and CD11b/CD18 are expressed at very low levels. In addition, cell surface receptor-ligand pairs such as CD200R/CD200 are present to maintain neuron-glia communication in the brain (Cardona et al. 2006). Microglial activation alters its surface receptor expression pattern (Tansey et al. 2007) and stimulates the expression of certain proteins like CD1, lymphocyte function-associated antigen 1 (LFA-1), intercellular adhesion molecule 1 (ICAM-1 or CD54), and vascular cell adhesion molecule (VCAM-1 or CD106). Activated microglia release a variety of inflammatory molecules including cytokines (TNF, and interleukins IL-1β and IL-6) and chemokines (macrophage inflammatory protein (MIP-1α), monocyte chemoattractant protein (MCP-1) and IFN inducible protein (IP-10)) that stimulate the inflammatory reactions. The morphology of the microglial cell changes from ramified to amoeboid as they become phagocytic in nature. The moderately active microglia are believed to accomplish helpful functions, like scavenging neurotoxins, eliminating dying cells and cellular debris and releasing trophic factors that stimulate neuronal survival. Long term activation of microglia could increase the penetrability of the BBB and promote augmented infiltration of peripheral immune cells comprising macrophages (Schmid et al. 2009).

6.3 Neuroinflammation in Neurodegenerative Diseases

6.3.1 Huntington's Disease (HD)

Huntington's Disease (HD) is an autosomal dominantly inherited progressive neurodegenerative disorder characterized by cognitive, motor and psychiatric symptoms. The disease is caused by abnormal expansion of CAG repeat (codes for glutamine) in the coding region of a gene called huntingtin. The CAG repeat length in normal individual varies from 6 to 20, while the disease is associated with more than 35 repeats. The onset and severity of the disease is inversely proportional to the CAG repeat length. Neuropathologically, HD is categorized by progressive degeneration of neurons in striatum, some layer of the cerebral cortex and hippocampus in addition to widespread atrophy in the common brain areas (Paulson and Fischbeck 1996; Orr and Zoghbi 2007). One of the most common pathological features of HD and other polyglutamine neurodegenerative disorders is the accumulation of mutant protein as insoluble aggregates. Mutant huntingtin and their aggregates has been shown to induce neuronal dysfunction and neurodegeneration in multiple ways including mitochondrial dysfunction and oxidative stress, aberrant interactions with multiple transcription factors and interference with gene transcription and disturbances in protein folding and clearance mechanisms (Paulson and Fischbeck 1996; Orr and Zoghbi 2007).

Multiple evidence now indicate that inflammation could be an important player in HD pathogenesis (Crotti and Glass 2015). Reactive astrocytes and activated microglia have been observed in the HD brain. The R6/2 transgenic mouse model of HD shows augmented serum IL-6 and its downstream effectors, such as alpha-2-macroglobulin and complement machineries (Dalrymple et al. 2007). Microarray analysis of different brain regions from HD patients and controls discovered augmented gliosis and increased expression of inflammation-related genes, including GFAP and complement proteins in the brain particularly in the caudate and putamen region (Hodges et al. 2006). Neurons and astrocytes of HD brain showed several fold increase in activators and regulators of the classical complement pathway (Singhrao et al. 1999). Several proinflammatory cytokines like IL5, IL6, and IL10 were found to be increased in the blood sample of HD patient even before the onset of clinical symptoms (Bjorkqvist et al. 2008).

Several studies also indicate that the altered levels of various proinflammatory cytokines in HD occurs prior to onset of clinical features of HD, signifying that inflammation could play crucial role in the degeneration of striatal and cortical neurons (Bjorkqvist et al. 2008; Chang et al. 2015). Activated microglia also detected in the HD brain before the inception of symptoms and increased microglial activation links with enhanced chance of developing HD symptoms in 5 years (Tai et al. 2007). In the 3-nitropropionic acid model of HD in rats, treatment with NSAIDs derived from plant origin, reduced the damage of the striatum (Cleren et al. 2005). However, treatment with commonly used NSAIDs like aspirin or

ibuprofen, was not found to be effective in either the R6/2 or N171-82Q transgenic mouse models of HD (Norflus et al. 2004).

Since, mutant huntingtin is known to cause a number of deleterious effects in the neuron, it is possible that inflammatory reactions in HD brains could be an inherent secondary response to neuronal death. However, several studies indicate that mutant huntingtin might itself trigger an inflammatory response, the side-effects of which could cause degeneration of neurons. For example, inflammation in HD could be a result of overactive immune cells, such as macrophages in the periphery and microglia in the brain. Monocytes from HD patients secrete abnormally high levels of the proinflammatory cytokine IL-6 in response to a combination of IFN-γ and LPS. Microglial cells from YAC128 and R6/2 mouse models of HD also secretes abnormally high levels of proinflammatory cytokines (Bjorkqvist et al. 2008).

Interestingly, mutant huntingtin has been shown to activate the IKK complex, the major kinase that drives the phosphorylation and subsequent degradation of IκBs through proteasome. This phenomenon results in nuclear translocation of NF-κB in mouse striatal cells (Khoshnan et al. 2004). The NF-κB stimulates expression of IL-6 and other inflammatory cytokines. Therefore, it is presumed that mutant huntingtin-induced activation of NF-κB pathway could be linked with the induction of inflammatory response in HD brain.

6.3.2 Alzheimer's Disease (AD)

Alzheimer's disease (AD) is the most common progressive neurodegenerative disorder affecting older people worldwide. It is also one of the major cause of dementia. AD is classically characterized by the presence of extracellular amyloid-beta (Aβ) plaque and intracellular neurofibrillary tangle in the brain. Familial form of AD is caused by mutations in amyloid precursor protein (APP) or components of its proteolytic processing machinery (γ and β-secretase) that results in excessive production of Aβ1-40 and Aβ1-42 peptides (Citron et al. 1992; Cai et al. 1993; Lemere et al. 1996). In the last two decades, role of microglia and neuroinflammation in pathophysiology of AD have been extensively investigated. In AD brain, microglial cells are the early responders to Aβ deposits, as they have been shown to be strongly attached with Aβ plaques and their phagocytosis (Varley et al. 2015; van Gool et al. 2003). Microglial cells are directly activated by most Aβ species via multiple mechanisms including pattern recognition receptors such as TLRs, and other receptors such as receptor for advanced end glycation products, LRP1, scavenger receptors and complement receptors (Lieberman et al. 1995).

The proinflammatory effects of Aβ deposition in the brain has been reiterated in aged mice transgenic for a familial AD mutation of APP, in which astrocytes and microglia expressing IL-1β, IL-6, and TNF have been found adjoining to amyloid plaques (Benzing et al. 1999). Interestingly, TGF-β cytokine has been shown to increase Aβ accumulation in the cerebral blood vessels of mice transgenic for human APP. This cytokine is also upregulated in the blood vessel of human patients

with cerebral amyloid angiopathy (Wyss-Coray et al. 1997). Presence of TGF-β1, 2 and 3 isoforms alongside with Aβ causes increased accumulation of Aβ in organotypic hippocampal slices (Harris-White et al. 1998). Together, these observations validate the link between microglia and amyloid plaque deposition and the capability of certain chronic inflammatory response to aggravate Aβ-plaque associated pathology. However, inflammatory response might also be crucial in preventing Aβ-plaque associated toxicity. For example, the stimulation of complement factor C3, the central component of the complement system, and a vital inflammatory protein may be necessary for plaque clearance by microglia in the AD brain (Wyss-Coray et al. 2002; Maier et al. 2008). The peroxisome proliferator-activated receptor-γ (PPARγ), a nuclear receptor which is activated by metabolites of prostaglandins generated by COX enzymes and by certain NSAIDs, is upregulated along with the COX enzymes in AD brain (Kitamura et al. 1999). As such, PPARγ activation exerts an anti-inflammatory effect, and PPARγ agonists have been shown to inhibit the production of cytokines and proinflammatory mediators in response to Aβ (Combs et al. 2000). The ratio of the proinflammatory cytokine IL-1β to the anti-inflammatory cytokine IL-10 is significantly elevated in the serum of AD patients (Remarque et al. 2001). Increased levels of IL-1β have been correlated with decreased long term potentiation in the hippocampus, resulting in impaired memory formation (Lynch 1998). In a recent study, IL-1β was shown to upregulate the α-secretase TACE, thus increasing non-amyloidogenic cleavage of APP and diminishing Aβ production (Tachida et al. 2008). Therefore, it is possible that IL-1β might be participating in neuroinflammatory reactions in AD brain by activating microglia to secrete other inflammatory molecules. It is probably not linked with Aβ deposition and may actually stimulate non-amyloidogenic pathways.

The role of inflammatory mediators in progression of AD is still poorly understood. But these inflammatory molecules could not only function as useful biomarkers but also might be the targets for drug development. For example, proinflammatory protein like cyclooxygenase 2 (COX2) and its homolog COX-1 are significantly elevated in AD brain (Lukiw and Bazan 2000). COX enzyme is one of the targets of NSAIDs. Epidemiological studies indicate a link between chronic use of NSAIDs and reduced risk for AD. A recent study exhibited that patients who had taken NSAIDs for more than 2 years showed the significant reduction in AD risk (Stewart et al. 1997). AD brain also exhibit about threefold decrease in the number of activated microglia upon long term treatment of NSAIDs (Mackenzie and Munoz 1998). In a recent study, chronic NSAIDs use, particularly ibuprofen, was shown to be effective to slow down the progression of AD (Vlad et al. 2008). These findings suggest that decrease in microglial activation upon chronic use of NSAIDs might lead to the beneficial effect in AD patients. However, so far, clinical trials using systemic administration of NSAIDs have yielded mixed or inconclusive results (van Gool et al. 2003; McGeer and McGeer 2007a, b), reflecting the need to identify and target the key inflammatory mediators that promote amyloid-associated neuropathology.

6.3.3 Parkinson's Disease (PD)

Parkinson's Disease (PD) is another progressive neurodegenerative disorders characterized by motor deficits including rigidity, resting tremor, and difficulty with walking and gait. Characteristic pathological features include loss of dopaminergic neurons in the substantia nigra and presence of eosinophilic Lewy body inclusions in the degenerated neurons. Both genetic and environmental factors are linked with the cause of PD. Mutations in α-synuclein gene is associated with autosomal dominant form of PD, while autosomal recessive form of PD is linked with mutations in parkin gene (Litvan et al. 2007). Several studies have established the increased levels of various inflammatory molecules (like TNF-α, IL-1β, IL-6, and IFNγ) in the CSF as well as in the degenerated substantia nigra region of post-mortem PD brain tissues (Hunot et al. 1999; Gerhard et al. 2006). Significantly elevated levels of TNF- α mRNA and protein can be seen in the rodent midbrain substantia nigra within few hours of administration of two neurotoxins widely used to model parkinsonism in rodents, 6-hydroxydopamine (Nagatsu and Sawada 2006) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Rousselet et al. 2002; Ferger et al. 2004). Very interestingly, plasma TNF-α levels have been found to remain elevated in MPTP-treated non-human primates even one year after administration of the neurotoxin (Barcia et al. 2005). However, studies involving mice deficient in TNF-α or its receptors showed conflicting outcome. There are studies that showed that lack of TNF-α receptors reduced dopamine metabolism and the survival of dopaminergic neurons (Rousselet et al. 2002). Other reports indicated that TNF-α-deficient mice exhibit reduced sensitivity to MPTP-induced neurotoxicity (Sriram et al. 2002; Ferger et al. 2004). It is possible that lack of TNF-α or its receptor during development might alter the activities of microglia or other immune cell populations that results in conflicting outcomes in these studies. The involvement of inflammatory cytokines, particularly TNF-α in the degeneration of dopaminergic neuron, is also observed in two endotoxin models. In the first model, chronic low dose of lipopolysaccharide (LPS) infusion into substantia nigra of rats results in delayed, selective and progressive loss of nigral dopaminergic neurons (Gao et al. 2002). In the second model, exposure of pregnant rats to LPS caused a loss of dopaminergic neurons in postnatal brains (Ling et al. 2002). Most importantly, chronic infusion of dominant negative TNF-α inhibitor into substantia nigra of adult rats protected nigral dopaminergic neurons from LPS and 6-hydroxydopamine-induced degeneration (McCoy et al. 2006). Similarly, a single injection (substantia nigra region) of a lentivirus encoding DN-TNF-α in 6-hydroxydopamine hemiparkinsonian rats rescued dopaminergic neuron degeneration (McCoy et al. 2008). Thus early genetic studies and the more recent chronic inflammation models of PD strongly implicate TNF-α and its downstream targets are strongly associated with neurotoxin and endotoxin-induced degeneration of nigral dopaminergic neurons. Nigrostriatal dopamine neurons are selectively susceptible to TNF-α-induced toxicity probably because of high level of expression of TNF receptors in these cells (Boka et al. 1994; Gayle et al. 2002; Carvey et al. 2005). TNF-α produced by brain-resident microglia might not be the only factor

causing the death of dopaminergic neurons. Other proinflammatory factors might also be involved that requires further investigation.

Another connection between inflammation and neurodegeneration in PD comes from studies of single nucleotide polymorphisms that are associated with excess formation of cytokines, chemokines, and acute phase proteins. These polymorphisms are overrepresented in explicit cohorts of persons affected with PD and may convene increased predisposition for the disease (Hakansson et al. 2005; Nishimura et al. 2005). However, a majority of these reports have not been replicated in independent studies. Lastly, PET studies detected significant increase in microglial activation in basal ganglia, pons and frontal and temporal cortex in patients with idiopathic PD in comparison with healthy age-matched controls (Gerhard et al. 2006). Continuous activation of the profuse number of microglia in the midbrain region could lead to cascading inflammatory cycle (Kim and Joh 2006; Block et al. 2007).

6.3.4 Amyotrophic Lateral Sclerosis (ALS)

Amyotrophic Lateral Sclerosis (ALS) is a progressive degenerative disease of motor neuron that results in muscles weakness and paralysis. Approximately 10 % of ALS are known to be familial in nature and the cause behind the 90 % cases of ALS are sporadic. Current evidence indicate that multiple genetic and environmental factors are associated with ALS pathogenesis. Both familial and sporadic ALS share common pathological features like cytoplasmic inclusions and abnormal neurofilament accumulations. So far, mutations in Cu/Zn superoxide dismutase gene (SOD1) have been reported the only proven cause of familial form of ALS and several other genes have been shown to be associated with either familial or sporadic form of ALS (Rosen 1993). Transgenic mice having mutation of SOD1 genes recapitulates many features of ALS thus serve as a typical animal model of ALS.

Several reports demonstrated that the areas surrounding the degenerating motor neurons in both ALS patients and in mouse model are noticeable by the presence of cytokines and immune cells, including T-cells, activated microglia, and astrocytes (Kawamata et al. 1992; Henkel et al. 2004). PET imaging analysis of ALS patients exhibited a significant increase in stimulated microglia in the motor cortex that well correlates with upper motor neuron symptoms (Turner et al. 2004). In transgenic mouse models of ALS, the disease severity is directly correlates with the presence of activated immune cells (Henkel et al. 2006). The MCP-1, a potent chemokines for microglia is elevated in several fold in the CSF of ALS patients (McManus et al. 2000; Kuhle et al. 2009), which strongly suggests that neuroinflammation could be one of the crucial factor leads to disease progression. Several other general markers of inflammation were also significantly increased in the serum of ALS patients and their levels positively correlate with the magnitude of symptoms (Keizman et al. 2009). SOD1 serves as an important antioxidant enzyme and there are evidence suggest that this enzyme helps to prevent protein aggregation. However, mutations in SOD1 increases its propensity to form aggregates in neurons that ultimately

contribute to inflammation and disease pathogenesis (Lino et al. 2002; Clement et al. 2003; Sargsyan et al. 2005). Removal of microglial expression of mutant SOD1 increases the survival rate of mutant SOD1 transgenic mice (Beers et al. 2006), reflecting that mutant SOD1 induces neuronal death by affecting glial rather than neuronal function. Similarly, mutant SOD1 transgenic mice stimulated with LPS release more inflammatory molecules, including TNF-α (Weydt et al. 2004), MCP-1, TGF-β and IFN-γ (Ferri et al. 2004), in comparison with control. More importantly, the level of TNF-α and its receptors were significantly correlated with the severity of motor neuron degeneration in the mouse model (Yoshihara et al. 2002; Poloni et al. 2000). Furthermore, treatments of NSAIDs have been demonstrated to increase the life span of ALS mice by more than 30 % (West et al. 2004) and also prevented neurotoxicity (Tikka et al. 2002).

6.4 Concluding Remarks

From the existing literature, it is very clear that neuroinflammation does take place in most age-related neurodegenerative disorders and microglia are the principal player in mediating the response. Neuroinflammatory responses might be directly linked with the toxic mutant disease proteins or their aggregates associated with diverse neurodegenerative disorders or it may be indirectly associated as a consequence of neurodegeneration. Neuroinflammatory response also could be beneficial or harmful. All these aspects needs extensive investigations in order to develop successful therapeutic strategies.

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References

Barcia C, de Pablos V et al (2005) Increased plasma levels of TNF-alpha but not of IL1-beta in MPTP-treated monkeys one year after the MPTP administration. Parkinsonism Relat Disord 11 (7):435–439

Beers DR, Henkel JS et al (2006) Wild-type microglia extend survival in PU.1 knockout mice with familial amyotrophic lateral sclerosis. Proc Natl Acad Sci USA 103(43):16021–16026

Benzing WC, Wujek JR et al (1999) Evidence for glial-mediated inflammation in aged APP(SW) transgenic mice. Neurobiol Aging 20(6):581–589

Bjorkqvist M, Wild EJ et al (2008) A novel pathogenic pathway of immune activation detectable before clinical onset in Huntington's disease. J Exp Med 205(8):1869–1877

Block ML, Hong JS (2005) Microglia and inflammation-mediated neurodegeneration: multiple triggers with a common mechanism. Prog Neurobiol 76(2):77–98

Block ML, Zecca L et al (2007) Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nat Rev Neurosci 8(1):57–69

Boka G, Anglade P et al (1994) Immunocytochemical analysis of tumor necrosis factor and its receptors in Parkinson's disease. Neurosci Lett 172(1–2):151–154
Cai XD, Golde TE et al (1993) Release of excess amyloid beta protein from a mutant amyloid beta protein precursor. Science 259(5094):514–516
Cardona AE, Huang D et al (2006) Isolation of murine microglial cells for RNA analysis or flow cytometry. Nat Protoc 1(4):1947–1951
Carvey PM, Chen EY et al (2005) Intra-parenchymal injection of tumor necrosis factor-alpha and interleukin 1-beta produces dopamine neuron loss in the rat. J Neural Transm 112(5):601–612
Chang KH, Wu YR et al (2015) Plasma inflammatory biomarkers for Huntington's disease patients and mouse model. Brain Behav Immun 44:121–127
Citron M, Oltersdorf T et al (1992) Mutation of the beta-amyloid precursor protein in familial Alzheimer's disease increases beta-protein production. Nature 360(6405):672–674
Clement AM, Nguyen MD et al (2003) Wild-type nonneuronal cells extend survival of SOD1 mutant motor neurons in ALS mice. Science 302(5642):113–117
Cleren C, Calingasan NY et al (2005) Celastrol protects against MPTP- and 3-nitropropionic acid-induced neurotoxicity. J Neurochem 94(4):995–1004
Combs CK, Johnson DE et al (2000) Inflammatory mechanisms in Alzheimer's disease: inhibition of beta-amyloid-stimulated proinflammatory responses and neurotoxicity by PPARgamma agonists. J Neurosci 20(2):558–567
Crotti A, Glass CK (2015) The choreography of neuroinflammation in Huntington's disease. Trends Immunol 36(6):364–373
Crutcher KA, Gendelman HE et al (2006) Debate: "is increasing neuroinflammation beneficial for neural repair?". J Neuroimmune Pharmacol 1(3):195–211
Dalrymple A, Wild EJ et al (2007) Proteomic profiling of plasma in Huntington's disease reveals neuroinflammatory activation and biomarker candidates. J Proteome Res 6(7):2833–2840
Ferger B, Leng A et al (2004) Genetic ablation of tumor necrosis factor-alpha (TNF-alpha) and pharmacological inhibition of TNF-synthesis attenuates MPTP toxicity in mouse striatum. J Neurochem 89(4):822–833
Ferri A, Nencini M et al (2004) Activity of protein phosphatase calcineurin is decreased in sporadic and familial amyotrophic lateral sclerosispatients. J Neurochem 90(5):1237–1242
Gao HM, Jiang J et al (2002) Microglial activation-mediated delayed and progressive degeneration of rat nigral dopaminergic neurons: relevance to Parkinson's disease. J Neurochem 81 (6):1285–1297
Gayle DA, Ling Z et al (2002) Lipopolysaccharide (LPS)-induced dopamine cell loss in culture: roles of tumor necrosis factor-alpha, interleukin-1beta, and nitric oxide. Brain Res Dev Brain Res 133(1):27–35
Gerhard A, Pavese N et al (2006) In vivo imaging of microglial activation with [11C](R)-PK11195 PET in idiopathic Parkinson's disease. Neurobiol Dis 21(2):404–412
Hakansson A, Westberg L et al (2005) Investigation of genes coding for inflammatory components in Parkinson's disease. Mov Disord 20(5):569–573
Hanisch UK (2002) Microglia as a source and target of cytokines. Glia 40(2):140–155
Hanisch UK, Kettenmann H (2007) Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat Neurosci 10(11):1387–1394
Harris-White ME, Chu T et al (1998) Effects of transforming growth factor-beta (isoforms 1-3) on amyloid-beta deposition, inflammation, and cell targeting in organotypic hippocampal slice cultures. J Neurosci 18(24):10366–10374
Henkel JS, Engelhardt JI et al (2004) Presence of dendritic cells, MCP-1, and activated microglia/macrophages in amyotrophic lateral sclerosis spinal cord tissue. Ann Neurol 55 (2):221–235
Henkel JS, Beers DR et al (2006) The chemokine MCP-1 and the dendritic and myeloid cells it attracts are increased in the mSOD1 mouse model of ALS. Mol Cell Neurosci 31(3):427–437
Hodges A, Strand AD et al (2006) Regional and cellular gene expression changes in human Huntington's disease brain. Hum Mol Genet 15(6):965–977

Hunot S, Dugas N et al (1999) FcepsilonRII/CD23 is expressed in Parkinson's disease and induces, in vitro, production of nitric oxide and tumor necrosis factor-alpha in glial cells. J Neurosci 19(9):3440–3447
Kawamata T, Akiyama H et al (1992) Immunologic reactions in amyotrophic lateral sclerosis brain and spinal cord tissue. Am J Pathol 140(3):691–707
Keizman D, Rogowski O et al (2009) Low-grade systemic inflammation in patients with amyotrophic lateral sclerosis. Acta Neurol Scand 119(6):383–389
Khoshnan A, Ko J et al (2004) Activation of the IkappaB kinase complex and nuclear factor-kappaB contributes to mutant huntingtin neurotoxicity. J Neurosci 24(37):7999–8008
Kim YS, Joh TH (2006) Microglia, major player in the brain inflammation: their roles in the pathogenesis of Parkinson's disease. Exp Mol Med 38(4):333–347
Kitamura Y, Shimohama S et al (1999) Increased expression of cyclooxygenases and peroxisome proliferator-activated receptor-gamma in Alzheimer's disease brains. Biochem Biophys Res Commun 254(3):582–586
Kuhle J, Lindberg RL et al (2009) Increased levels of inflammatory chemokines in amyotrophic lateral sclerosis. Eur J Neurol 16(6):771–774
Lemere CA, Lopera F et al (1996) The E280A presenilin 1 Alzheimer mutation produces increased A beta 42 deposition and severe cerebellar pathology. Nat Med 2(10):1146–1150
Lieberman J, Schleissner L et al (1995) Serum alpha 1-antichymotrypsin level as a marker for Alzheimer-type dementia. Neurobiol Aging 16(5):747–753
Ling Z, Gayle DA et al (2002) In utero bacterial endotoxin exposure causes loss of tyrosine hydroxylase neurons in the postnatal rat midbrain. Mov Disord 17(1):116–124
Lino MM, Schneider C et al (2002) Accumulation of SOD1 mutants in postnatal motoneurons does not cause motoneuron pathology or motoneuron disease. J Neurosci 22(12):4825–4832
Litvan I, Halliday G et al (2007) The etiopathogenesis of Parkinson disease and suggestions for future research. Part I. J Neuropathol Exp Neurol 66(4):251–257
Lukiw WJ, Bazan NG (2000) Neuroinflammatory signaling upregulation in Alzheimer's disease. Neurochem Res 25(9–10):1173–1184
Lynch MA (1998) Age-related impairment in long-term potentiation in hippocampus: a role for the cytokine, interleukin-1 beta? Prog Neurobiol 56(5):571–589
Mackenzie IR, Munoz DG (1998) Nonsteroidal anti-inflammatory drug use and Alzheimer-type pathology in aging. Neurology 50(4):986–990
Maier M, Peng Y et al (2008) Complement C3 deficiency leads to accelerated amyloid beta plaque deposition and neurodegeneration and modulation of the microglia/macrophage phenotype in amyloid precursor protein transgenic mice. J Neurosci 28(25):6333–6341
McCoy MK, Martinez TN et al (2006) Blocking soluble tumor necrosis factor signaling with dominant-negative tumor necrosis factor inhibitor attenuates loss of dopaminergic neurons in models of Parkinson's disease. J Neurosci 26(37):9365–9375
McCoy MK, Ruhn KA et al (2008) Intranigral lentiviral delivery of dominant-negative TNF attenuates neurodegeneration and behavioral deficits in hemiparkinsonian rats. Mol Ther 16(9): 1572–1579
McGeer EG, McGeer PL (2007a) The role of anti-inflammatory agents in Parkinson's disease. CNS Drugs 21(10):789–797
McGeer PL, McGeer EG (2007b) NSAIDs and Alzheimer disease: epidemiological, animal model and clinical studies. Neurobiol Aging 28(5):639–647
McManus CM, Liu JS et al (2000) Differential induction of chemokines in human microglia by type I and II interferons. Glia 29(3):273–280
Nagatsu T, Sawada M (2006) Cellular and molecular mechanisms of Parkinson's disease: neurotoxins, causative genes, and inflammatory cytokines. Cell Mol Neurobiol 26(4–6):781–802
Nimmerjahn A, Kirchhoff F et al (2005) Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308(5726):1314–1318
Nishimura M, Kuno S et al (2005) Glutathione-S-transferase-1 and interleukin-1beta gene polymorphisms in Japanese patients with Parkinson's disease. Mov Disord 20(7):901–902

Norflus F, Nanje A et al (2004) Anti-inflammatory treatment with acetylsalicylate or rofecoxib is not neuroprotective in Huntington's disease transgenic mice. Neurobiol Dis 17(2):319–325
Orr HT, Zoghbi HY (2007) Trinucleotide repeat disorders. Annu Rev Neurosci 30:575–621
Paulson HL, Fischbeck KH (1996) Trinucleotide repeats in neurogenetic disorders. Annu Rev Neurosci 19:79–107
Poloni M, Facchetti D et al (2000) Circulating levels of tumour necrosis factor-alpha and its soluble receptors are increased in the blood of patients with amyotrophic lateral sclerosis. Neurosci Lett 287(3):211–214
Popovich PG, Longbrake EE (2008) Can the immune system be harnessed to repair the CNS? Nat Rev Neurosci 9(6):481–493
Remarque EJ, Bollen EL et al (2001) Patients with Alzheimer's disease display a pro-inflammatory phenotype. Exp Gerontol 36(1):171–176
Rivest S (2009) Regulation of innate immune responses in the brain. Nat Rev Immunol 9(6): 429–439
Rosen DR (1993) Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 364(6435):362
Rousselet E, Callebert J et al (2002) Role of TNF-alpha receptors in mice intoxicated with the parkinsonian toxin MPTP. Exp Neurol 177(1):183–192
Sargsyan SA, Monk PN et al (2005) Microglia as potential contributors to motor neuron injury in amyotrophic lateral sclerosis. Glia 51(4):241–253
Schmid CD, Melchior B et al (2009) Differential gene expression in LPS/IFNgamma activated microglia and macrophages: in vitro versus in vivo. J Neurochem 109(Suppl 1):117–125
Singhrao SK, Neal JW et al (1999) Increased complement biosynthesis by microglia and complement activation on neurons in Huntington's disease. Exp Neurol 159(2):362–376
Sriram K, Matheson JM et al (2002) Mice deficient in TNF receptors are protected against dopaminergic neurotoxicity: implications for Parkinson's disease. FASEB J 16(11):1474–1476
Stewart WF, Kawas C et al (1997) Risk of Alzheimer's disease and duration of NSAID use. Neurology 48(3):626–632
Tachida Y, Nakagawa K et al (2008) Interleukin-1 beta up-regulates TACE to enhance alpha-cleavage of APP in neurons: resulting decrease in Abeta production. J Neurochem 104(5):1387–1393
Tai YF, Pavese N et al (2007) Microglial activation in presymptomatic Huntington's disease gene carriers. Brain 130(Pt 7):1759–1766
Tansey MG, McCoy MK et al (2007) Neuroinflammatory mechanisms in Parkinson's disease: potential environmental triggers, pathways, and targets for early therapeutic intervention. Exp Neurol 208(1):1–25
Tikka TM, Vartiainen NE et al (2002) Minocycline prevents neurotoxicity induced by cerebrospinal fluid from patients with motor neurone disease. Brain 125(Pt 4):722–731
Turner MR, Cagnin A et al (2004) Evidence of widespread cerebral microglial activation in amyotrophic lateral sclerosis: an [11C](R)-PK11195 positron emission tomography study. Neurobiol Dis 15(3):601–609
van Gool WA, Aisen PS et al (2003) Anti-inflammatory therapy in Alzheimer's disease: is hope still alive? J Neurol 250(7):788–792
Varley J, Vincent A et al (2015) Clinical and experimental studies of potentially pathogenic brain-directed autoantibodies: current knowledge and future directions. J Neurol 262(4): 1081–1095
Vlad SC, Miller DR et al (2008) Protective effects of NSAIDs on the development of Alzheimer disease. Neurology 70(19):1672–1677
West M, Mhatre M et al (2004) The arachidonic acid 5-lipoxygenase inhibitor nordihydroguaiaretic acid inhibits tumor necrosis factor alpha activation of microglia and extends survival of G93A-SOD1 transgenic mice. J Neurochem 91(1):133–143
Weydt P, Yuen EC et al (2004) Increased cytotoxic potential of microglia from ALS-transgenic mice. Glia 48(2):179–182

Wyss-Coray T, Masliah E et al (1997) Amyloidogenic role of cytokine TGF-beta1 in transgenic mice and in Alzheimer's disease. Nature 389(6651):603–606

Wyss-Coray T, Yan F et al (2002) Prominent neurodegeneration and increased plaque formation in complement-inhibited Alzheimer's mice. Proc Natl Acad Sci USA 99(16):10837–10842

Yoshihara T, Ishigaki S et al (2002) Differential expression of inflammation- and apoptosis-related genes in spinal cords of a mutant SOD1 transgenic mouse model of familial amyotrophic lateral sclerosis. J Neurochem 80(1):158–167

Neuroinflammation During Parkinson's Disease: Key Cells and Molecules Involved in It

7

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Abstract

Parkinson's disease (PD), an idiopathic neurodegenerative disorder, is characterized by dopaminergic neuronal degeneration in the substantia nigra pars compacta of brain. Recent findings suggest the multifactorial etiology of the disease. Neuroinflammation and infiltration of peripheral inflammatory cells, chemokines, and cytokines may have a crucial role in PD pathogenesis. During the onset of the disease acute inflammation sets in to prevent dopaminergic neuronal death, but as the disease progresses neuroinflammation becomes chronic and promotes neurodegeneration. Rampant release of proinflammatory cytokines (TNF-α and IFN-γ), infiltration of peripheral CD4 and CD8 lymphocytes and augmented rate of reactive oxygen species (ROS) and reactive nitrogen species (RNS) generation act as key players in disease progression. These proinflammatory factors are released by astrocytes stimulated by neuron derived α-synuclein which in turn recruit and activate microglia. These activated microglia contribute hugely to the progressive neurodegeneration in PD. In this chapter, we have summarized and discussed the findings on the neuroinflammarotry status in PD patients emphasizing on the role of cells and molecules involved. In addition, we have also discussed the plausible therapeutic interventions that may prove to be beneficial to PD patients.

Priyobrata and Nabanita contributes to this review equally.

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7.1 Introduction

Parkinson's disease (PD), one of the most commonly occurring age related progressive neurodegenerative disorder, is characterized by the death of dopaminergic (DA) neurons in substantia nigra pars compacta and striatum, cytoplasmic inclusions of aggregated proteins (Lewy bodies (LBs), and chronic neuroinflammation (McGeer and McGeer 2008; Moore et al. 2005). Neuroinflammation is marked by the presence of activated microglia and reactive astrocytes in the central nervous system (CNS) and augmented expression of proinflammatory factors like cytokines, chemokines, prostaglandins, complement cascade proteins, reactive oxygen and nitrogen species (ROS/RNS) which sometimes result in disruption of the blood–brain barrier (BBB) causing direct participation of the adaptive immune system in the disease pathogenesis (Ransohoff and Perry 2009). The exact extent to which chronic neuroinflammation and peripheral immune response contribute to the severity of PD remains elusive till date. However, it is evident from the literature review that by modulating the BBB permeability of peripheral macrophage and blood leukocytes in the brain parenchyma, the brain homeostasis and neuronal damage can be tightly regulated (Ransohoff and Perry 2009; Rezai-Zadeh et al. 2009). Neuroinflammation may not be the primary trigger for neurodegeneration, but, according to epidemiological and preclinical data of age related neurodegenerative diseases, chronic neuroinflammation may be referred to as the "silent driver" of neuronal dysfunction and death during the progression of such neurological disorders (like PD, AD, HD, etc.). So in the following sections, we will discuss about the key players (both cellular and molecular) of neuroinflammation in Parkinson's disease.

Animal Models of Parkinson's disease: To understand the mechanisms underlying neurodegeneration and neuroinflammation in Parkinson's disease, various animal models of the disease have been established. Several pesticides (Paraquat and Rotenone) and neurotoxins (MPTP and 6-OHDA) have been used to induce Parkinson-like symptoms in animal models. A detailed mechanism of their cellular entry and mode of action is given in Fig. 7.1.

7.2 Role of Resident Brain Cells in Neuroinflammation

7.2.1 Microglia

1. Brain contains three distinct types of Glial cells named microglia, astrocytes, and oligodendrocytes (Teismann and Schulz 2004). All these cell types perform various functions to maintain overall brain homeostasis. Among them, microglia being the most abundant resident macrophages of brain, are meant for overall immune surveillance (Perry 2012). Microglia keep a strict vigilance by continuously examining the brain microenvironment (Nimmerjahn et al. 2005). Microglia reside in two main forms: ramified microglia and activated microglia.

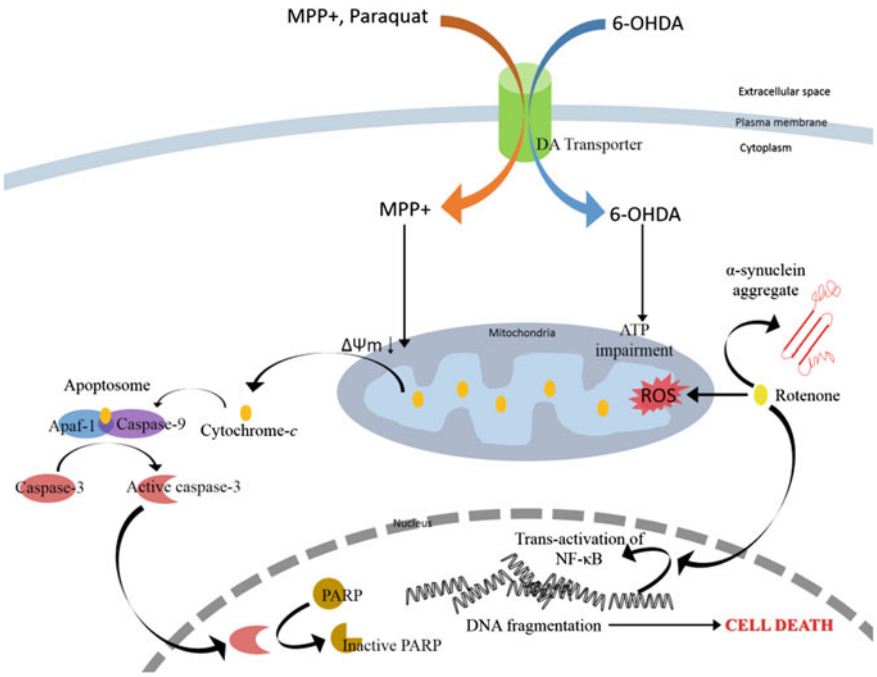

Fig. 7.1 Experimental models in PD. Pesticides paraquat or Rotenone and neurotoxins 6; OHDA or MPP+ (active metabolite of MPTP) easily cross cell membrane and enter the cell through the dopamine transporter (DA) resulting in aggregation of α-synuclein and mitochondrial dysregulation followed by excessive ROS generation. Rotenone, being hydrophobic, easily penetrates neurons and astrocytes and results in subsequent translocation of NF-κB in the nucleus. Rotenone and MPP+ being mitochondrial complex I inhibitor, causes the ATP impairment, generates more ROS and the release of proapoptotic molecules, that induce apoptosis by triggering caspases 3, 6, 7 caspase 9 action

Ramified microglia are resting type which function as scavengers in developing brain by removing the excess unwanted cells of the neocortex and other brain regions that is crucial to form a fully developed fetal brain (Voyvodic 1996). On the other hand, resting microglia react rigorously during neurotoxic insult. They proliferate, become hypertrophic, and show heightened expression of marker molecules like CD68, CD11b, MHC-I and II molecules and further achieve more macrophage-like morphology and function in PD patients (McGeer and McGeer 2004). However, in response to any sort of neurotoxic stress, ramified microglia have unique ability to change its morphology to reactive form, release an array of mediators, and augment expression of specific receptor types in defense of the damage to the brain. Though it is an established fact that chronic microglial activation leads to neuroinflammation, still not all types of microglia promote neurodegeneration. Distinct stimuli can transform microglia into M1 or M2 type performing different functions (Gordon and Taylor 2005).

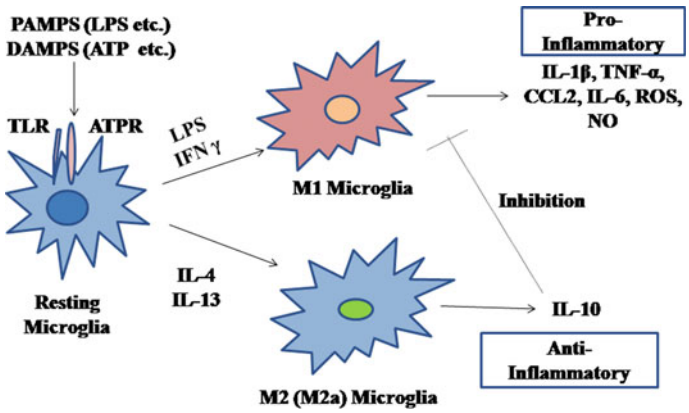

Fig. 7.2 Depending on different activation signals microglia are polarized to M1 and M2 fate. Ramified microglia undergo modifications to M1 microglial form upon stimulation by LPS, IFN-gamma from Th1 and Th17 cells which promotes neurodegeneration by releasing proinflammatory cytokines. M2 microglia polarization occurs if stimulation comes from Th2 or Treg cells (Il-4, Il-13) which promotes neuroprotection and repair by releasing anti-inflammatory cytokines

M1 microglia are proinflammatory in function releasing proinflammatory cytokines (TNF-α, Il-1β, Il-6, etc.) sending proapoptotic signals to neurons. On the contrary, M2 type microglia are neuroprotective in action releasing anti-inflammatory cytokines (Il-10, TGF-β, BDNF, NGF, etc.) and providing anti-apoptotic signal to damaged neurons (Nakagawa and Chiba 2014) (Fig. 7.2).

Activated microglia are considered a key cell-type in defense against inflammatory diseases and infections of the CNS, both in vitro and in vivo (Banati 2003; Rock et al. 2004). The changes in brain homeostasis by toxic insults, aggregated proteins or pathogens, etc., provide the trigger for microglial activation. They then promptly react to the altered condition by means of chemokine receptors, Toll-like receptors, ion channels, cytokines, etc., (Stone et al. 2009) to bring back the brain homeostasis.

Among other brain regions, the substantia nigra is relatively rich in microglia. The nigral dopaminergic neurons are more susceptible to microglia-mediated injury and oxidative stress because of reduced level of intracellular glutathione (Kim and Joh 2006). Thus it is evident that in PD patients, nigral dopaminergic neurons are mostly affected (Qian et al. 2010). In various animal models of PD, selective degeneration of dopaminergic neurons in the SN is induced by 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine(MPTP), paraquat, and rotenone. All these toxins result in death of dopaminergic cells of the SN over a period of few days. Other degenerative changes also occur along with sustained neuroinflammation. According to a recent study using intrastriatal injection of 6-OHDA, not only did nigral dopaminergic cell died, but it was accompanied by

activation of microglia identified by upregulation of the complement type 3 receptor (Walsh et al. 2011). Similarly, MPTP-induced neurodegeneration is also associated with activated microglia (McGeer et al. 2003; Sugama et al. 2003). Recent research indicates that toxin-induced activation of NADPH oxidase causes microglial activation and consequent neurotoxicity (Qian et al. 2010). Other immunological insults that are capable of activating microglia include IFN-γ, TGF-β, brain-derived neurotrophic factor (BDNF), CX3CL1, CD22, neurotrophin-3, and neurotransmitters. Substantia nigra of PD patients has been found to produce significantly high amount of iNOS that promotes persistent and excessive release of NO and other superoxide radicals resulting in prolonged neuroinflammation. Other factors produced by activated microglia that contribute to the neurodegenation in PD are COX2, PGE2, etc., (Knott et al. 2000; Koppula et al. 2012; Wang et al. 2005).

So, in a nutshell, under normal physiological conditions, microglia reside in quiescent state by well-orchestrated action of neurons and astrocytes. They are activated when neuronal homeostasis and integrity is disturbed in PD, under influence of activation signals from affected neurons and also due to absence of neuronal inhibitory signals (Kim et al. 2000) (Fig. 7.3).

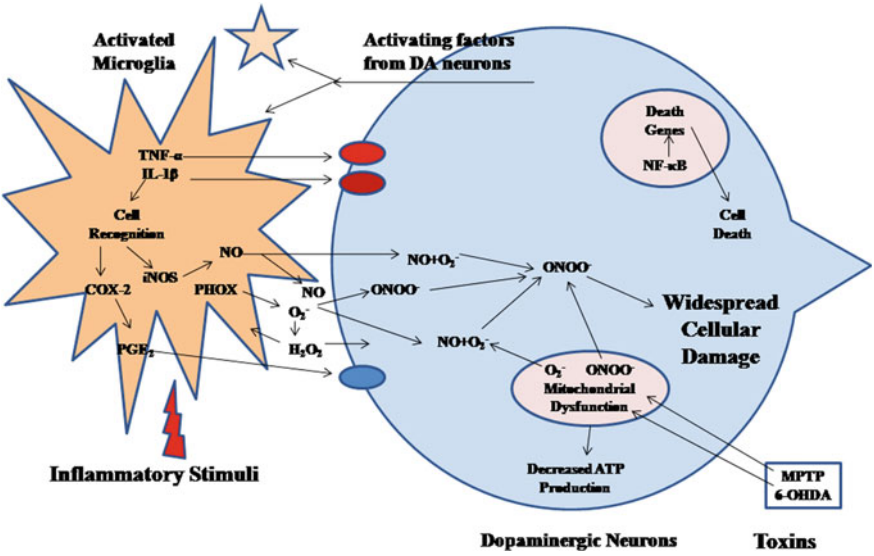

Fig. 7.3 Diagram depicting microglial activation and its relation to death of dopaminergic neurons. Activated microglia releases TNF-α and IL-1β and thus activates the NF-κB pathway leading to activation of death genes and neuronal death. These activated microglia release (i) iNOS, (ii) COX-2, (iii) PHOX (gene responsible for NADPH oxidase), (iv) H_2O_2. iNOS releases nitric oxide which is oxidized to peroxinitrite ion by PHOX. This peroxinitrite ($ONOO^-$) and hydrogen peroxide cause mitochondrial dysfunction in DA neurons leading to decreased energy production. COX-2 induces formation of PGE-2 which exerts direct toxicity to DA neurons

7.2.2 Astrocyte

Astrocytes, the most widely distributed glial cell in the mammalian brain (Chen et al. 2010), are characterized by intermediate filaments glial fibrillary acidic protein (GFAP) and vimentin (Vim). Astrocytes transport nutrients and metabolic precursors to the neurons by the malate–aspartate shuttle and other transporters and thus are important for brain metabolism (Maragakis and Rothstein 2006). The two main types of astrocytes found in the CNS are protoplasmic astrocytes (envelope neuronal bodies and synapses) and fibrous astrocytes (interact with the nodes of Ranvier and oligodendroglia) (Halliday and Stevens 2011). According to recent report, only protoplasmic astrocytes show increased α-synuclein accumulation, but not fibrous astrocytes (Braak et al. 2006; Halliday and Stevens 2011).

Astrocytes in healthy adult brain play important role in development and maintenance of blood–brain barrier, promoting neurovascular coupling, attracting different cells by releasing chemokines, ionic buffering, release of gliotransmitters and glutamate by calcium signaling, maintaining general metabolism, controlling brain pH, production of antioxidants, regulation of dopamine metabolism and other substrates by monoamine oxidases and uptake of glutamate and γ-aminobutyric acid (GABA) by respective transporters (Chinta and Andersen 2008; Hamby and Sofroniew 2010; Parpura et al. 2011; Volterra and Meldolesi 2005).

During any type of brain damage (neurodegenerative disease or oxidative stress), the above-mentioned normal astrocytic function is temporarily or permanently impaired, which may lead to a pathological condition and prolonged neuroinflammation in neurodegenerative diseases (Hamby and Sofroniew 2010; Kimelberg and Nedergaard 2010). In case of any insult neurons are more susceptible to injury than astrocytes, as they have limited antioxidant capacity. Neurons are metabolically coupled with astrocytes and fully depend on them to survive any type of oxidative stress (Hamby and Sofroniew 2010). But as the inflammation proceeds for a prolonged period in case of neurodegenerative diseases like PD, astrocytes become nonfunctional or die resulting in chronic neurodegeneration (Greve and Zink 2009).

It is widely reported that astrocytes have both protective and degenerative function in brain depending on the condition of the microenvironment and factors released in response to it. Such a condition (reactive astrogliosis) may be seen in several neurodegenerative diseases including PD. The triggers behind astrogliosis may be manifold, such as, infection, trauma (Barreto et al. 2009), α-synuclein accumulation (Gu et al. 2010), ischemia (Adelson et al. 2012; Wu et al. 2003), and any type of neurotoxic insults (Barreto et al. 2007). Astrogliosis brings about both morphological and molecular changes in brain resident astrocytes. Activated astrocytes are marked by heightened expression of GFAP, vimentin, increased uptake of glutamate (excitotoxic). It provides protection from oxidative stress by producing GSH, and provides neuroprotection by releasing adenosine, facilitating blood–brain barrier, degrading amyloid-beta peptides, increasing gap junctions between astrocytes. By release of inflammatory cytokines (TNF-α, Il-1β, Il-6, etc.),

and ROS generation (Gu et al. 2010; Hamby and Sofroniew 2010; Kang and Hebert 2011; Ridet et al. 1997), reactive astrocytes contribute to neurodegeneration.

The chronic neurodegeneration in PD patients is an outcome of reactive astrogliosis and microgliosis in substantia nigra of Parkinson patients (Hirsch et al. 2003). The gliosis in experimental models (both in vitro and in vivo) can be induced by environmental and biological toxins like lipopolysaccharides (LPS), pesticides like rotenone or MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). It is accompanied by mitochondrial dysfunction, neuronal death, and nuclear fragmentation (Herrera et al. 2000; Langston et al. 1999; Samantaray et al. 2007). Chronic gliosis increases iNOS production and causes oxidative stress in brain resulting in neurodegeneration resembling that in Alzheimer's disease (AD) and PD (Hirsch et al. 2003; Sugaya et al. 1998). Astrocyte released cytokines may induce increased activation of caspase 3, caspase 8, and cytochrome c in dopaminergic neurons by binding to their respective receptors, such as TNFR1 and 2, resulting in their premature apoptosis (Hirsch et al. 2003). Also, excessive uptake of neuronal α-synuclein aggregates by astrocytes, leads to its accumulation and causes increased expression of TNF-α, IL-1α, IL-1β and IL-6 (Rappold and Tieu 2010). The above-mentioned results suggest that the inhibition of astrogliosis and microgliosis may be a promising therapeutic tool to arrest neurodegeneration during PD (Hirsch et al. 2003).

So on a whole, astrocytes secrete pro-inflammatory, anti-inflammatory, neurotrophic, and pro- survival factors affecting health of neurons depending on different triggers, and may have important role in modulating microglial activity. Most importantly, GFAP-expressing astrocytes have ability to contribute to cell genesis, both as stem cells and as important cellular elements of the neurogenic microenvironment, indicating its role in neuronal repair and self-recovery. So astrocytes may be a potent therapeutic target in healing neurodegeneration (Fig. 7.4).

7.2.3 Oligodendrocyte

Another important member of the neuroglia family, oligodendrocyte, is widely studied for its role in myelination of neurons. But its status in neurodegeneration did not receive much attention. Consequently, the knowledge about their functions in neurodegenerative processes is relatively limited and fragmentary. It is known that oligodendrocytes are the end product of a cell lineage which undergo a complex process of proliferation, migration, differentiation, and myelination to finally produce the insulating sheath of axons. Roles of oligodendrocytes in other protein aggregation related neurodegenerative diseases, such as tauopathies and synucleinopathy, have also been reported recently. Recently, it is documented that clathrin-mediated α-synuclein aggregation is seen in oligodendrocytes in vivo (Kisos et al. 2012). In recent times disturbance in myelin synthesis and their putative roles in the initiation and progression of Alzheimer's disease AD and Huntington's disease (HD) has been topic of great interest among scientists (Desai et al. 2010; Mitew et al. 2010; Valenza and Cattaneo 2011; Valenza et al. 2010).

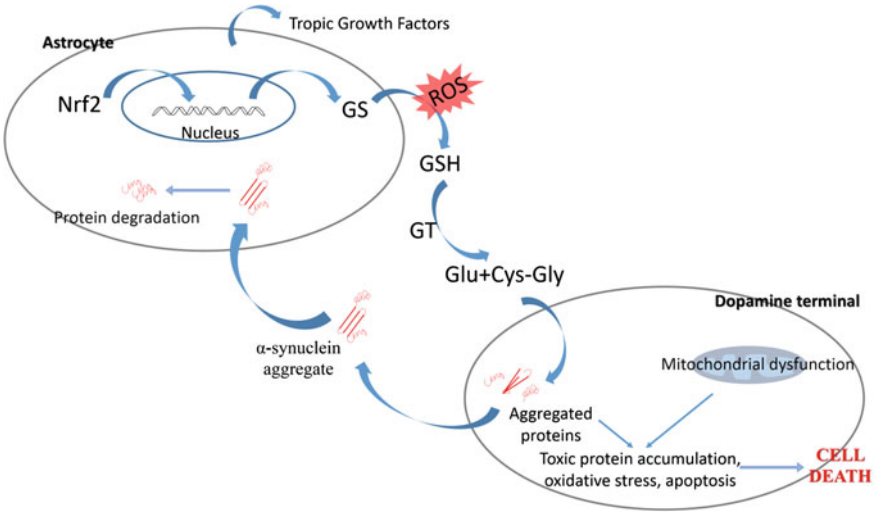

Fig. 7.4 This figure describes astrocyte mediated neuroprotection to nigral dopaminergic neurons. Nigral dopaminergic neurotoxicity by mitochondrial dysfunction and insufficient degradation of misfolded proteins (alpha-synuclein) can be a result of genetic mutations or environmental toxic insults or both. In response to these stimuli, astrocytes may attempt to protect the damaged neurons by release of trophic growth factors, release of glutathione (GSH) cleaved by γ-glutamyltranspeptidase on astrocytic plasma membrane to generate glutamate and cysteinyl-glycine which is precursors for neuronal GSH synthesis; by activating transcription factor Nrf2 which promotes transcription of protective genes antioxidant response element (ARE), including γ-glutamylcysteinesynthetase (GS) resulting in GSH synthesis; also by removal and degradation of cytotoxic molecules such as α-synuclein

Oligodendrocytes are among the most vulnerable CNS cells because of their complex differentiation program, and unique metabolism and physiology. Therefore, the primary onset of PD where the dopaminergic neurons are degenerated by ROS must be started with the degeneration of oligodendrocytes and demyelination. Exact contribution of oligodendrocyte in PD pathology is still in debate.

7.3 Peripheral T-Cell Infiltration Playing Pivotal Role in Neuroinflammation in PD

The T-cells play a major role in pathophysiology of Parkinson's disease. Among them δT^{+} cells have been identified to express CD25 majorly especially in Parkinsonian patients rather than healthy individuals (Fiszer et al. 1994). This is relevant with another study conducted with PD patients and normal human beings where CD4+: CD8+ ratio and CD4+: CD25+ ratio depleted, but IFN-γ producing T-cells increased in number than IL-4 producing T-cells in PD patients compared to the normal individuals (Baba et al. 2005). The upregulation of adhesion molecules by proinflammatory cytokines lead to the recruitment of passing T-cells and

monocytes, which express the counter receptors, and continue releasing more cytokines. TLRs stimulation upregulates MHC class II molecule that causes activation of quiescent microglia and costimulatory molecules that present myelin antigens to CD4+ T-cells (Olson and Miller 2004). It has been observed that T-cell proliferation was more robust when mice were immunized with alpha-synuclein slightly modified with the nitrotyrosine in the C-terminal tail fragment but not the native protein. The proinflammatory secretory responses are specific only for the modified antigen and not for the unmodified protein. One interesting fact is that mice which lacks T and B lymphocytes became resistant to the MPTP-induced Parkinson's disease. So this robust neuroinflammatory response accelerated dopaminergic cell loss in the mice immunized with the modified α-synuclein. However, this modified α-synuclein may have another interesting role of recruiting of peripheral leukocytes to the cervical lymph nodes in MPTP-induced mouse model of Parkinsonism (Benner et al. 2008). PET study of the mid brain has already unveiled the dysfunction in the Blood–Brain Barrier (BBB) of PD patient (Kortekaas et al. 2005). In case of PD patient and MPTP-induced animal model of Parkinsonism it has been observed that CD4+ and CD8+ T-cells from systemic circulation invaded the blood–brain barrier to enter into the substantia nigra indicating the BBB dysfunction in that particular disease condition (Brochard et al. 2009) giving rise to the possibility of a modulatory role of that infiltrated peripheral T-cells over the inflammatory response in the CNS lacking the molecular trigger mediating this response. So, the dysfunctional, leaky BBB is a matter of concern in the context of genetic as well as toxin-induced PD as it may recruit peripheral T-cells easily but it is not sufficient enough for disease etiology. Nevertheless, the collective data from recent in vivo studies suggested the protective effect of CD4+/CD25+ regulatory T-cells (Tregs) by inhibiting reactive microgliosis and inducing microglia apoptosis over CD4+/CD25− effector T-cells that promote microglia activation with other neurotoxic activities in response to nitrated α-synuclein (Reynolds et al. 2009). So these reports suggests that the microglia effector functions in the context of PD pathogenesis can be modulated by adaptive immune system. Hence peripheral T-cell infiltration along with the other protective molecules in the CNS during PD pathogenesis would probably make it a tremendous treatment strategy to stop progressive neurodegeneration (Fig. 7.5).

7.4 Immunomodulatory Molecules Affecting Inflammation in PD

Parkison's disease is associated with neuroinflammation and microglia provides a first line defense system by protecting the brain parenchymal cell in the neuroinflamation pathway though the activated microglia and active astrocytes are associated with the production of ROS/RNS, chemokines, cytokines leading the brain towards neurodegeneration (More et al. 2013). It is a well-known fact that different regions of brain are responsible for different types cognitive works (summative effect of learning memory, recognition, perception, etc.). So any

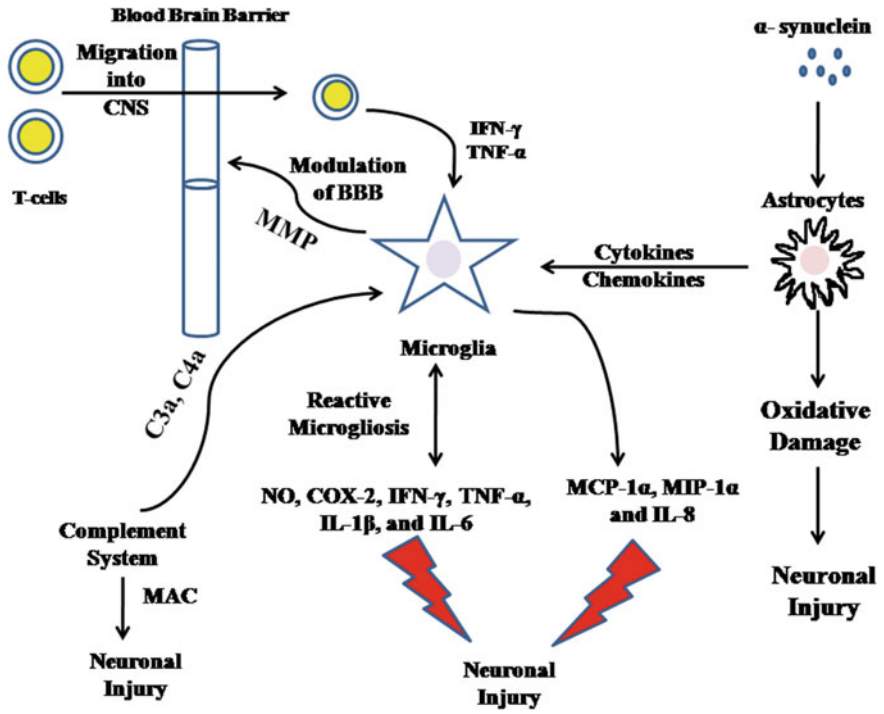

Fig. 7.5 Different cell types involved in PD pathogenesis. Peripheral T-cells that infiltrate in CNS by disrupting BBB contribute to microglial activation and subsequent release of proinflammatory factors resulting in neuronal damage and death. Reactive astrocytes produce huge amount of ROS which worsens the scenario and augments neuronal loss. Adding to it is the activation of complement system which targets the injured neurons by MAC and kills those

neurodegenerative disease that hampers the summative effect means the disease affecting different regions of brain. We have found that dopaminergic neuronal status changed differentially and neuroinflammation took place with or without participation of activated microglia during paraquat treatment in three different regions of brain. Now these differential changes indicate separate signaling phenomenon and different time frames for initiation of neurodegeneration might involve in the substantia nigra, frontal cortex and hippocampus of mouse brain. The exact cause of such changes and their correlation is yet to reveal (Mitra et al. 2011). MPTP-induced neurotoxicity in association with neuroinflammation is linked to microglial activation (Gao et al. 2002; McGeer et al. 2003). Under the circumstances of neuroinflammation microglial release of proinflammatory cytokines to act on the endothelium cells of BBB to stimulate upregulation of VCAM-1 and ICAM-1, which leads to the recruitment of passing T-cells and monocytes expressing the counter receptors, including CD11a/CD18 (LFA-1) and very late antigen-4 (Neumann et al. 2009), which in turn release more cytokines. In a recent study the proinflammatory cytokines Il-1β, TNF-α, IL-8, RANTES, MCP-1,

MIP-1α were found to be higher in PD patients. Elevated levels of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin (IL-1β and IL-6) in the cerebrospinal fluid (CSF), strital and dopaminergic regions of patients brain suffered with PD have also been demonstrated (Blum-Degen et al. 1995; Mogi et al. 1994; Muller et al. 1998). Activated glial cells might exert detrimental effect by releasing proinflammatory cytokines, such as TNF-α, IL-1β, and IFN-γ as well as iNOS which in turn activate the other glial cells in the substantia nigra in PD has been reported (Hirsch et al. 2003; Hisahara and Shimohama 2010). Furthermore, elevated levels of several interleukins, EGF, TGF-β, etc., along with the apoptotic proteins and low levels of neurotrophins have been found in the striatum of post-mortem PD brain and ventricular and cerebrospinal fluid (Nagatsu 2002; Nagatsu and Sawada 2006). LPS is widely used known toxin to induce Parkinson's like syndrome in cells as well as in rodent. LPS also induces microglial activation to give rise to IL-1β levels and dopeminergic neuronal loss consequently in mice whereas administration of anti-IL-1β neutralizing antibody could reverse the condition (Arai et al. 2004, 2006). The released cytokines from the dying dopaminergic neurons and microglia seem to amplify and sustain the neuroinflammation leading to persistent nigral dopaminergic neurons destruction (Orr et al. 2002). Recent study in our lab has demonstrated the high increment in the IL-1β expression in the hippocampus and the frontal cortex region of the male mouse brain after exposure to paraquat but lack of expression or, disperses immunoreactivity of IL-1β was observed in the substantia nigra (Mitra et al. 2011). ICAM-1 which is important for the persistence of neuroinflammation has been found to be overexpressed in activated astrocytes in the substantia nigra of PD patients as its counter receptor LFA-1 (CD11a/CD18) in the microglia found in the tissue matrix of the substantia nigra. In these patients, ICAM-1 expression is high particularly in the residual neuronal area where extensive cell loss has occurred. This ICAM-1 and LFA-1 interactions sustain inflammation in PD patients as well as in MPTP-treated monkeys (Miklossy et al. 2006). So in short molecules like TNF-α, IL-1, IL-6, and NO are toxic to neurons (Allan and Rothwell 2001; Fisher et al. 2001; Gayle et al. 2002; Liu and Hong 2003; Ma and Ma 2002; Sriram et al. 2002). Gene expression of Toll-like receptor 2, IFN-γ, COX-2, IL-6, and IL-6 receptor has been reported to be increased in the CNS of LPS-induced rodent model of PD (Laflamme et al. 2001; Vallieres and Rivest 1997) which may be significant in the context of that particular toxin-induced PD. Furthermore, COX-2 (Feng et al. 2002), nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Wu et al. 2002) and both TNF-α receptors (Sriram et al. 2002) genes inactivation inhibit the synthesis of proinflammatory molecules to protect DA neurons against MPTP-induced neurotoxicity, implicating important role that inflammation plays during MPTP mediated as well as in other types of nigrostriatal neurodegeneration. Additionally, COX-2 produces Prostaglandin E2 (PGE-2) from arachidonic acid induce dopaminergic neurotoxicity directly (Gao et al. 2003b). Increase in the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in the amoeboid microglia in nigral region of PD patient has been observed but not in the control subjects (Knott et al. 2000). The upregulation of iNOS is also generally found in

experimental PD models (Iravani et al. 2002; Liberatore et al. 1999), however inhibition of it stalled or inhibit 75 % of LPS-induced toxicity or LPS and IFN-γ activated microglial detrimental effect on dopaminergic neurons in vitro (Hemmer et al. 2001; Le et al. 2001). So now it is quite clear from the evidence that inflammatory cytokines, such as TNF-α, IL-1, IL-6 and the signaling molecule NO are toxic to neurons (Sriram et al. 2002). Depino et al. (Depino et al. 2003) reported that subacute 6-OHDA dose administration is linked with the atypical cytokine response in the nigral region. Upregulation of the gene expression of IL-1α and -1β has been found after 1 month of 6-OHDA injection lacking IL-1α or -β protein induction. However, a bacterial toxin can induce not only both mRNA at similar levels of these cytokines but at similar protein level also, although TNF-α mRNA was barely detectable in the nigral region. They concluded that death of the neurons itself does not induce proinflammatory cytokines secretion but requires an additional stimulus. However, Nagatsu et al. (2000) showed an elevation in the levels of plenty of proinflamatory cytokines, including TNF-α, IL-1β, and IL-6 along with the decreased neurotrophin levels of such as brain-derived neurotrophic factor (BDNF) in the CSF of PD patients and the nigral region of 6-OHDA treated rats. MHC class 1 and II antigens and iNOS were increased in the striatum and substantia nigra of MPTP-treated mice (Kurkowska-Jastrzebska et al. 1999) as well as increases in proinflammatory cytokines such as IL-1β and IL-6 (Nagatsu and Sawada 2006). Administration of MPTP also modifies the expression of numerous genes including IL-1, IL-6, IL-10, and TNF-α linked with the inflammation (Mandel et al. 2003). Although MPTP and its active metabolite MPP^+ have not reported yet to activate microglia (Gao et al. 2003a), but their toxicity in mice is decreased significantly with very low production of inflammation inducer such as superoxide (Wu et al. 2002), prostaglandins (Feng et al. 2002; Teismann et al. 2003a, b), and TNF-α (Sriram et al. 2002). TNF-α, IL-1β, and IFN-γ potentially activates the iNOS in the glial cells of rodent (Hunot et al. 1996) but failed to do so in humans (Ding et al. 1997; Peterson et al. 1994). The anti-inflammatory steroidal drug dexamethasone inhibits microglial reaction by decreasing the proinflammatory cytokines and NO production, hence reducing the MPTP-induced degeneration of DA neurons (Kurkowska-Jastrzebska et al. 1999). It is well known that NF-κB pathway is linked with the inflammation so any modulation in that pathway could lead to the alterations in the inflammation. So the above-stated (Kurkowska-Jastrzebska et al. 1999) fact could make NF-κB as potential therapeutic target in the context of PD. Not only this drugs but antibiotics such as a tetracycline derivative minocycline has been shown to decrease the inflammatory cytokines production like IL-1β as well as to inhbit iNOS and NADPH oxidase in comparison the control animals not treated with MPTP (Winklhofer and Haass 2010).

Collectively, the use of potent anti-inflammatory drugs depicts inhibition of inflammation during the course of PD. However, it has been found in a study with LPS activated microglia that IL-13 an inflammatory cytokines induces death to the microglia by upregulating the COX-2 expression and PGE-2 formation, whether the other inflammatory like cytokines TGF-β, IL-10 failed to do so (Yamada et al. 1992) although sodium salicylate, the nonselective COX-2 inhibitor, shown to be

protective to neurons in either MPTP-induced (Liu et al. 2002) or the 6-OHDA rat model of Parkinsonism (Sanchez-Pernaute et al. 2004). This would be due to the different microenvironment in in vivo condition compare to the in vitro condition. However, the neuroprotective effect of COX-2 inhibitors against MPTP in vivo might be due to the inhibition of COX-induced DA oxidation (Sanchez-Pernaute et al. 2004; Teismann et al. 2003a, b) rather than microglial activation. The NFκB promotes apoptosis by activating gene transcription of Bax and p53 and increasing the TGFβ1 and cyclopentenone prostaglandins expression (Lawrence et al. 2001). Important role is also played by Chemokine and its receptor in PD. The protective role of chemokine receptor CX3CR1has been established in the animal models of amyotrophic lateral sclerosis and PD. Microglial cells are attracted towards the chemokine ligands of CX3CR1 and supported the neurons at risk but lack of this receptor resulted in severe neurodegeneration (Cardona et al. 2006). But the mechanisms of action of CX3CR1-positive microglia assisting the entangled neurons have yet to be determined, although assumptions are made about the release of neuroprotective and trophic factors exert protection to the neurons (Neumann and Wekerle 1998). Fractalkine (CX3CL1) is a chemokine that is highly conservative and expressed in the surface of neurons and neuron and suppress the effect microglia activation. It was evident in the 6-OHDA induced rat model of Parkinsonism and also in in vitro study that CX3CL1 induction causes decrease in the lesion volume and protect the neurons in striatum via inhibiting the Fas ligand mediated apoptosis (Boehme et al. 2000; Pabon et al. 2011).

7.5 Complement System Involvement in Aggravating Neurodegeneration in PD

The complement system, one of the most primitive segments of innate immune system, enhances the potency of both the nonspecific and specific immunologic response. On activation of complement cascade, an array of molecules are produced which directly or indirectly contribute to reduce the insult. Among them, anaphylatoxins promote further inflammation, active counterparts of complement proteins (e.g., C3b) opsonizes target components and direct them for phagocytosis and ultimate destruction by producing membrane attack complex (MAC) (Bonifati and Kishore 2007; McGeer and McGeer 2002). Interestingly, complement proteins and components of MAC have been localized intracellularly on Lewy bodies and on oligodendroglia in substantia nigra of both sporadic (Xiong et al. 2011) and familial PD patients. Complement proteins have been reported to promote inflammatory cytokines synthesis from glial cells. Additionally, MAC has been identified in PD brains (Bonifati and Kishore 2007; McGeer and McGeer 2002) along with enhanced levels of C-reactive protein and inflammation markers (McGeer and McGeer 2005). On a whole, role of complement system in inflammation-mediated neurodegeneration and PD seems to be quite evident (Bonifati and Kishore 2007; McGeer and McGeer 2005). As the inflammation progresses in later stages, it becomes uncontrolled and chronic. Several reports indicate that therapeutic

targeting and inhibition of inflammatory response may prove to be successful in reducing the dopaminergic neurodegeneration in different models of PD (Gao et al. 2003b).

7.6 Contribution of Oxidative Stress in PD related Neuroinflammation

Parkison's disease is a common progressive neurodegenerative disease and is associated with the nigrostriatal dopaminergic neuronal loss. Oxidative stress, mitochondrial dysfunction could contribute to the progression of the disease (Hisahara and Shimohama 2010). The most affected region in Parkinson's disease of the CNS is substantia nigra and the excessive ROS production and deposition of lewy bodies (α-synucleiopathy), mitochondrial dysfunction leading to apoptosis are the main cause of nigral cell abolition (Kones 2010). Microglial activation by α-synuclein and activation of NADPH oxidase along with ROS production plays a vital role in the disease progression in case of PD (Yang et al. 2006). Lipopolysaccharide (LPS) is a common bacterial endotoxin that has been used as a neurotoxin to produce the deleterious effects like PD. Activation of PHOX, the gene responsible for NADPH oxidase contributes most of the LPS mediated intracellular ROS production that leads to the activation of microglia and proinflammatory mediators like TNF-α (Qin et al. 2004) or, Prostaglandin E2 (PGE2) (Block and Hong 2005). So NADPH oxidase could be a potent target in the pharmaceutical aspects in α-synuclein mediated neurotoxicity during PD. Although LPS increased the proinflammatory molecules, iNOS, and MAP kinase expression the inhibition of phosphorylation of NF-kB can be achieved by NADPH oxidase inhibitors and catalase (Pawate et al. 2004). Gao et al. (2003a, b, c) have shown that lack functional NADPH oxidase decreased DA toxicity in neurons and glia of mice when treated with MPTP and MPP^+ suggesting the contribution of extracellular superoxide in MPTP and its metabolite mediated toxicity (Gao et al. 2003a). Not only that, the very involvement of NADPH oxidase in the course of neurotoxicity by microglia either generating extracellular ROS or the enzyme may increase ROS production within the microglia which activates the generation of mediators of inflammation that subsequently exert neuronal toxicity. T-cells influence the microglia to produce ROS (Wu et al. 2002), proinflammatory prostaglandins and cytokines, causing progressive neurodegeneration consequently (Arai et al. 2004, 2006; Lucas et al. 2006). Several studies have shown that mitochondria are the arsenal of reactive oxygen species (ROS). Overproduction of ROS and defective ROS removal by mitochondrial defense systems in mitochondria cause severe oxidative damage to mitochondrial DNA, proteins and lipids and affect mainly the electron transport chain. Lots of evidence suggested that ROS production at some extent is not all bad but have physiological roles as signaling molecules in different cellular signaling. Moreover, an adequate production of ROS may be protective toward stress. The complex I inhibition in different toxin-induced models of PD is not well understood due to the lack of proper cause. In studies it is assumed that

mutations in the complex I in the mitochondrial or nuclear genome contributed to the abnormality in complex I function. The toxin rotenone which is also a Parkinson-like syndrome inducer inhibits complex I and ROS formation by binding to proximity to the quinone-binding site. Introduction of pyruvate or glutamate plus malate that helps forward in electron transport chain induces rotenone to block proton pumping and increase superoxide generation. However, rotenone can block superoxide formation in presence of succinate when electron transport is reverse. The proton-motive force (ΔpH and Δψ) component across the mitochondrial inner membrane highly implicated in superoxide production at high alkaline pH or membrane potential leads to consecutive formation of ROS. In absence of histone proteins and defected DNA repair mechanism mitochondrial DNA (mt-DNA) are more vulnerable to mutations (Barrientos and Moraes 1999; Zhang et al. 2005; Zhou et al. 2008b). ROS affects the mt-DNA mainly due to the closeness of the respiratory chain. The deficiency of cytochrome c oxidase (COX, complex IV) in neurons of which three catalytic subunits are mainly encoded by the mt-DNA make the other mt-DNA's susceptible for deletion. The observations suggested that a critical doorway of the extent of mt-DNA deletions exist above which respiratory chain deficiency occur. The occurrence of mt-DNA deletions is slightly higher in dopaminergic neurons in PD patients compared to the age matched controls. The highly specific deletion event of Mt-DNA has seen to be missing in hippocampal neurons and pyramidal neurons of the cerebral cortex or cerebellar Purkinje cells in aging individuals except the eventual deletions in the nigral region in both the age group (Bender et al. 2006; Zhang et al. 2005; Zhou et al. 2008b). The mechanism of action of ROS and RNS contributing to the pathogenesis of PD could be extracted from the fact of modification of the molecular factors by oxidative stress important to cellular function and survival. Some of the symptoms addressing the oxidative stress have been observed in PD like the decrease in the concentration of PUFAs in the SN, production of ROS, increase in the malondialdehyde concentration confirming lipid peroxidation, the elevated levels of free and bound nitrotyrosine a molecule of the RNS (reactive nitrogen species) entity, etc. These have been shown to be elevated in damaged areas during the course of MPTP in mouse model. DNA is not also out of the list of 'oxidative stress prey' as deoxyguanosine is converted to 8 hydroxydeoxyguanosine (8-OHdG) by oxidative agents in PD and 8-OHdG has been found to be markedly increased like other markers of in postmortem samples of substantianigra. But most of the abnormalities of ROS and RNS in PD stated above might be nonspecific proof and features of the cells going to die as the actual cause of neurodegeneration in PD remains elusive. Thereare neurons and other cells where the necessary, disciplined balance is maintained in our body between the accumulation and removal of ROS and RNS occur to keep them at very low non toxic levels. Superoxide dismutase (SOD), a ROS-scavenging enzyme, is assumed to be the potential defense system against an elevating ROS level. The enzyme has two isoform- SOD1 (cytosolic) and SOD2 (mitochondrial). Cytosolic isofom, i.e., SOD1 has found to be unchanged whereas its mitochondrial isoform, i.e., SOD2 has found to be activated despite of its inducible feature by excess ROS in PD brains indicating the mitochondrial compartment as the site of high ROS

production. This highly studied result are in contrast with the small degree of changes of catalase and glutathione peroxidase low activities in PD brains which might not be so significant in comparison to SOD (Zhang et al. 2005). Interestingly, synergistic of MPTP and LPS to mediate nigral dopaminergic neurotoxicity has also been revealed influencing the superoxide free radical entity (Gao et al. 2003c). L-DOPA like dopamine which is used as a drug in case of aged people having PD can be readily auto-oxidized and can give rise to ROS production and worsen the condition. In normal condition, small amounts of molecular oxygen that is consumed majorly by the mitochondrial electron transport chain have been converted to ROS as superoxide radicals despite of converting into water. It is due to the SOD2 and other antioxidant enzymes that keep the basal levels of ROS by products of mitochondrial respiratory chain are minimal inside the mitochondria (Marttila et al. 1988; Poirier et al. 1994; Saggu et al. 1989; Zhang et al. 2005). Mitochondrial respiratory defect, however, has been reported to exist in PD to give rise to a vis-à-vis regulation of high amount of ROS production with the defective electron transport chain, wiping out the neuroprotection. Glial cells are mainly protective and perform their normal function in brain, but upon activation of astrocytes and microglia can produce cytotoxic molecules including RNS and ROS in the CNS. In this occasion, the participation of microglia is greater than that of the astrocytes. The gene expression of inflammatory enzyme NADPH oxidase is increased and the enzyme remains activated in PD postmortem tissues and in the degenerating brain areas of MPTP-induced mice model of PD. Inactivation of the catalytic subunit of this enzyme complex causes dopaminergic neurotoxicity to be stalled in mice. This ROS and RNS, produced by activated glial cells; damage proteins can harm the neighboring dopaminergic neurons also. Another ROS species hydrogen peroxide can kill cells by direct toxicity; modulates a transcriptional factors such as NFκB and its signaling pathway and protein kinases, such as c-Jun N-terminal kinases (JNKs). In PD during the course of inflammation, a very destructive agent of ROS, i.e., H_2O_2 is produced from dopamine (DA) by the remaining dopaminergic neurons by oxidative deamination in the striatum. Incubation of rat striatal synaptosomes with levodopa causes increment in oxidized glutathione (GSSG) level in short-term exposure, but repetitive treatment did not alter the GSSG level despite a marked increase in DA turnover in case of Parkinson's like syndrome in rat (Loeffler et al. 2006). So the study concluded that the rat striatum defended the oxidative insult that is produced by DA turnover in in vivo condition than in synaptosomes in the course of neurodegeneration in PD. Malondialdehyde (MDA) the product of lipid peroxidation and ROS level increased, SOD level was decreased in the hippocampus of the mouse brain after treatment of paraquat. It also causes the increment of 8-OHG level in the neuronal mt-DNA, which implies mt-DNA defects (Chen and Swanson 2003). It was shown by Kutker et al. that repeated and systematic administration of low dose of paraquat may result in the oxidative damage and that leads to slow disease progression in the brain without affecting the peripheral tissue (Kuter et al. 2010). Prx2 is an antioxidant protein that reduces hydrogen peroxide to water. Its activity loss leads to greater intracellular concentration of ROS and eventual loss of dopaminergic neurons (Zhou et al. 2008a).

We reported in our paper that PQ-mediates neurotoxicity via ROS generation with the differential pattern α-synuclein aggregation in Substantia Nigra, Frontal Cortex and hippocampus of mouse brain (Mitra et al. 2011). Thus, neuronal death could arise from malfunctioning of many of these pathways mentioned above rather than the toxic effects exerted by the ROS or RNS directly. Despite extensive research on oxidative stress in several neurodegenerative diseases and its effect on their pathology, its exact role in cross-talk of different apoptotic pathways (intrinsic and extrinsic) is far from clear till date (Sinha et al. 2013).

7.7 Probable Therapeutic Interventions in PD

Several strategies have been found in the previous literature which may reflect counter balancing effect against PD. NADPH oxidase may be a potent target as stated earlier (Yang et al. 2006). It is a vital enzyme whose activation could produce oxidative damage maximally in the CNS. We stated earlier that the goal could be achieved to survive the neuron by inhibition of its catalytic subunit gp91PHOX (Zhou et al. 2008a). Not only that we have also described above that how its inhibition could cause the stoppage of proinflammatory cytokine release as well as NF-κB-mediated cell death (Pawate et al. 2004). Other therapeutic strategies are there to protect the CNS during PD. T-cell infiltration might be a good approach. It was stated above that the peripheral T-cells can protrude the BBB during PD (Tansey and Goldberg 2010). Use of anti-inflammatory drugs which can activate the anti-inflammatory cytokine IL-13 which could cause death to aged microglia by upregulating the COX-2 expression and PGE-2 formation (Yamada et al. 1992). Overexpression of human wild type α-synuclein and mutant A-53T synuclein which show a similar type of aggregation like α-synuclein in PD brain, but could protect the neuron by increasing the HSP-70 expression (Manning-Bog et al. 2003) which might refold the misfolded α-synuclein. So α-synuclein overexpression could be a potent therapeutic target. N-acetylcysteine (NAC) which is generally used as an inhibitor of ROS by many researchers. It was observed that NAC cause less oedema and cellular infiltration in paraquat affected rat. So NAC might have a role in the context of therapeutic intervention in toxin-induced Parkinsonian model (Wegener et al. 1988). Another agent ethyl pyruvate was assumed to be a potent agent in attenuating the oxidant and inflammatory response in rat after pre or, post treatment of paraquat (Lee et al. 2008). So these are a few probable therapeutic approaches that could prevent neurodegeneration and lead to repair and survival of the neurons during disease progression (Fig. 7.6).

7.8 Conclusion and Future Direction

Many symptoms and probable cause of PD have been documented till date but any early diagnostic marker of the disease onset has not yet been identified. So the therapeutic interventions suggested above lacks the approach which could protect the neuron at the early stage or, inhibit the neurodegeneration at the onset of the

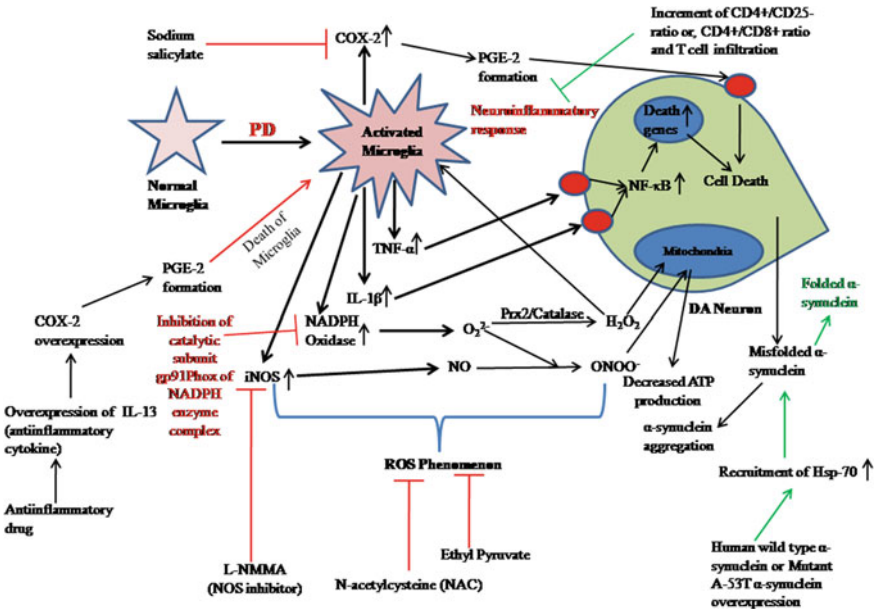

Fig. 7.6 Several therapeutic approaches can be undertaken to inhibit the disease progression in case of toxin-induced PD (a) Increment of CD4+/CD25− or, CD4+/CD8+ T-cell ratio and the infiltration of these peripheral T-cell, (b) Sodium salicylate introduction to inhibit the COX-2 released from microglia in the PD brain, (c) Introduction of anti-inflammatory drug that could lead to the production of anti-inflammatory cytokine which in turn lead to the overexpression of COX-2 and and PGE-2 formation and ultimately aged microglial death, (d) inhibition of the catalytic subunit of NADPH oxidase complex gp91phox, (e) inhibition of inducible nitric oxide synthase by $L\text{-}N^G$-monomethy arginine (LMMA) or, some other drugs, (f) inhibition of whole ROS formation by drug like ethyl pyruvate or, N-acetyl cysteine (NAC), (g) Overexpressing human wild type or, mutant A-53T α-synuclein and recruiting the Hsp-70, the molecular chaperone, to fold the misfolded α-synuclein

disease. So the future direction of PD research should be directed towards finding a potent early diagnostic marker of the disease, so that the disease progression can be arrested before it is too late.

References

Adelson JD, Barreto GE, Xu L et al (2012) Neuroprotection from stroke in the absence of MHCI or PirB. Neuron 73:1100–1107

Allan SM, Rothwell NJ (2001) Cytokines and acute neurodegeneration. Nat Rev Neurosci 2: 734–744

Arai H, Furuya T, Yasuda T, Miura M, Mizuno Y, Mochizuki H (2004) Neurotoxic effects of lipopolysaccharide on nigral dopaminergic neurons are mediated by microglial activation, interleukin-1beta, and expression of caspase-11 in mice. J Biol Chem 279:51647–51653

Arai H, Furuya T, Mizuno Y, Mochizuki H (2006) Inflammation and infection in Parkinson's disease. Histol Histopathol 21:673–678

Baba Y, Kuroiwa A, Uitti RJ, Wszolek ZK, Yamada T (2005) Alterations of T-lymphocyte populations in Parkinson disease. Parkinsonism Relat Disord 11:493–498

Banati RB (2003) Neuropathological imaging: in vivo detection of glial activation as a measure of disease and adaptive change in the brain. Br Med Bull 65:121–131

Barreto G, Veiga S, Azcoitia I, Garcia-Segura LM, Garcia-Ovejero D (2007) Testosterone decreases reactive astroglia and reactive microglia after brain injury in male rats: role of its metabolites, oestradiol and dihydrotestosterone. Eur J Neurosci 25:3039–3046

Barreto G, Santos-Galindo M, Diz-Chaves Y et al (2009) Selective estrogen receptor modulators decrease reactive astrogliosis in the injured brain: effects of aging and prolonged depletion of ovarian hormones. Endocrinology 150:5010–5015

Barrientos A, Moraes CT (1999) Titrating the effects of mitochondrial complex I impairment in the cell physiology. J Biol Chem 274:16188–16197

Bender A, Krishnan KJ, Morris CM et al (2006) High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. Nat Genet 38:515–517

Benner EJ, Banerjee R, Reynolds AD et al (2008) Nitrated alpha-synuclein immunity accelerates degeneration of nigral dopaminergic neurons. PLoS One 3:e1376

Block ML, Hong JS (2005) Microglia and inflammation-mediated neurodegeneration: multiple triggers with a common mechanism. Prog Neurobiol 76:77–98

Blum-Degen D, Muller T, Kuhn W, Gerlach M, Przuntek H, Riederer P (1995) Interleukin-1 beta and interleukin-6 are elevated in the cerebrospinal fluid of Alzheimer's and de novo Parkinson's disease patients. Neurosci Lett 202:17–20

Boehme SA, Lio FM, Maciejewski-Lenoir D, Bacon KB, Conlon PJ (2000) The chemokine fractalkine inhibits Fas-mediated cell death of brain microglia. J Immunol 165:397–403

Bonifati DM, Kishore U (2007) Role of complement in neurodegeneration and neuroinflammation. Mol Immunol 44:999–1010

Braak H, de Vos RA, Bohl J, Del Tredici K (2006) Gastric alpha-synuclein immunoreactive inclusions in Meissner's and Auerbach's plexuses in cases staged for Parkinson's disease-related brain pathology. Neurosci Lett 396:67–72

Brochard V, Combadiere B, Prigent A et al (2009) Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. J Clin Invest 119:182–192

Cardona AE, Pioro EP, Sasse ME et al (2006) Control of microglial neurotoxicity by the fractalkine receptor. Nat Neurosci 9:917–924

Chen Y, Swanson RA (2003) Astrocytes and brain injury. J Cereb Blood Flow Metab 23:137–149

Chen Q, Niu Y, Zhang R et al (2010) The toxic influence of paraquat on hippocampus of mice: involvement of oxidative stress. Neurotoxicology 31:310–316

Chinta SJ, Andersen JK (2008) Redox imbalance in Parkinson's disease. Biochim Biophys Acta 1780:1362–1367

Depino AM, Earl C, Kaczmarczyk E et al (2003) Microglial activation with atypical proinflammatory cytokine expression in a rat model of Parkinson's disease. Eur J Neurosci 18:2731–2742

Desai MK, Mastrangelo MA, Ryan DA, Sudol KL, Narrow WC, Bowers WJ (2010) Early oligodendrocyte/myelin pathology in Alzheimer's disease mice constitutes a novel therapeutic target. Am J Pathol 177:1422–1435

Ding M, St Pierre BA, Parkinson JF et al (1997) Inducible nitric-oxide synthase and nitric oxide production in human fetal astrocytes and microglia. A kinetic analysis. J Biol Chem 272:11327–11335

Feng ZH, Wang TG, Li DD et al (2002) Cyclooxygenase-2-deficient mice are resistant to 1-methyl-4-phenyl1, 2, 3, 6-tetrahydropyridine-induced damage of dopaminergic neurons in the substantia nigra. Neurosci Lett 329:354–358

Fisher J, Mizrahi T, Schori H et al (2001) Increased post-traumatic survival of neurons in IL-6-knockout mice on a background of EAE susceptibility. J Neuroimmunol 119:1–9

Fiszer U, Mix E, Fredrikson S, Kostulas V, Link H (1994) Parkinson's disease and immunological abnormalities: increase of HLA-DR expression on monocytes in cerebrospinal fluid and of CD45RO + T cells in peripheral blood. Acta Neurol Scand 90:160–166

Gao HM, Jiang J, Wilson B, Zhang W, Hong JS, Liu B (2002) Microglial activation-mediated delayed and progressive degeneration of rat nigral dopaminergic neurons: relevance to Parkinson's disease. J Neurochem 81:1285–1297

Gao HM, Liu B, Zhang W, Hong JS (2003a) Critical role of microglial NADPH oxidase-derived free radicals in the in vitro MPTP model of Parkinson's disease. Faseb j 17:1954–1956

Gao HM, Liu B, Zhang W, Hong JS (2003b) Novel anti-inflammatory therapy for Parkinson's disease. Trends Pharmacol Sci 24:395–401

Gao HM, Liu B, Zhang W, Hong JS (2003c) Synergistic dopaminergic neurotoxicity of MPTP and inflammogen lipopolysaccharide: relevance to the etiology of Parkinson's disease. Faseb j 17:1957–1959

Gayle DA, Ling Z, Tong C, Landers T, Lipton JW, Carvey PM (2002) Lipopolysaccharide (LPS)-induced dopamine cell loss in culture: roles of tumor necrosis factor-alpha, interleukin-1beta, and nitric oxide. Brain Res Dev Brain Res 133:27–35

Gordon S, Taylor PR (2005) Monocyte and macrophage heterogeneity. Nat Rev Immunol 5: 953–964

Greve MW, Zink BJ (2009) Pathophysiology of traumatic brain injury. Mt Sinai J Med 76:97–104

Gu XL, Long CX, Sun L, Xie C, Lin X, Cai H (2010) Astrocytic expression of Parkinson's disease-related A53T alpha-synuclein causes neurodegeneration in mice. Mol Brain 3:12

Halliday GM, Stevens CH (2011) Glia: initiators and progressors of pathology in Parkinson's disease. Mov Disord 26:6–17

Hamby ME, Sofroniew MV (2010) Reactive astrocytes as therapeutic targets for CNS disorders. Neurotherapeutics 7:494–506

Hemmer K, Fransen L, Vanderstichele H, Vanmechelen E, Heuschling P (2001) An in vitro model for the study of microglia-induced neurodegeneration: involvement of nitric oxide and tumor necrosis factor-alpha. Neurochem Int 38:557–565

Herrera AJ, Castano A, Venero JL, Cano J, Machado A (2000) The single intranigral injection of LPS as a new model for studying the selective effects of inflammatory reactions on dopaminergic system. Neurobiol Dis 7:429–447

Hirsch EC, Breidert T, Rousselet E, Hunot S, Hartmann A, Michel PP (2003) The role of glial reaction and inflammation in Parkinson's disease. Ann N Y Acad Sci 991:214–228

Hisahara S, Shimohama S (2010) Toxin-induced and genetic animal models of Parkinson's disease. Parkinsons Dis 2011:951709

Hunot S, Boissiere F, Faucheux B et al (1996) Nitric oxide synthase and neuronal vulnerability in Parkinson's disease. Neuroscience 72:355–363

Iravani MM, Kashefi K, Mander P, Rose S, Jenner P (2002) Involvement of inducible nitric oxide synthase in inflammation-induced dopaminergic neurodegeneration. Neuroscience 110:49–58

Kang W, Hebert JM (2011) Signaling pathways in reactive astrocytes, a genetic perspective. Mol Neurobiol 43:147–154

Kim YS, Joh TH (2006) Microglia, major player in the brain inflammation: their roles in the pathogenesis of Parkinson's disease. Exp Mol Med 38:333–347

Kim WG, Mohney RP, Wilson B, Jeohn GH, Liu B, Hong JS (2000) Regional difference in susceptibility to lipopolysaccharide-induced neurotoxicity in the rat brain: role of microglia. J Neurosci 20:6309–6316

Kimelberg HK, Nedergaard M (2010) Functions of astrocytes and their potential as therapeutic targets. Neurotherapeutics 7:338–353

Kisos H, Pukass K, Ben-Hur T, Richter-Landsberg C, Sharon R (2012) Increased neuronal alpha-synuclein pathology associates with its accumulation in oligodendrocytes in mice modeling alpha-synucleinopathies. PLoS One 7:e46817

Knott C, Stern G, Wilkin GP (2000) Inflammatory regulators in Parkinson's disease: iNOS, lipocortin-1, and cyclooxygenases-1 and -2. Mol Cell Neurosci 16:724–739

Kones R (2010) Parkinson's disease: mitochondrial molecular pathology, inflammation, statins, and therapeutic neuroprotective nutrition. Nutr Clin Pract 25:371–389
Koppula S, Kumar H, Kim IS, Choi DK (2012) Reactive oxygen species and inhibitors of inflammatory enzymes, NADPH oxidase, and iNOS in experimental models of Parkinson's disease. Mediators Inflamm 2012:823902
Kortekaas R, Leenders KL, van Oostrom JC et al (2005) Blood-brain barrier dysfunction in parkinsonian midbrain in vivo. Ann Neurol 57:176–179
Kurkowska-Jastrzebska I, Wronska A, Kohutnicka M, Czlonkowski A, Czlonkowska A (1999) The inflammatory reaction following 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine intoxication in mouse. Exp Neurol 156:50–61
Kuter K, Nowak P, Golembiowska K, Ossowska K (2010) Increased reactive oxygen species production in the brain after repeated low-dose pesticide paraquat exposure in rats. A comparison with peripheral tissues. Neurochem Res 35:1121–1130
Laflamme N, Soucy G, Rivest S (2001) Circulating cell wall components derived from gram-negative, not gram-positive, bacteria cause a profound induction of the gene-encoding Toll-like receptor 2 in the CNS. J Neurochem 79:648–657
Langston JW, Forno LS, Tetrud J, Reeves AG, Kaplan JA, Karluk D (1999) Evidence of active nerve cell degeneration in the substantia nigra of humans years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine exposure. Ann Neurol 46:598–605
Lawrence T, Gilroy DW, Colville-Nash PR, Willoughby DA (2001) Possible new role for NF-kappaB in the resolution of inflammation. Nat Med 7:1291–1297
Le W, Rowe D, Xie W, Ortiz I, He Y, Appel SH (2001) Microglial activation and dopaminergic cell injury: an in vitro model relevant to Parkinson's disease. J Neurosci 21:8447–8455
Lee J, Kwon W, Jo Y, Suh G, Youn Y (2008) Protective effects of ethyl pyruvate treatment on paraquat-intoxicated rats. Hum Exp Toxicol 27:49–54
Liberatore GT, Jackson-Lewis V, Vukosavic S et al (1999) Inducible nitric oxide synthase stimulates dopaminergic neurodegeneration in the MPTP model of Parkinson disease. Nat Med 5:1403–1409
Liu B, Hong JS (2003) Role of microglia in inflammation-mediated neurodegenerative diseases: mechanisms and strategies for therapeutic intervention. J Pharmacol Exp Ther 304:1–7
Liu B, Gao HM, Wang JY, Jeohn GH, Cooper CL, Hong JS (2002) Role of nitric oxide in inflammation-mediated neurodegeneration. Ann N Y Acad Sci 962:318–331
Loeffler DA, Camp DM, Conant SB (2006) Complement activation in the Parkinson's disease substantia nigra: an immunocytochemical study. J Neuroinflammation 3:29
Lucas SM, Rothwell NJ, Gibson RM (2006) The role of inflammation in CNS injury and disease. Br J Pharmacol 147(Suppl 1):S232–S240
Ma JY, Ma JK (2002) The dual effect of the particulate and organic components of diesel exhaust particles on the alteration of pulmonary immune/inflammatory responses and metabolic enzymes. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 20:117–147
Mandel S, Weinreb O, Youdim MB (2003) Using cDNA microarray to assess Parkinson's disease models and the effects of neuroprotective drugs. Trends Pharmacol Sci 24:184–191
Manning-Bog AB, McCormack AL, Purisai MG, Bolin LM, Di Monte DA (2003) Alpha-synuclein overexpression protects against paraquat-induced neurodegeneration. J Neurosci 23:3095–3099
Maragakis NJ, Rothstein JD (2006) Mechanisms of Disease: astrocytes in neurodegenerative disease. Nat Clin Pract Neurol 2:679–689
Marttila RJ, Lorentz H, Rinne UK (1988) Oxygen toxicity protecting enzymes in Parkinson's disease. Increase of superoxide dismutase-like activity in the substantia nigra and basal nucleus. J Neurol Sci 86:321–331
McGeer PL, McGeer EG (2002) Innate immunity, local inflammation, and degenerative disease. Sci Aging Knowl Environ 2002:re3
McGeer PL, McGeer EG (2004) Inflammation and neurodegeneration in Parkinson's disease. Parkinsonism Relat Disord 10(Suppl 1):S3–S7

McGeer EG, McGeer PL (2005) Pharmacologic approaches to the treatment of amyotrophic lateral sclerosis. BioDrugs 19:31–37
McGeer PL, McGeer EG (2008) Glial reactions in Parkinson's disease. Mov Disord 23:474–483
McGeer PL, Schwab C, Parent A, Doudet D (2003) Presence of reactive microglia in monkey substantia nigra years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine administration. Ann Neurol 54:599–604
Miklossy J, Doudet DD, Schwab C, Yu S, McGeer EG, McGeer PL (2006) Role of ICAM-1 in persisting inflammation in Parkinson disease and MPTP monkeys. Exp Neurol 197:275–283
Mitew S, Kirkcaldie MT, Halliday GM, Shepherd CE, Vickers JC, Dickson TC (2010) Focal demyelination in Alzheimer's disease and transgenic mouse models. Acta Neuropathol 119:567–577
Mitra S, Chakrabarti N, Bhattacharyya A (2011) Differential regional expression patterns of alpha-synuclein, TNF-alpha, and IL-1beta; and variable status of dopaminergic neurotoxicity in mouse brain after Paraquat treatment. J Neuroinflammation 8:163
Mogi M, Harada M, Kondo T et al (1994) Interleukin-1 beta, interleukin-6, epidermal growth factor and transforming growth factor-alpha are elevated in the brain from parkinsonian patients. Neurosci Lett 180:147–150
Moore DJ, West AB, Dawson VL, Dawson TM (2005) Molecular pathophysiology of Parkinson's disease. Annu Rev Neurosci 28:57–87
More SV, Kumar H, Songs SY, Choi DK (2013) Cellular and molecular mediators of neuroinflammation in the pathogenesis of Parkinson's disease. Mediators Inflamm 2013:952375
Muller T, Blum-Degen D, Przuntek H, Kuhn W (1998) Interleukin-6 levels in cerebrospinal fluid inversely correlate to severity of Parkinson's disease. Acta Neurol Scand 98:142–144
Nagatsu T (2002) Parkinson's disease: changes in apoptosis-related factors suggesting possible gene therapy. J Neural Transm 109:731–745
Nagatsu T, Sawada M (2006) Cellular and molecular mechanisms of Parkinson's disease: neurotoxins, causative genes, and inflammatory cytokines. Cell Mol Neurobiol 26:781–802
Nagatsu T, Mogi M, Ichinose H, Togari A (2000) Cytokines in Parkinson's disease. J Neural Transm Suppl 143–51
Nakagawa Y, Chiba K (2014) Role of microglial m1/m2 polarization in relapse and remission of psychiatric disorders and diseases. Pharmaceuticals (Basel) 7:1028–1048
Neumann H, Wekerle H (1998) Neuronal control of the immune response in the central nervous system: linking brain immunity to neurodegeneration. J Neuropathol Exp Neurol 57:1–9
Neumann H, Kotter MR, Franklin RJ (2009) Debris clearance by microglia: an essential link between degeneration and regeneration. Brain 132:288–295
Nimmerjahn A, Kirchhoff F, Helmchen F (2005) Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308:1314–1318
Olson JK, Miller SD (2004) Microglia initiate central nervous system innate and adaptive immune responses through multiple TLRs. J Immunol 173:3916–3924
Orr CF, Rowe DB, Halliday GM (2002) An inflammatory review of Parkinson's disease. Prog Neurobiol 68:325–340
Pabon MM, Bachstetter AD, Hudson CE, Gemma C, Bickford PC (2011) CX3CL1 reduces neurotoxicity and microglial activation in a rat model of Parkinson's disease. J Neuroinflammation 8:9
Parpura V, Grubisic V, Verkhratsky A (2011) Ca(2+) sources for the exocytotic release of glutamate from astrocytes. Biochim Biophys Acta 1813:984–991
Pawate S, Shen Q, Fan F, Bhat NR (2004) Redox regulation of glial inflammatory response to lipopolysaccharide and interferongamma. J Neurosci Res 77:540–551
Perry VH (2012) Innate inflammation in Parkinson's disease. Cold Spring Harb Perspect Med 2: a009373
Peterson PK, Hu S, Anderson WR, Chao CC (1994) Nitric oxide production and neurotoxicity mediated by activated microglia from human versus mouse brain. J Infect Dis 170:457–460

Poirier J, Dea D, Baccichet A, Thiffault C (1994) Superoxide dismutase expression in Parkinson's disease. Ann N Y Acad Sci 738:116–120

Qian L, Flood PM, Hong JS (2010) Neuroinflammation is a key player in Parkinson's disease and a prime target for therapy. J Neural Transm 117:971–979

Qin L, Liu Y, Wang T et al (2004) NADPH oxidase mediates lipopolysaccharide-induced neurotoxicity and proinflammatory gene expression in activated microglia. J Biol Chem 279:1415–1421

Ransohoff RM, Perry VH (2009) Microglial physiology: unique stimuli, specialized responses. Annu Rev Immunol 27:119–145

Rappold PM, Tieu K (2010) Astrocytes and therapeutics for Parkinson's disease. Neurotherapeutics 7:413–423

Reynolds AD, Stone DK, Mosley RL, Gendelman HE (2009) Nitrated {alpha}-synuclein-induced alterations in microglial immunity are regulated by CD4+ T cell subsets. J Immunol 182:4137–4149

Rezai-Zadeh K, Gate D, Town T (2009) CNS infiltration of peripheral immune cells: D-Day for neurodegenerative disease? J Neuroimmune Pharmacol 4:462–475

Ridet JL, Malhotra SK, Privat A, Gage FH (1997) Reactive astrocytes: cellular and molecular cues to biological function. Trends Neurosci 20:570–577

Rock RB, Gekker G, Hu S et al (2004) Role of microglia in central nervous system infections. Clin Microbiol Rev 17:942–964 (table of contents)

Saggu H, Cooksey J, Dexter D et al (1989) A selective increase in particulate superoxide dismutase activity in parkinsonian substantia nigra. J Neurochem 53:692–697

Samantaray S, Knaryan VH, Guyton MK, Matzelle DD, Ray SK, Banik NL (2007) The parkinsonian neurotoxin rotenone activates calpain and caspase-3 leading to motoneuron degeneration in spinal cord of Lewis rats. Neuroscience 146:741–755

Sanchez-Pernaute R, Ferree A, Cooper O, Yu M, Brownell AL, Isacson O (2004) Selective COX-2 inhibition prevents progressive dopamine neuron degeneration in a rat model of Parkinson's disease. J Neuroinflammation 1:6

Sinha K, Das J, Pal PB, Sil PC (2013) Oxidative stress: the mitochondria-dependent and mitochondria-independent pathways of apoptosis. Arch Toxicol 87:1157–1180

Sriram K, Matheson JM, Benkovic SA, Miller DB, Luster MI, O'Callaghan JP (2002) Mice deficient in TNF receptors are protected against dopaminergic neurotoxicity: implications for Parkinson's disease. Faseb J 16:1474–1476

Stone DK, Reynolds AD, Mosley RL, Gendelman HE (2009) Innate and adaptive immunity for the pathobiology of Parkinson's disease. Antioxid Redox Signal 11:2151–2166

Sugama S, Yang L, Cho BP et al (2003) Age-related microglial activation in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced dopaminergic neurodegeneration in C57BL/6 mice. Brain Res 964:288–294

Sugaya K, Chou S, Xu SJ, McKinney M (1998) Indicators of glial activation and brain oxidative stress after intraventricular infusion of endotoxin. Brain Res Mol Brain Res 58:1–9

Tansey MG, Goldberg MS (2010) Neuroinflammation in Parkinson's disease: its role in neuronal death and implications for therapeutic intervention. Neurobiol Dis 37(3):510–518

Teismann P, Schulz JB (2004) Cellular pathology of Parkinson's disease: astrocytes, microglia and inflammation. Cell Tissue Res 318:149–161

Teismann P, Tieu K, Choi DK et al (2003a) Cyclooxygenase-2 is instrumental in Parkinson's disease neurodegeneration. Proc Natl Acad Sci USA 100:5473–5478

Teismann P, Tieu K, Cohen O et al (2003b) Pathogenic role of glial cells in Parkinson's disease. Mov Disord 18:121–129

Valenza M, Cattaneo E (2011) Emerging roles for cholesterol in Huntington's disease. Trends Neurosci 34:474–486

Valenza M, Leoni V, Karasinska JM et al (2010) Cholesterol defect is marked across multiple rodent models of Huntington's disease and is manifest in astrocytes. J Neurosci 30:10844–10850

Vallieres L, Rivest S (1997) Regulation of the genes encoding interleukin-6, its receptor, and gp130 in the rat brain in response to the immune activator lipopolysaccharide and the proinflammatory cytokine interleukin-1beta. J Neurochem 69:1668–1683

Volterra A, Meldolesi J (2005) Astrocytes, from brain glue to communication elements: the revolution continues. Nat Rev Neurosci 6:626–640

Voyvodic JT (1996) Cell death in cortical development: How much? Why? So what? Neuron 16:693–696

Walsh S, Finn DP, Dowd E (2011) Time-course of nigrostriatal neurodegeneration and neuroinflammation in the 6-hydroxydopamine-induced axonal and terminal lesion models of Parkinson's disease in the rat. Neuroscience 175:251–261

Wang T, Pei Z, Zhang W et al (2005) MPP+—induced COX-2 activation and subsequent dopaminergic neurodegeneration. Faseb j 19:1134–1136

Wegener T, Sandhagen B, Chan KW, Saldeen T (1988) N-acetylcysteine in paraquat toxicity: toxicological and histological evaluation in rats. Ups J Med Sci 93:81–89

Winklhofer KF, Haass C (2010) Mitochondrial dysfunction in Parkinson's disease. Biochim Biophys Acta 1802(1):29–44

Wu DC, Jackson-Lewis V, Vila M et al (2002) Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson disease. J Neurosci 22:1763–1771

Wu DC, Teismann P, Tieu K et al (2003) NADPH oxidase mediates oxidative stress in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of Parkinson's disease. Proc Natl Acad Sci USA 100:6145–6150

Xiong X, Barreto GE, Xu L, Ouyang YB, Xie X, Giffard RG (2011) Increased brain injury and worsened neurological outcome in interleukin-4 knockout mice after transient focal cerebral ischemia. Stroke 42:2026–2032

Yamada T, McGeer PL, McGeer EG (1992) Lewy bodies in Parkinson's disease are recognized by antibodies to complement proteins. Acta Neuropathol 84(1):100–104

Yang MS, Ji KA, Jeon SB et al (2006) Interleukin-13 enhances cyclooxygenase-2 expression in activated rat brain microglia: implications for death of activated microglia. J Immunol 177:1323–1329

Zhang S, Boyd J, Delaney K, Murphy TH (2005) Rapid reversible changes in dendritic spine structure in vivo gated by the degree of ischemia. J Neurosci 25:5333–5338

Zhou C, Huang Y, Przedborski S (2008a) Oxidative stress in Parkinson's disease: a mechanism of pathogenic and therapeutic significance. Ann N Y Acad Sci 1147:93–104

Zhou C, Rao Y, Rao Y (2008b) A subset of octopaminergic neurons are important for Drosophila aggression. Nat Neurosci 11:1059–1067

Molecular and Cellular Insights: Neuroinflammation and Amyotrophic Lateral Sclerosis

8

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Abstract

Accumulation of misfolded and abnormal proteins generates probably a common and complex pathomechanism in various neurodegenerative diseases (Alzheimer's, Parkinson's, Huntington's, Amyotrophic Lateral Sclerosis, and Prion) and in aging. In amyotrophic lateral sclerosis (ALS), neuroinflammation appears in the form of T-lymphocyte infiltration, presence of reactive astroglial and microglial cells. Most likely, end stage of this toxic cascade results in death of motor neurons in the cortex, brainstem, and spinal cord. More than 10 different genetic causes of familial ALS are known; but still it is a challenge to prevent the loss of descending motor tracts by suppressing the degeneration of motor neurons. This chapter will focus on the precise understanding of neuroinflammatory responses in molecular pathomechanism of ALS and it also discusses new potential therapeutic strategies to improve neuroprotection and to alleviate proteotoxicity in ALS linked motor neurodegeneration.

Abbreviations

ALS	Amyotrophic lateral sclerosis
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANG	Angiogenin
BBB	Blood brain barrier
Bcl-2	B-cell lymphoma 2
C9orf72	Chromosome 9 open reading frame 72
CHIP	Carboxy terminus of Hsp70-interacting protein

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CNS	Central nervous system
EAAT2	Excitatory amino acid transporter 2
fALS	Familial amyotrophic lateral sclerosis
FTD	Frontotemporal dementia
FUS	Fused in sarcoma
GDNF	Glial cell-derived neurotrophic factor
GFAP	Glial fibrillary acidic protein
Hsp70	Heat shock protein70
IFN-γ	Interferon-γ
IL	Interleukins
MRI	Magnetic resonance imaging
$NG2^+$	Neuron-glial antigen 2-positive
NMDA	*N*-methyl-D-aspartic acid
PET	Positron emission tomography
PNS	Peripheral nervous system
PGC-1α	Peroxisome proliferator-activated receptor gamma (PPAR-γ) coactivator-1α
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SETX	Senataxin
SOD-1	Superoxide dismutase 1
TDP-43	Transactive response DNA binding protein-43
TNF-α	Tumor necrosis factor-α
UPS	Ubiquitin proteasome system
VAPB	Vesicle-associated membrane protein-associated protein B

8.1 Introduction

Amyotrophic lateral sclerosis (ALS) or classical motor neuron disease is a fatal neurodegenerative disorder caused due to motor neurons degeneration. Death of motor neurons in ALS affects the transmission of motor signals and hence results in gradual loss of muscle movements. In 1860s, French neurologist Jean-Martin Charcot observed myelin pallor in the lateral portion of spinal cord, and is known to be the first who identified this disease (Charcot and Joffroy 1869). In the United States, this disease is familiarly known as Lou Gehrig's disease, named after the famous baseball player Lou Gehrig, who died because of this disease at an age of 38 years (Cleveland and Rothstein 2001). Prevalence rate for ALS in the United States is 4–6 per 100,000 with its manifestation at a median age of 55 years (Pasinelli and Brown 2006). Patients suffering with ALS have relatively shorter life span and within 3 years of symptoms onset, 50 % of patients do not survive (Deng et al. 2006). Every year,

approximately two new cases out of 100,000 people are reported in Europe (Logroscino et al. 2010).

Sporadic and familial (inherited) are the two basic forms of this disease, but there are few other variants also, such as Western Pacific ALS, Juvenile ALS, and Hiramayas disease (Foster et al. 2015). Sporadic ALS includes around 90 % incidence as compared to familial ALS (Wijesekera and Leigh 2009; Al-Chalabi et al. 2012; Boillee et al. 2006; Chen et al. 2013). Etiology of ALS is not well known; but studies show that more than 25 genetic mutations and 18 gene loci are associated with the pathology of ALS (Marangi and Traynor 2015). The major symptoms of disease are degeneration of neurons in brainstem, cortex, and spinal cord which results in muscle twitching, atrophy on both sides of the body and difficulty in the process of speaking, breathing which ultimately leads to complete paralysis (Abrahams et al. 2014). Several mechanisms have been proposed to be the causing factors of ALS, such as ubiquitin proteasome system (UPS) impairment, protein aggregation, disruption in protein quality control machinery, neuroinflammation, neurofilament accumulation, and glutamate excitotoxicity (Goodall and Morrison 2006).

Cellular protein quality control machinery removes partially and incorrectly folded proteins from the cells. To perform this function, UPS and chaperones, both systems work independently as well as synergistically (Chhangani and Mishra 2013; Chhangani et al. 2013). In patients of ALS, protein quality control machinery is severely damaged, which consequently causes accumulation of proteins such as superoxide dismutase 1 (SOD1), fused in sarcoma (FUS) and transactive response DNA binding protein-43 (TDP-43) as ubiquitinated inclusions in the affected brain regions (Kabashi and Durham 2006). Along with the death of motor neurons, ALS has found to be consociated with changes in the normal responses of astrocytes, microglia, oligodendrocytes, and natural killer cells (Boillee et al. 2006; Rothstein 2009). Proteotoxic insult generated inside central nervous system (CNS) activates microglia which further releases cytokines, reactive oxygen species (ROS), and reactive nitrogen species (RNS) responsible for inflammatory responses, whereas increased astrocyte activation results in the production of nerve growth factor causing degeneration of motor neurons (Henkel et al. 2009; Pehar et al. 2004; Nakamura et al. 1999).

Natural killer cells were found to raise the levels of cytokines such as interleukins IL-4, IL-10, Tumor necrosis factor alpha (TNF-α), and Interferon gamma (IFN-γ) (Finkelstein et al. 2011). $NG2^+$ oligodendrocyte progenitor cells (the glial precursor cells known for generating myelinated cells of CNS) proliferation is also enhanced in disease mouse model of ALS (Kang et al. 2013). All these responses along with certain other changes taking place simultaneously inside the CNS constitute a process, which was certainly under question for a long time, known as neuroinflammation (details in box 1). It is a cellular immune response to the changes occurring in CNS and its microenvironment. In ALS, it is associated with invasion of CNS through inflammatory molecules, phagocytic cells, and various proteins (Weydt and Möller 2005).

Box 1. Neuroinflammation and Brain Inflammation is a well-programmed spontaneous response of our body against any perturbation or unusual situation generated inside, due to external triggers. Research in past few years have significantly revised the historical idea of brain being immune-privileged (Aloisi 2001). Current advancements have clearly established the notion of well-structured immune system inside our central nervous system (CNS), which can respond to a variety of stresses such as injuries, infections, microhemorrhage, and a number of diseases (Tracey 2002; Nance and Sanders 2007). The term 'neuroinflammation' has drawn remarkable attention in recent past. It refers to a collective response of different brain cells against any kind of insult generated from environment or other neurotoxicants. Glial cells play primary role in executing all such immune responses. They release a number of proinflammatory cytokines viz. IL-1β and TNF-α either directly inside the CNS or sometimes indirectly released from their counterparts of peripheral nervous system (PNS) by crossing the blood brain barrier (BBB) (Lasiene and Yamanaka 2011). Inflammation is considered to be a safeguard tactic against a number of intrusions and infections, but following the phrase 'excess of everything is bad', chronic state of neuroinflammation has found to play a crucial role in progression of various neurodegenerative diseases. Activation of immune cells leads to release of a number of inflammatory factors, which causes breakdown of BBB, loss of neuroprotective mechanisms, increased risk of neuronal tissue damage and thus creating a positive feedback for invasion of other kinds of cells inside the CNS (Carson et al. 2006; Ransohoff et al. 2003).

The chief mediators of neuroinflammation in ALS are microglial cells, which are the main phagocytic cells of brain along with other accessory participants, such as astrocytes, oligodendrocytes, T-lymphocytes, some inflammatory proteins, and various other molecules (Endo and Yamanaka 2014). The sequence of neuroinflammatory events in ALS starts with the activation of microglial cells and their accumulation in brain and spinal cord affected regions (McGeer and McGeer 2002). Activation of microglial cells is associated with secretion of various inflammatory proteins such as chemokines, cytokines, prostaglandins, complement activation molecules, anaphylatoxins, integrins, and other acute phase proteins, which are further involved in the process of neuroinflammation (Streit et al. 2004). These biochemical molecules attract other important immune cells such as astrocytes, T-lymphocytes, oligodendrocytes and inflammatory molecules to the site of injury in ALS patients; producing stress, cellular damage, and finally leading to neuronal death (Rizzo et al. 2014). Activation of microglia, movement of inflammatory cells to site of injury and release of the other inflammatory molecules aggravate whole process of neuroinflammation in patients of ALS.

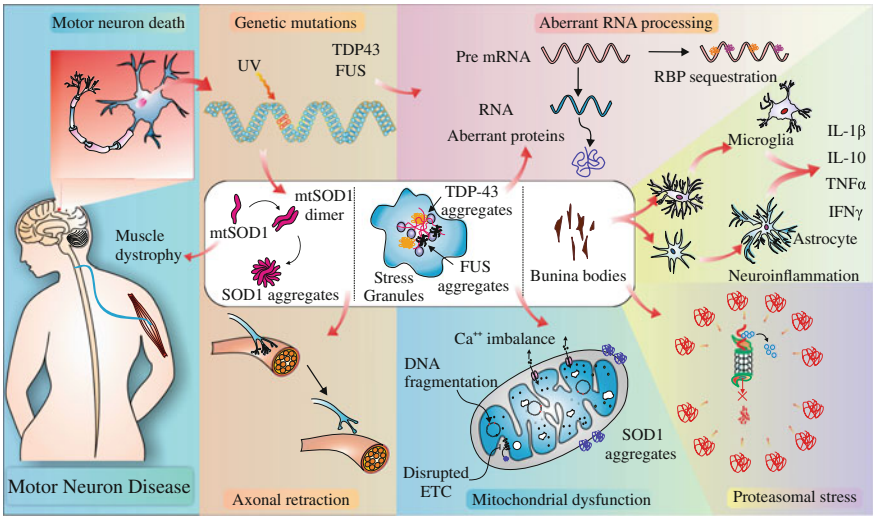

Fig. 8.1 Schematic representation of ALS molecular pathogenesis: the diagram here represents the causes and symptoms of ALS disease. Various genetic mutations and aberrant RNA processing may cause the onset of motor neuron disease, which leads to several pathological changes, taking place simultaneously. These changes include: neuroinflammation, proteasomal stress, mitochondrial stress, axonal retraction, and finally death of motor neurons. The central part of the diagram depicts various kinds of protein aggregates, which have been reported in ALS brain

8.2 Etiology

ALS is associated with the phenotypic heterogeneity and considered as a complex disease with various mechanisms causing the death of motor neurons as shown in Fig. 8.1. This diseases is mainly caused by genetic defects, but there are few other reasons also, including environmental factors such as exposure to heavy metals like mercury, arsenic, and viral infections (Soriani and Desnuelle 2009; Alfahad and Nath 2013). Sometimes, it is also considered as a prion disease because of prion like properties of ALS associated proteins (Lee and Kim 2015). There is a claim about the involvement of more than 25 genes in causing ALS by their genetic variations. Few profoundly studied genes and their mutations are *SOD1, TDP-43, Chromosome 9 open reading frame 72, Angiogenin, Amyotrophic lateral sclerosis2, Senataxin,* and *Vesicle-associated membrane protein-associated protein B* (Marangi and Traynor 2015). Defective glutamate metabolism, free radical injuries, mitochondrial dysfunction, programmed cell death, cytoskeleton protein defects, autoimmune dysfunction, and protein aggregations are few other causes of ALS disease (Rossi et al. 2013).

8.3 Various Cellular Pathways Involved in ALS

Till now, the proposed hypotheses for the pathogenesis of ALS include accumulation of intracellular aggregates, oxidative damage, mitochondrial dysfunction, glutamate excitotoxicity, growth factor deficiency, and defect in axonal transport. In oxidative damage hypothesis, the mutation in superoxide dismutase 1 (*SOD1*) leads to increase in the free radical and thus contributes to neuronal cell degeneration (Bunton-Stasyshyn et al. 2014). Intracellular aggregates of SOD1 mutants also ensure toxicity in motor neurons (Shaw and Valentine 2007). Pathological studies of ALS suggest an increase in the volume of mitochondria of muscle cells and elevation of calcium inside the mitochondria (Dupuis et al. 2004). There are many shreds of evidences for an involvement of apoptosis in ALS by balancing out the B-cell lymphoma 2 (Bcl-2) oncoproteins, in addition to the elevated levels of the caspases, the proteases involved in apoptosis (Iaccarino et al. 2011). Another hallmark of ALS is the aberrant accumulation of neurofilaments in the cell body and proximal axons, causing defect in axonal transport (Rao and Weiss 2004).

8.4 Abnormal Functions of Protein Quality Control Machinery in ALS

The abnormal functioning of UPS causes accumulation of SOD1 mutant protein in motor neurons and leads to their degeneration (Kabashi and Durham 2006). Impairment of UPS leads to accumulation of misfolded proteins inside the cells. These toxic proteinaceous inclusions are bona fide causative factor of a number of neurodegenerative disorders. ALS is also one such disease, which is induced due to aberrant accumulation of misfolded proteins (Blokhuis et al. 2013; Lee and Kim 2015; Scotter et al. 2015). In most of the cases, overexpression of mutant proteins causes an overload on UPS for removal of defective proteins from the neuronal cell, producing accumulation of mutant proteins, which leads to pathological conditions (Lehman 2009). In both, familial and sporadic ALS, alterations in the UPS machinery leads to formation of toxic proteinaceous aggregates (Cheroni et al. 2009; Bendotti et al. 2012; Urushitani et al. 2002). The first gene to be linked with ALS discovered in fALS patient was *SOD1*. The product of *SOD1* gene, i.e., SOD1 protein was shown to be present in the ubiquitinated inclusions of ALS patients brain sections (Kato et al. 2000). TDP-43 and FUS are two recently discovered proteins in the aggregated ubiquitinated inclusions of SOD1 negative ALS patients (Mackenzie et al. 2010).

Along with UPS (as explained in the previous section), chaperones are also severely affected in patients of ALS. It is being observed that the chaperoning capacity of chaperones viz. Heat shock protein 70 (Hsp70) associated with protein quality control is severely reduced in the patients of ALS (Shinder et al. 2001; Tummala et al. 2005). Evidences indicate that a direct interaction occurs between Hsc70 and aggregated proteins in mouse model of ALS, as compared with normal mouse (Watanabe et al. 2001). An increase in activity of these chaperones is found to reduce the toxicity of aggregated proteins in a cell culture model of ALS,

indicating the importance of chaperones regulating proteotoxicity in ALS (Bruening et al. 1999).

8.5 Neurofilament Protein Accumulation

Neurofilament proteins in motor neurons are expressed primarily in axons (Llorens 2013). Many changes such as dysregulated protein synthesis of neurofilamentary protein subunits, ineffective axonal transport, irregularity in phosphorylation/glycosylation and oxidation, can cause accumulation of neurofilamentary proteins in motor neurons (Wong et al. 2000; Boylan et al. 2009; Ludemann et al. 2005; Niebroj-Dobosz et al. 2004; Liem and Messing 2009; Dale and Garcia 2012). This affects various neuronal processes such as dendritic arborization, axonal transport, deficit in neuronal signal processing, and finally progressing toward neuronal loss and death (Smith et al. 2003; Stamer et al. 2002).

8.6 Glutamate Excitotoxicity

Another mechanism implicated in ALS pathogenesis is glutamate excitotoxicity (the process of excessive stimulation of glutamate receptors that permits the large amount of calcium influx). In neuronal synapses, glutamate is released from the presynaptic terminal and activates *N*-methyl-D-aspartic acid (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors of the postsynaptic neurons (Traynelis et al. 2010; Kakizawa et al. 2005). Activation of both receptors causes influx of Na^+ and Ca^{2+} ions in postsynaptic cell, further causes neuronal depolarization and generates action potential (Foran and Trotti 2009; Lau and Tymianski 2010). Glutamate excitotoxicity is induced by increased release and impaired uptake of glutamate ions by the postsynaptic neuron. In ALS, a higher concentration of glutamate causes overstimulation of glutamate receptors on postsynaptic neurons and leads to increased intracellular Ca^{2+} influx, thus producing a higher Ca^{2+} concentration in the neurons (Van Den Bosch et al. 2006). Increased amount of intracellular Ca^{2+} causes detrimental harm to motor neurons in ALS and results in neuronal death (Gleichmann and Mattson 2011; Leal et al. 2013).

8.7 Proteomics of ALS Disease

Researchers have shown great interest in recent years in revealing the underlying causes of the lethal motor neuron disease. Last one decade in the field has led to an increase in discovery of ALS-associated proteins. The most studied of these, SOD1 mutant protein causes an increase in free radicals which lend up in neuronal cell degeneration (Bunton-Stasyshyn et al. 2014). Another important and most recent of these proteins is TDP-43, which forms immunoreactive inclusions (also known as tau negative neuronal inclusions) in the cytoplasm of ALS patients neurons (Arai et al.

2006). TDP-43 inclusions are present in the cytoplasm of glial cells and neurons within the spinal cord and throughout brain. There are about 38 nonsynonymous mutations identified in both sporadic and familial ALS (Sreedharan et al. 2008). There is some strong mechanistic basis that suggests the prion like behavior of TDP-43 inclusions (Smethurst et al. 2014). Mutations of TDP-43 also cause frontotemporal dementia (FTD) with ALS (DeJesus-Hernandez et al. 2011).

Microtubule-associated tau protein is another protein found in neuronal inclusions in early stages of ALS (Yang et al. 2003). Peroxisome proliferator-activated receptor gamma (PPAR-γ) coactivator (PGC)-1α is a transcription coactivator that improves motor function and survival of SOD1-G93A mice by regulating mitochondrial biogenesis and oxidative metabolism (Zhao et al. 2011). There are few evidences of enhanced activity of S100 beta, a calcium binding protein in the spinal cord of ALS mice which further leads to neurodegeneration through calcium pathways (Shobha et al. 2010). Proteomics analysis of an ALS mouse model proposed changes in the levels of near about 50 proteins in spinal cord (Bergemalm et al. 2009). In another study, it was shown that mutated SOD1 causes accumulation of other proteins, such as inactivation of specific chaperones, including Hsp70, its co chaperones CHIP, Hsp40, Hsp105, and others (Jain et al. 2008). Recently, it was discovered that increased levels of galectin 3 (Gal3), a protein involved in many biological processes such as cell adhesion, cell cycle, apoptosis, etc., causes onset of ALS symptoms in mice (Zhou et al. 2010). Various other types of inclusion bodies are also found in glia, neuronal soma, and proximal dendrites in the brain of ALS patients. Table 8.1 summarizes the most studied types of such inclusion bodies, which are found in ALS patients.

Table 8.1 Various types of inclusion bodies in ALS

S. No.	Inclusion bodies or aggresomes like structures	Remark
1	Ubiquitylated inclusions	Proteins involved are ubiquitin, peripherin, Cu/Zn SOD1 and dorfin (Matsumoto et al. 1993)
2	TDP-43	Ubiquitin positive but tau negative inclusions (Arai et al. 2006)
3	Fused in sarcoma protein (FUS)	Inclusions present immunorectivity for TDP-43 and ubiquitin (Vance et al. 2009)
4	Bunina bodies	Immunoreactive for cystatin (Piao et al. 2003)
5	Hyaline conglomerate inclusions	Intermediate filament proteins especially peripherin and hyperphosphorylated neurofilament subunits (Troost et al. 1992)
6	Astrocytic hyaline inclusions	Formed by SOD1, ubiquitin, and cytoskeletal protein (Kato et al. 1997)
7	Axonal spheroids	Positive for phosphorylated neurofilament, ubiquitin, synaptophysin (Takahashi et al. 1997)
8	Basophilic inclusions	Consist mainly of thick filamentous structures associated with granules (Ito 2014)

8.8 Animal Models to Study ALS Disease

The transgenic animal models for diseases having aggregation of mutant proteins as a prominent phenotype provide better understanding of disease pathology. ALS and Huntington's disease are two such diseases. Animal models produced till now for ALS disease are Swine, Mouse, Zebrafish, *Caenorhabditis elegans*, and *Drosophila* (Islam et al. 2014). The animal models were developed majorly for mutations in three genes *SOD1*, *FUS*, and *TDP-43*. All five animal models are available for *SOD1* with different mutations as well as for *TDP-43* (Joyce et al. 2011). Transgenic rat models are also developed recently to study *SOD1* and *TDP-43* mutations for characteristic phenotype and pathophysiology of ALS (McGoldrick et al. 2013). FUS is a DNA/RNA binding protein that plays a significant role in RNA metabolism; animal models for FUS open a new path for studying the molecular mechanism of ALS (Lanson and Pandey 2012). Despite the availability of so many animal models, an intrinsic difference in genetics and anatomy causes the limitation in the application of animal models for ALS.

8.9 Immune Responses in ALS and Related Inflammatory Pathways

In 1919, Del Rio Hortega was the first who identified phagocytic, mesenchymal cells, microglia, in the brain. This view was under questions till the development of some advanced techniques. Now, molecular biology has given so many evidences of microglial cells as the very first line of defense inside the CNS. Microglia are the resident macrophages present throughout the CNS. ALS is a movement disorder, characterized by loss of upper and lower motor neurons in motor cortex, brain stem and spinal cord, followed by increased population of activated glial cells in affected areas of the brain (Baumer et al. 2014). Arborizations (branching) of cytoplasmic processes provide microglial cells an ability to patrol the nervous tissues inside the brain, whereas initiation of glial responses leads to enlargement of cell body with shrinkage of processes giving them amoeboid shape (Kettenmann et al. 2011; Moisse and Strong 2006; Henkel et al. 2009).

In recent years, scientists have shown clear relationship between upregulation of glial activation and progress of various neurodegenerative disorders, e.g., Alzheimer's disease, Parkinson's disease, Multiple sclerosis, and ALS, etc. (Mandrekar-Colucci and Landreth 2010; Dewil et al. 2007; Muzio et al. 2007; Rogers et al. 2007). ALS, in general, is characterized by accumulation of activated amoeboid microglia, reactive astrocytes, and marginating leucocytes within degenerating brain areas viz. spinal cord, brain stem, and motor cortex (McGeer and McGeer 2002; Turner et al. 2004). Diversion of microglia from their housekeeping functions like eliminating dysfunctional synapses generates a vicious feed forward cycle of inflammatory responses inside the CNS, which ultimately leads to accelerated progression of the diseased condition.

Astrocytes, ectodermal cells, are nonimmune cells, providing nutrition to nearby neurons and maintaining neurotransmitters concentrations in extracellular environment. But during gliosis, they play significant roles (Julien 2007; Farina et al. 2007). Activation of astrocytes can be seen by marked increase in expression of glial fibrillary acidic protein (GFAP), a marker of astrocytes. T-cell infiltration seems to add additional inflammatory reactions inside the brain tissues. Roles of oligodendrocytes and other brain cells are still to be understood properly. In various post-mortem studies, tissue sections from ALS brains have shown presence of microgliosis in motor nuclei of the brainstem, in the ventral horn of spinal cord as well as in the motor cortex of brain. Astrocytosis has also been reported in both, dorsal as well as ventral horns of the spinal cord (Kawamata et al. 1992; Schiffer et al. 1996). Studies on mice have shown the increased populations of other immune cells like CD4+ and CD8+ cytotoxic T cells at early stage of ALS (Chiu et al. 2008).

Microglia cells get activated by several factors, as happens in case of various other diseases. In case of ALS, accumulation of extracellular inclusions and subsequent degeneration of motor neurons acts as the trigger for their activation. Mutations of Cu^{2+}/Zn^{2+} superoxide dismutase 1 (SOD1) add deteriorating effects several folds (Rosen et al. 1993). Degenerating neurons release various trigger factors like ATP and extracellular SOD1, which activates microglial cells. As shown in Fig. 8.2, activated

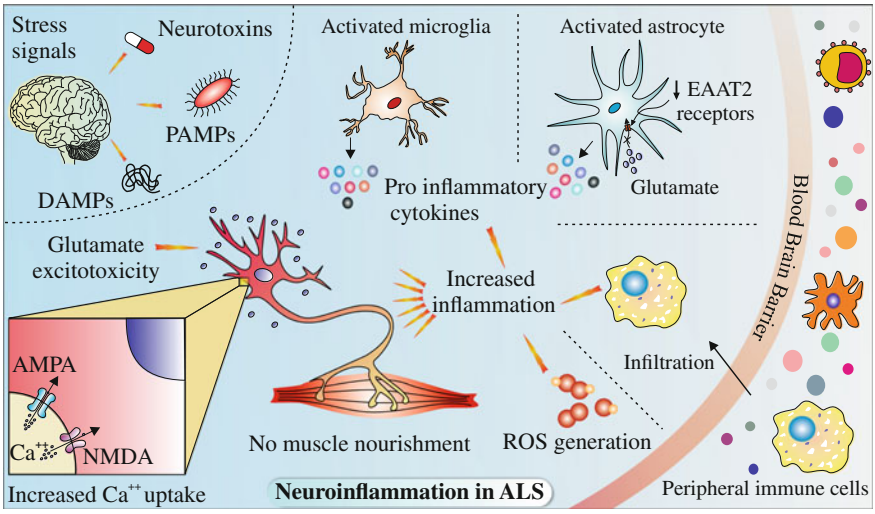

Fig. 8.2 Depiction of activated microenvironment of central nervous system in amyotrophic lateral sclerosis: external or internal stimuli including neurotoxins, pathogen-associated molecular patterns (PAMPs), and damage-associated molecular pattern molecules (DAMPs) trigger up the cells inside CNS to counter the changes and act accordingly. Microglia are the chief immune cells, which gets activated first, followed by astrocytes, releasing various kinds of proinflammatory cytokines. These cytokines and elevated oxidative stress accelerates inflammatory reactions causing damage in BBB, leading to infiltration of peripheral immune cells, e.g., macrophages, NK cells, and cytotoxic T-cells. Increased glutamate release further increases the toxicity by activating AMPA and NMDA receptors, causing excess calcium uptake, and thus death of motor neurons

microglia cells further release a variety of proinflammatory molecules, e.g., IL-1β, IL-12, interferon-γ, TNF-α; ROS species like superoxides and peroxides; other chemokines and mitogenic factors (Almer et al. 1999; Elliott 2001; Yoshihara et al. 2002; Hensley et al. 2003). All these factors, in turn generate a feed forward loop for sustained neuroinflammatory processes.

Activation of microenvironment inside the CNS weakens the blood brain barrier (BBB), allowing passage of several other factors and ingression of other peripheral immune cells, e.g., macrophages, NK cells, and cytotoxic T cells (Li et al. 2014). Adding to all these, death of brain cells leads to release of glutamate into the extracellular environment, which elevates its extracellular concentration from normal (0.6 μM) to more than 2 μM. This concentration of glutamate is sufficient to cause lethal damage to neuronal cells by causing excitotoxicity in the brain tissues (Benveniste et al. 1984; Meldrum and Garthwaite 1990). Housekeeping functions of astrocytes are also modulated in ALS patients and in mutant SOD1 mice. Neurotoxic insults lead to decreased transcription of glutamate receptors excitatory amino acid transporters 2 (EAAT2) or glutamate transporter (GLT1) in mice, causing decreased neuronal signaling (Howland et al. 2002; Rothstein et al. 1992). Elevated concentration of glutamate in synaptic clefts gives rise to activation of AMPA and NMDA receptors present on motor neurons. Increased entry of calcium leads to death of these cells (Yang et al. 2009; Lipton and Rosenberg 1994).

Despite evidences of an array of pathological features associated with motor neuron disease, the question of what actually switches the disease 'on' remains unanswered. Although neuroinflammatory processes provide a substantial contribution in progression and sustainability of various neurodegenerative diseases, still they are not considered to be the initiating factors of these diseases. All these evidences lead to a common conclusion that mechanism of selective deaths of motor neurons in ALS is not cell autonomous. Roles of neighboring nonneuronal cells significantly add deteriorating effects to the whole process of neuroinflammation. Release of various proinflammatory chemicals and aggregation of different kinds of protein inclusions also cause significant damage. These stresses lead to cell death due to loss of various intracellular organelles like mitochondria. Tremendous efforts have been made to treat or slow down the disease progression, but very little success has been observed in recent past. Riluzole (2-amino-6-(trifluoromethoxy) benzothiazole), the only available drug, is a proved inhibitor of glutamate release, has shown some neuroprotection in ALS patients (Bellingham 2011).

8.10 Therapeutical Aspects in ALS Treatment

Due to the complex nature and partial understanding of underlying molecular mechanisms, ALS has become an important challenge from diagnostic perspective. Currently, no single test is there that can detect ALS. Clinicians often rely on various tests such as electromyography, nerve conduction study, blood and urine test, magnetic resonance imaging (MRI), and spinocerebral fluid analysis for ruling

out possibilities for presence of other symptomatically similar diseases. Thus, from symptom onset to correct diagnosis, it takes ample amount of time (Chio 1999) causing significant delay in starting early stage treatment. Due to this delay many research studies and clinical trials targeted on mechanisms and treatment strategies at early stage ALS are also negatively affected, imposing limitation in developing new therapeutics. Looking for markers of disease could be an effective solution in reducing diagnosis time. Studies have shown association of TDP-43 (Neumann et al. 2006), SOD1 mutation (Rosen et al. 1993), FUS (Vance et al. 2009), C9orf72 hexanucleotide expansions (DeJesus-Hernandez et al. 2011), ratio of phosphorylated tau to normal tau in cerebrospinal fluid (Grossman et al. 2014) and light chain neurofilaments (Gaiottino et al. 2013) with ALS, but in-depth studies are needed from application point of view.

Advances in imaging techniques have also aided in finding new potential biomarkers for ALS. Techniques like voxel and surface-based morphometry; magnetic resonance spectroscopy, positron emission tomography (PET), and diffusion tensor imaging have shown promise in getting new insights of disease. Some important findings include thinning of primary motor cortex (Mezzapesa et al. 2013), decrease in ratio of N-acetylaspartate to creatinine in primary motor cortex (Abe et al. 2001) reduction in fractional anisotropy in corticospinal tract (Zhang et al. 2011) and loss of corpus callosum integrity (Chapman et al. 2014). Rapid diagnosis and finding new markers for disease will not only help clinically but will also help researchers in understanding the disease more clearly at various levels of progression.

Like many other neurodegenerative diseases, there is no curative therapy for ALS. Riluzole is the only drug, which slows down the progression of disease, but has side effects (Bellingham 2011). Various molecules are being tested, which targets the neuroinflammatory events of ALS; they include compounds such as minocycline, thalidomide, and lenalidomide (Zhu et al. 2002; Kiaei et al. 2006). But, except riluzole, no other medication is approved for treatment of ALS. Riluzole is a benzothiazole class drug with unknown mechanism of action. Studies have shown that riluzole slows down progression of disease and extends the survival of patients for several months (Bensimon et al. 1994; Lacomblez et al. 1996). Its beneficiary effect in ALS could probably be due to its pharmacological properties which include inhibition of glutamate release and inactivation of voltage dependent sodium channels (Doble 1996).

Due to limitations in treatment options for ALS, the focus of current medication restricts to symptom control and maintaining quality life of patients. For this purpose, advances in techniques such as noninvasive ventilation for ALS respiratory treatment complications and gain in understanding of nutritional aspect of this motor neuron disease have helped a lot. Various studies have proposed different methods and targets that could prove to be helpful in developing effective treatment strategy against this disease. These include: (i) adeno-associated virus-mediated delivery of single chain antibody D3H5 that binds specifically to misfolded SOD1 (Patel et al. 2014); (ii) antibody (GSK577548) to inhibit neurite outgrowth inhibitor (Nogo A) in SOD1 mutant mice (Bros-Facer et al. 2014); (iii) induction of tropic

factors (Tovar et al. 2014); (iv) reduction of oxidative stress and mitochondrial dysfunction (Carri et al. 2015); (v) autophagy upregulation (Bucchia et al. 2015); (vi) modulation of monocytes activity (Butovsky et al. 2012); (vii) targeting aberrant RNA metabolism (Droppelmann et al. 2014); (viii) intrathecal and intracerebroventrical drug delivery to overcome passage problems through BBB (Van Damme and Robberecht 2014) (ix) gene therapy (Federici and Boulis 2012) and last but not the least (x) stem cell therapy (Mazzini et al. 2015). Stem cell therapy is one of the major advancements that have shown its potential in ALS treatment, which will be further discussed in our next section.

8.11 Stem Cell Therapy and ALS

Stem cell therapy has emerged as a new treatment avenue for ALS. In past, studies have shown potential of stem cells in restoring functions in animals with motor neuron disease (Deshpande et al. 2006). The focus of the stem cell therapy basically remains either on replacement of degenerated neuronal cells or to support existing dying neuronal cells. Embryonic stem cells, mesenchymal stem cells, and progenitor cells have been studied in the past for their potential in ALS therapy. In past, successful survival and integration of glial cell derived neurotrophic factor (GDNF) releasing modified human neural progenitor cells have shown potential in case of glial replacement and trophic factor delivery (Klein et al. 2005).

Intravascular transplantation of c-kit (+) stem/progenitor cells in SOD1G93A mutant mice has shown prolonged disease duration and life span (Corti et al. 2010). Distribution, differentiation, and survival of neural stem cells with positive outcomes have also been concluded (Mitrecic et al. 2010). A recent study in SOD1G93A mice have shown delayed disease onset and extended life span on intrathecal transplantation of motor neurons derived from neural stem cells (Lee et al. 2014). Such studies have provided a ray of hope to use stem cells as a promising strategy in ALS therapy. However, various issues such as proper-defined protocol for administration and dose of cells, proper migration and integration of cells, survival of the transplanted cells, controlling the oncogenic transformation of those cells, immune reactivity, and rejections are still needed to be addressed for successful clinical translation.

8.12 Key Questions

Here, in this chapter, we represent a comprehensive overview of the pathomechanism of motor neurons death in ALS, discuss crucial points and their convergence towards multifactorial ALS pathogenesis. Till now, we are not able to develop a reliable test that can clearly distinguish ALS disease at early stages as compared to other neurodegenerative diseases. Previous reports indicate the accumulation of misfolded proteinaceous inclusions or disordered structures in ALS and other neurodegenerative pathology (Chhangani and Mishra 2013; Chhangani et al. 2013, 2015); but still

we do not know how to design biomarkers to target or detect specific stages of ALS disease progression. It is important to search beneficiary biological (Upadhyay et al. 2015a, b; Chhangani et al. 2014) or chemical agents and their effective mode of delivery by which clearance of abnormal protein aggregation in motor neurons can be possible. We know that multiple pathways and numerous causative factors are involved in death of motor neurons in ALS disease. Our current understanding of how perturbations in cellular quality control mechanism and neuroinflammation linked with ALS and other neurodegenerative diseases is still not well developed. Therefore, in near future, it is crucial to align or pool maximum available data or existing findings on a large scale to design an effective and potential therapeutic strategy against the proteotoxic insults in ALS treatment approaches.

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References

Abe K, Takanashi M, Watanabe Y, Tanaka H, Fujita N, Hirabuki N, Yanagihara T (2001) Decrease in N-acetylaspartate/creatine ratio in the motor area and the frontal lobe in amyotrophic lateral sclerosis. Neuroradiology 43(7):537–541

Abrahams S, Newton J, Niven E, Foley J, Bak TH (2014) Screening for cognition and behaviour changes in ALS. Amyotroph Lateral Scler Frontotemporal Degener 15(1–2):9–14. doi:10.3109/21678421.2013.805784

Al-Chalabi A, Jones A, Troakes C, King A, Al-Sarraj S, van den Berg LH (2012) The genetics and neuropathology of amyotrophic lateral sclerosis. Acta Neuropathol 124(3):339–352. doi:10.1007/s00401-012-1022-4

Alfahad T, Nath A (2013) Retroviruses and amyotrophic lateral sclerosis. Antiviral Res 99(2):180–187. doi:10.1016/j.antiviral.2013.05.006

Almer G, Vukosavic S, Romero N, Przedborski S (1999) Inducible nitric oxide synthase up-regulation in a transgenic mouse model of familial amyotrophic lateral sclerosis. J Neurochem 72(6):2415–2425

Aloisi F (2001) Immune function of microglia. Glia 36(2):165–179

Arai T, Hasegawa M, Akiyama H, Ikeda K, Nonaka T, Mori H, Mann D, Tsuchiya K, Yoshida M, Hashizume Y, Oda T (2006) TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Biochem Biophys Res Commun 351(3):602–611. doi:10.1016/j.bbrc.2006.10.093

Baumer D, Talbot K, Turner MR (2014) Advances in motor neurone disease. J R Soc Med 107(1):14–21. doi:10.1177/0141076813511451

Bellingham MC (2011) A review of the neural mechanisms of action and clinical efficiency of riluzole in treating amyotrophic lateral sclerosis: what have we learned in the last decade? CNS Neurosci Ther 17(1):4–31. doi:10.1111/j.1755-5949.2009.00116.x

Bendotti C, Marino M, Cheroni C, Fontana E, Crippa V, Poletti A, De Biasi S (2012) Dysfunction of constitutive and inducible ubiquitin-proteasome system in amyotrophic lateral sclerosis: implication for protein aggregation and immune response. Prog Neurobiol 97(2):101–126. doi:10.1016/j.pneurobio.2011.10.001

Bensimon G, Lacomblez L, Meininger V (1994) A controlled trial of riluzole in amyotrophic lateral sclerosis. ALS/Riluzole Study Group. N Engl J Med 330(9):585–591. doi:10.1056/NEJM199403033300901

Benveniste H, Drejer J, Schousboe A, Diemer NH (1984) Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. J Neurochem 43(5):1369–1374

Bergemalm D, Forsberg K, Jonsson PA, Graffmo KS, Brannstrom T, Andersen PM, Antti H, Marklund SL (2009) Changes in the spinal cord proteome of an amyotrophic lateral sclerosis murine model determined by differential in-gel electrophoresis. Mol Cell Proteomics 8 (6):1306–1317. doi:10.1074/mcp.M900046-MCP200

Blokhuis AM, Groen EJ, Koppers M, van den Berg LH, Pasterkamp RJ (2013) Protein aggregation in amyotrophic lateral sclerosis. Acta Neuropathol 125(6):777–794. doi:10.1007/s00401-013-1125-6

Boillee S, Vande Velde C, Cleveland DW (2006) ALS: a disease of motor neurons and their nonneuronal neighbors. Neuron 52(1):39–59. doi:10.1016/j.neuron.2006.09.018

Boylan K, Yang C, Crook J, Overstreet K, Heckman M, Wang Y, Borchelt D, Shaw G (2009) Immunoreactivity of the phosphorylated axonal neurofilament H subunit (pNF-H) in blood of ALS model rodents and ALS patients: evaluation of blood pNF-H as a potential ALS biomarker. J Neurochem 111(5):1182–1191. doi:10.1111/j.1471-4159.2009.06386.x

Bros-Facer V, Krull D, Taylor A, Dick JR, Bates SA, Cleveland MS, Prinjha RK, Greensmith L (2014) Treatment with an antibody directed against Nogo-A delays disease progression in the SOD1G93A mouse model of Amyotrophic lateral sclerosis. Hum Mol Genet 23(16):4187–4200. doi:10.1093/hmg/ddu136

Bruening W, Roy J, Giasson B, Figlewicz DA, Mushynski WE, Durham HD (1999) Up-regulation of protein chaperones preserves viability of cells expressing toxic Cu/Zn-superoxide dismutase mutants associated with amyotrophic lateral sclerosis. J Neurochem 72(2):693–699

Bucchia M, Ramirez A, Parente V, Simone C, Nizzardo M, Magri F, Dametti S, Corti S (2015) Therapeutic development in amyotrophic lateral sclerosis. Clin Ther 37(3):668–680. doi:10.1016/j.clinthera.2014.12.020

Bunton-Stasyshyn RK, Saccon RA, Fratta P, Fisher EM (2014) SOD1 function and its implications for amyotrophic lateral sclerosis pathology: new and renascent themes. Neuroscientist. doi:10.1177/1073858414561795

Butovsky O, Siddiqui S, Gabriely G, Lanser AJ, Dake B, Murugaiyan G, Doykan CE, Wu PM, Gali RR, Iyer LK, Lawson R, Berry J, Krichevsky AM, Cudkowicz ME, Weiner HL (2012) Modulating inflammatory monocytes with a unique microRNA gene signature ameliorates murine ALS. J Clin Invest 122(9):3063–3087. doi:10.1172/JCI62636

Carri MT, Valle C, Bozzo F, Cozzolino M (2015) Oxidative stress and mitochondrial damage: importance in non-SOD1 ALS. Front Cell Neurosci 9:41. doi:10.3389/fncel.2015.00041

Carson MJ, Doose JM, Melchior B, Schmid CD, Ploix CC (2006) CNS immune privilege: hiding in plain sight. Immunol Rev 213:48–65. doi:10.1111/j.1600-065X.2006.00441.x

Chapman MC, Jelsone-Swain L, Johnson TD, Gruis KL, Welsh RC (2014) Diffusion tensor MRI of the corpus callosum in amyotrophic lateral sclerosis. J Magn Reson Imaging 39(3):641–647. doi:10.1002/jmri.24218

Charcot J-M, Joffroy A (1869) Deux cas d'atrophie musculaire progressive avec lésions de la substance grise et des faisceaux antérolatéraux de la moelle épinière. Masson, Paris

Chen S, Sayana P, Zhang X, Le W (2013) Genetics of amyotrophic lateral sclerosis: an update. Mol Neurodegener 8:28. doi:10.1186/1750-1326-8-28

Cheroni C, Marino M, Tortarolo M, Veglianese P, De Biasi S, Fontana E, Zuccarello LV, Maynard CJ, Dantuma NP, Bendotti C (2009) Functional alterations of the ubiquitin-proteasome

system in motor neurons of a mouse model of familial amyotrophic lateral sclerosis. Hum Mol Genet 18(1):82–96. doi:10.1093/hmg/ddn319

Chhangani D, Mishra A (2013) Mahogunin ring finger-1 (MGRN1) suppresses chaperone-associated misfolded protein aggregation and toxicity. Sci Rep 3:1972. doi:10.1038/srep01972

Chhangani D, Jana NR, Mishra A (2013) Misfolded proteins recognition strategies of E3 ubiquitin ligases and neurodegenerative diseases. Mol Neurobiol 47(1):302–312. doi:10.1007/s12035-012-8351-0

Chhangani D, Upadhyay A, Amanullah A, Joshi V, Mishra A (2014) Ubiquitin ligase ITCH recruitment suppresses the aggregation and cellular toxicity of cytoplasmic misfolded proteins. Sci Rep 4:5077. doi:10.1038/srep05077

Chhangani D, Endo F, Amanullah A, Upadhyay A, Watanabe S, Mishra R, Yamanaka K, Mishra A (2015) Mahogunin ring finger 1 confers cytoprotection against mutant SOD1 aggresomes and is defective in an ALS mouse model. Neurobiol Dis 86:16–28. doi:10.1016/j.nbd.2015.11.017

Chio A (1999) ISIS survey: an international study on the diagnostic process and its implications in amyotrophic lateral sclerosis. J Neurol 246(Suppl 3):III1-5

Chiu IM, Chen A, Zheng Y, Kosaras B, Tsiftsoglou SA, Vartanian TK, Brown RH Jr, Carroll MC (2008) T lymphocytes potentiate endogenous neuroprotective inflammation in a mouse model of ALS. Proc Natl Acad Sci USA 105(46):17913–17918. doi:10.1073/pnas.0804610105

Cleveland DW, Rothstein JD (2001) From Charcot to Lou Gehrig: deciphering selective motor neuron death in ALS. Nat Rev Neurosci 2(11):806–819. doi:10.1038/35097565

Corti S, Nizzardo M, Nardini M, Donadoni C, Salani S, Simone C, Falcone M, Riboldi G, Govoni A, Bresolin N, Comi GP (2010) Systemic transplantation of c-kit+ cells exerts a therapeutic effect in a model of amyotrophic lateral sclerosis. Hum Mol Genet 19(19):3782–3796. doi:10.1093/hmg/ddq293

Dale JM, Garcia ML (2012) Neurofilament phosphorylation during development and disease: which came first, the phosphorylation or the accumulation? J Amino Acids 2012:382107. doi:10.1155/2012/382107

DeJesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ, Nicholson AM, Finch NA, Flynn H, Adamson J, Kouri N, Wojtas A, Sengdy P, Hsiung GY, Karydas A, Seeley WW, Josephs KA, Coppola G, Geschwind DH, Wszolek ZK, Feldman H, Knopman DS, Petersen RC, Miller BL, Dickson DW, Boylan KB, Graff-Radford NR, Rademakers R (2011) Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron 72(2):245–256. doi:10.1016/j.neuron.2011.09.011

Deng HX, Shi Y, Furukawa Y, Zhai H, Fu R, Liu E, Gorrie GH, Khan MS, Hung WY, Bigio EH, Lukas T, Dal Canto MC, O'Halloran TV, Siddique T (2006) Conversion to the amyotrophic lateral sclerosis phenotype is associated with intermolecular linked insoluble aggregates of SOD1 in mitochondria. Proc Natl Acad Sci USA 103(18):7142–7147. doi:10.1073/pnas.0602046103

Deshpande DM, Kim YS, Martinez T, Carmen J, Dike S, Shats I, Rubin LL, Drummond J, Krishnan C, Hoke A, Maragakis N, Shefner J, Rothstein JD, Kerr DA (2006) Recovery from paralysis in adult rats using embryonic stem cells. Ann Neurol 60(1):32–44. doi:10.1002/ana.20901

Dewil M, Van Den Bosch L, Robberecht W (2007) Microglia in amyotrophic lateral sclerosis. Acta Neurol Belg 107(3):63–70

Doble A (1996) The pharmacology and mechanism of action of riluzole. Neurology 47(6 Suppl 4):S233–S241

Droppelmann CA, Campos-Melo D, Ishtiaq M, Volkening K, Strong MJ (2014) RNA metabolism in ALS: when normal processes become pathological. Amyotroph Lateral Scler Frontotemporal Degener 15(5–6):321–336. doi:10.3109/21678421.2014.881377

Dupuis L, Gonzalez de Aguilar JL, Oudart H, de Tapia M, Barbeito L, Loeffler JP (2004) Mitochondria in amyotrophic lateral sclerosis: a trigger and a target. Neurodegener Dis 1 (6):245–254. doi:10.1159/000085063

Elliott JL (2001) Cytokine upregulation in a murine model of familial amyotrophic lateral sclerosis. Brain Res Mol Brain Res 95(1–2):172–178

Endo F, Yamanaka K (2014) Neuroinflammation in amyotrophic lateral sclerosis. Rinsho Shinkeigaku 54(12):1128–1131. doi:10.5692/clinicalneurol.54.1128

Farina C, Aloisi F, Meinl E (2007) Astrocytes are active players in cerebral innate immunity. Trends Immunol 28(3):138–145. doi:10.1016/j.it.2007.01.005

Federici T, Boulis NM (2012) Gene therapy for amyotrophic lateral sclerosis. Neurobiol Dis 48 (2):236–242. doi:10.1016/j.nbd.2011.08.018

Finkelstein A, Kunis G, Seksenyan A, Ronen A, Berkutzki T, Azoulay D, Koronyo-Hamaoui M, Schwartz M (2011) Abnormal changes in NKT cells, the IGF-1 axis, and liver pathology in an animal model of ALS. PLoS ONE 6(8):e22374. doi:10.1371/journal.pone.0022374

Foran E, Trotti D (2009) Glutamate transporters and the excitotoxic path to motor neuron degeneration in amyotrophic lateral sclerosis. Antioxid Redox Signal 11(7):1587–1602. doi:10.1089/ars.2009.2444

Foster E, Tsang BK, Kam A, Storey E, Day B, Hill A (2015) Hirayama disease. J Clin Neurosci 22 (6):951–954. doi:10.1016/j.jocn.2014.11.025

Gaiottino J, Norgren N, Dobson R, Topping J, Nissim A, Malaspina A, Bestwick JP, Monsch AU, Regeniter A, Lindberg RL, Kappos L, Leppert D, Petzold A, Giovannoni G, Kuhle J (2013) Increased neurofilament light chain blood levels in neurodegenerative neurological diseases. PLoS ONE 8(9):e75091. doi:10.1371/journal.pone.0075091

Gleichmann M, Mattson MP (2011) Neuronal calcium homeostasis and dysregulation. Antioxid Redox Signal 14(7):1261–1273. doi:10.1089/ars.2010.3386

Goodall EF, Morrison KE (2006) Amyotrophic lateral sclerosis (motor neuron disease): proposed mechanisms and pathways to treatment. Expert Rev Mol Med 8(11):1–22. doi:10.1017/S1462399406010854

Grossman M, Elman L, McCluskey L, McMillan CT, Boller A, Powers J, Rascovsky K, Hu W, Shaw L, Irwin DJ, Lee VM, Trojanowski JQ (2014) Phosphorylated tau as a candidate biomarker for amyotrophic lateral sclerosis. JAMA Neurol 71(4):442–448. doi:10.1001/jamaneurol.2013.6064

Henkel JS, Beers DR, Zhao W, Appel SH (2009) Microglia in ALS: the good, the bad, and the resting. J Neuroimmune Pharmacol 4(4):389–398. doi:10.1007/s11481-009-9171-5

Hensley K, Fedynyshyn J, Ferrell S, Floyd RA, Gordon B, Grammas P, Hamdheydari L, Mhatre M, Mou S, Pye QN, Stewart C, West M, West S, Williamson KS (2003) Message and protein-level elevation of tumor necrosis factor alpha (TNF alpha) and TNF alpha-modulating cytokines in spinal cords of the G93A-SOD1 mouse model for amyotrophic lateral sclerosis. Neurobiol Dis 14(1):74–80

Howland DS, Liu J, She Y, Goad B, Maragakis NJ, Kim B, Erickson J, Kulik J, De Vito L, Psaltis G, De Gennaro LJ, Cleveland DW, Rothstein JD (2002) Focal loss of the glutamate transporter EAAT2 in a transgenic rat model of SOD1 mutant-mediated amyotrophic lateral sclerosis (ALS). Proc Natl Acad Sci USA 99(3):1604–1609. doi:10.1073/pnas.032539299

Iaccarino C, Mura ME, Esposito S, Carta F, Sanna G, Turrini F, Carri MT, Crosio C (2011) Bcl2-A1 interacts with pro-caspase-3: implications for amyotrophic lateral sclerosis. Neurobiol Dis 43(3):642–650. doi:10.1016/j.nbd.2011.05.013

Islam AT, Kwak J, Jung Y, Kee Y (2014) Animal models of amyotrophic lateral sclerosis and Huntington's disease. Genes Genomics 36(4):399–413. doi:10.1007/s13258-014-0188-7

Ito H (2014) Basophilic inclusions and neuronal intermediate filament inclusions in amyotrophic lateral sclerosis and frontotemporal lobar degeneration. Neuropathology 34(6):589–595

Jain MR, Ge WW, Elkabes S, Li H (2008) Amyotrophic lateral sclerosis: protein chaperone dysfunction revealed by proteomic studies of animal models. Proteomics Clin Appl 2(5):670–684. doi:10.1002/prca.200780023

Joyce PI, Fratta P, Fisher EM, Acevedo-Arozena A (2011) SOD1 and TDP-43 animal models of amyotrophic lateral sclerosis: recent advances in understanding disease toward the development of clinical treatments. Mamm Genome 22(7–8):420–448. doi:10.1007/s00335-011-9339-1

Julien JP (2007) ALS: astrocytes move in as deadly neighbors. Nat Neurosci 10(5):535–537. doi:10.1038/nn0507-535

Kabashi E, Durham HD (2006) Failure of protein quality control in amyotrophic lateral sclerosis. Biochimica et Biophysica Acta (BBA)—Mol Basis Dis 1762(11–12):1038–1050. doi:10.1016/j.bbadis.2006.06.006

Kakizawa S, Miyazaki T, Yanagihara D, Iino M, Watanabe M, Kano M (2005) Maintenance of presynaptic function by AMPA receptor-mediated excitatory postsynaptic activity in adult brain. Proc Natl Acad Sci USA 102(52):19180–19185. doi:10.1073/pnas.0504359103

Kang SH, Li Y, Fukaya M, Lorenzini I, Cleveland DW, Ostrow LW, Rothstein JD, Bergles DE (2013) Degeneration and impaired regeneration of gray matter oligodendrocytes in amyotrophic lateral sclerosis. Nat Neurosci 16(5):571–579. doi:10.1038/nn.3357

Kato S, Hayashi H, Nakashima K, Nanba E, Kato M, Hirano A, Nakano I, Asayama K, Ohama E (1997) Pathological characterization of astrocytic hyaline inclusions in familial amyotrophic lateral sclerosis. Am J Pathol 151(2):611–620

Kato S, Horiuchi S, Liu J, Cleveland DW, Shibata N, Nakashima K, Nagai R, Hirano A, Takikawa M, Kato M, Nakano I, Ohama E (2000) Advanced glycation endproduct-modified superoxide dismutase-1 (SOD1)-positive inclusions are common to familial amyotrophic lateral sclerosis patients with SOD1 gene mutations and transgenic mice expressing human SOD1 with a G85R mutation. Acta Neuropathol 100(5):490–505

Kawamata T, Akiyama H, Yamada T, McGeer PL (1992) Immunologic reactions in amyotrophic lateral sclerosis brain and spinal cord tissue. Am J Pathol 140(3):691–707

Kettenmann H, Hanisch UK, Noda M, Verkhratsky A (2011) Physiology of microglia. Physiol Rev 91(2):461–553. doi:10.1152/physrev.00011.2010

Kiaei M, Petri S, Kipiani K, Gardian G, Choi DK, Chen J, Calingasan NY, Schafer P, Muller GW, Stewart C, Hensley K, Beal MF (2006) Thalidomide and lenalidomide extend survival in a transgenic mouse model of amyotrophic lateral sclerosis. J Neurosci 26(9):2467–2473. doi:10.1523/JNEUROSCI.5253-05.2006

Klein SM, Behrstock S, McHugh J, Hoffmann K, Wallace K, Suzuki M, Aebischer P, Svendsen CN (2005) GDNF delivery using human neural progenitor cells in a rat model of ALS. Hum Gene Ther 16(4):509–521. doi:10.1089/hum.2005.16.509

Lacomblez L, Bensimon G, Leigh PN, Guillet P, Meininger V (1996) Dose-ranging study of riluzole in amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis/Riluzole Study Group II. Lancet 347(9013):1425–1431

Lanson NA Jr, Pandey UB (2012) FUS-related proteinopathies: lessons from animal models. Brain Res 1462:44–60. doi:10.1016/j.brainres.2012.01.039

Lasiene J, Yamanaka K (2011) Glial cells in amyotrophic lateral sclerosis. Neurol Res Int 2011:718987. doi:10.1155/2011/718987

Lau A, Tymianski M (2010) Glutamate receptors, neurotoxicity and neurodegeneration. Pflugers Arch 460(2):525–542. doi:10.1007/s00424-010-0809-1

Leal SS, Cardoso I, Valentine JS, Gomes CM (2013) Calcium ions promote superoxide dismutase 1 (SOD1) aggregation into non-fibrillar amyloid: a link to toxic effects of calcium overload in amyotrophic lateral sclerosis (ALS)? J Biol Chem 288(35):25219–25228. doi:10.1074/jbc.M113.470740

Lee S, Kim HJ (2015) Prion-like mechanism in amyotrophic lateral sclerosis: are protein aggregates the key? Exp Neurobiol 24(1):1–7. doi:10.5607/en.2015.24.1.1

Lee HJ, Kim KS, Ahn J, Bae HM, Lim I, Kim SU (2014) Human motor neurons generated from neural stem cells delay clinical onset and prolong life in ALS mouse model. PLoS ONE 9(5):e97518. doi:10.1371/journal.pone.0097518

Lehman N (2009) The ubiquitin proteasome system in neuropathology. Acta Neuropathol 118 (3):329–347. doi:10.1007/s00401-009-0560-x

Li P, Gan Y, Mao L, Leak R, Chen J, Hu X (2014) The critical roles of immune cells in acute brain injuries. In: The critical roles of immune cells in acute brain injuries. Springer. doi:10.1007/978-1-4614-8915-3_2

Liem RK, Messing A (2009) Dysfunctions of neuronal and glial intermediate filaments in disease. J Clin Invest 119(7):1814–1824. doi:10.1172/JCI38003

Lipton SA, Rosenberg PA (1994) Excitatory amino acids as a final common pathway for neurologic disorders. N Engl J Med 330(9):613–622. doi:10.1056/NEJM199403033300907

Llorens J (2013) Toxic neurofilamentous axonopathies—accumulation of neurofilaments and axonal degeneration. J Intern Med 273(5):478–489. doi:10.1111/joim.12030

Logroscino G, Traynor BJ, Hardiman O, Chio A, Mitchell D, Swingler RJ, Millul A, Benn E, Beghi E, Eurals (2010) Incidence of amyotrophic lateral sclerosis in Europe. J Neurol Neurosurg Psychiatry 81(4):385–390. doi:10.1136/jnnp.2009.183525

Ludemann N, Clement A, Hans VH, Leschik J, Behl C, Brandt R (2005) O-glycosylation of the tail domain of neurofilament protein M in human neurons and in spinal cord tissue of a rat model of amyotrophic lateral sclerosis (ALS). J Biol Chem 280(36):31648–31658. doi:10.1074/jbc.M504395200

Mackenzie IR, Rademakers R, Neumann M (2010) TDP-43 and FUS in amyotrophic lateral sclerosis and frontotemporal dementia. Lancet Neurol 9(10):995–1007. doi:10.1016/S1474-4422(10)70195-2

Mandrekar-Colucci S, Landreth GE (2010) Microglia and inflammation in Alzheimer's disease. CNS Neurol Disord Drug Targets 9(2):156–167

Marangi G, Traynor BJ (2015) Genetic causes of amyotrophic lateral sclerosis: new genetic analysis methodologies entailing new opportunities and challenges. Brain Res 1607:75–93. doi:10.1016/j.brainres.2014.10.009

Matsumoto S, Goto S, Kusaka H, Imai T, Murakami N, Hashizume Y, Okazaki H, Hirano A (1993) Ubiquitin-positive inclusion in anterior horn cells in subgroups of motor neuron diseases: a comparative study of adult-onset amyotrophic lateral sclerosis, juvenile amyotrophic lateral sclerosis and Werdnig-Hoffmann disease. J Neurol Sci 115(2):208–213

Mazzini L, Gelati M, Profico DC, Sgaravizzi G, Projetti Pensi M, Muzi G, Ricciolini C, Rota Nodari L, Carletti S, Giorgi C, Spera C, Domenico F, Bersano E, Petruzzelli F, Cisari C, Maglione A, Sarnelli MF, Stecco A, Querin G, Masiero S, Cantello R, Ferrari D, Zalfa C, Binda E, Visioli A, Trombetta D, Novelli A, Torres B, Bernardini L, Carriero A, Prandi P, Servo S, Cerino A, Cima V, Gaiani A, Nasuelli N, Massara M, Glass J, Soraru G, Boulis NM, Vescovi AL (2015) Human neural stem cell transplantation in ALS: initial results from a phase I trial. J Transl Med 13(1):17. doi:10.1186/s12967-014-0371-2

McGeer PL, McGeer EG (2002) Inflammatory processes in amyotrophic lateral sclerosis. Muscle Nerve 26(4):459–470. doi:10.1002/mus.10191

McGoldrick P, Joyce PI, Fisher EM, Greensmith L (2013) Rodent models of amyotrophic lateral sclerosis. Biochim Biophys Acta 1832(9):1421–1436. doi:10.1016/j.bbadis.2013.03.012

Meldrum B, Garthwaite J (1990) Excitatory amino acid neurotoxicity and neurodegenerative disease. Trends Pharmacol Sci 11(9):379–387

Mezzapesa DM, D'Errico E, Tortelli R, Distaso E, Cortese R, Tursi M, Federico F, Zoccolella S, Logroscino G, Dicuonzo F, Simone IL (2013) Cortical thinning and clinical heterogeneity in amyotrophic lateral sclerosis. PLoS ONE 8(11):e80748. doi:10.1371/journal.pone.0080748

Mitrecic D, Nicaise C, Gajovic S, Pochet R (2010) Distribution, differentiation, and survival of intravenously administered neural stem cells in a rat model of amyotrophic lateral sclerosis. Cell Transplant 19(5):537–548. doi:10.3727/096368910X498269

Moisse K, Strong MJ (2006) Innate immunity in amyotrophic lateral sclerosis. Biochim Biophys Acta 1762(11–12):1083–1093. doi:10.1016/j.bbadis.2006.03.001

Muzio L, Martino G, Furlan R (2007) Multifaceted aspects of inflammation in multiple sclerosis: the role of microglia. J Neuroimmunol 191(1–2):39–44. doi:10.1016/j.jneuroim.2007.09.016

Nakamura Y, Si QS, Kataoka K (1999) Lipopolysaccharide-induced microglial activation in culture: temporal profiles of morphological change and release of cytokines and nitric oxide. Neurosci Res 35(2):95–100

Nance DM, Sanders VM (2007) Autonomic innervation and regulation of the immune system (1987–2007). Brain Behav Immun 21(6):736–745. doi:10.1016/j.bbi.2007.03.008

Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, Bruce J, Schuck T, Grossman M, Clark CM, McCluskey LF, Miller BL, Masliah E, Mackenzie IR, Feldman H, Feiden W, Kretzschmar HA, Trojanowski JQ, Lee VM (2006) Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 314(5796):130–133. doi:10.1126/science.1134108

Niebroj-Dobosz I, Dziewulska D, Kwiecinski H (2004) Oxidative damage to proteins in the spinal cord in amyotrophic lateral sclerosis (ALS). Folia Neuropathol 42(3):151–156

Pasinelli P, Brown RH (2006) Molecular biology of amyotrophic lateral sclerosis: insights from genetics. Nat Rev Neurosci 7(9):710–723. doi:10.1038/nrn1971

Patel P, Kriz J, Gravel M, Soucy G, Bareil C, Gravel C, Julien JP (2014) Adeno-associated virus-mediated delivery of a recombinant single-chain antibody against misfolded superoxide dismutase for treatment of amyotrophic lateral sclerosis. Mol Ther 22(3):498–510. doi:10.1038/mt.2013.239

Pehar M, Cassina P, Vargas MR, Castellanos R, Viera L, Beckman JS, Estevez AG, Barbeito L (2004) Astrocytic production of nerve growth factor in motor neuron apoptosis: implications for amyotrophic lateral sclerosis. J Neurochem 89(2):464–473. doi:10.1111/j.1471-4159.2004.02357.x

Piao YS, Wakabayashi K, Kakita A, Yamada M, Hayashi S, Morita T, Ikuta F, Oyanagi K, Takahashi H (2003) Neuropathology with clinical correlations of sporadic amyotrophic lateral sclerosis: 102 autopsy cases examined between 1962 and 2000. Brain Pathol 13(1):10–22

Ransohoff RM, Kivisakk P, Kidd G (2003) Three or more routes for leukocyte migration into the central nervous system. Nat Rev Immunol 3(7):569–581. doi:10.1038/nri1130

Rao SD, Weiss JH (2004) Excitotoxic and oxidative cross-talk between motor neurons and glia in ALS pathogenesis. Trends Neurosci 27(1):17–23

Rizzo F, Riboldi G, Salani S, Nizzardo M, Simone C, Corti S, Hedlund E (2014) Cellular therapy to target neuroinflammation in amyotrophic lateral sclerosis. Cell Mol Life Sci 71(6):999–1015. doi:10.1007/s00018-013-1480-4

Rogers J, Mastroeni D, Leonard B, Joyce J, Grover A (2007) Neuroinflammation in Alzheimer's disease and Parkinson's disease: are microglia pathogenic in either disorder? Int Rev Neurobiol 82:235–246. doi:10.1016/S0074-7742(07)82012-5

Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, Hentati A, Donaldson D, Goto J, O'Regan JP, Deng HX et al (1993) Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 362(6415):59–62. doi:10.1038/362059a0

Rossi FH, Franco MC, Estevez AG (2013) Pathophysiology of Amyotrophic Lateral Sclerosis. In: Estévez AG (ed) Current Advances in Amyotrophic Lateral Sclerosis. ISBN: 978-953-51-1195-5. doi:10.5772/56562. http://www.intechopen.com/books/current-advances-in-amyotrophic-lateral-sclerosis/pathophysiology-ofamyotrophic-lateral-sclerosis

Rothstein JD (2009) Current hypotheses for the underlying biology of amyotrophic lateral sclerosis. Ann Neurol 65(Suppl 1):S3–S9. doi:10.1002/ana.21543

Rothstein JD, Martin LJ, Kuncl RW (1992) Decreased glutamate transport by the brain and spinal cord in amyotrophic lateral sclerosis. N Engl J Med 326(22):1464–1468. doi:10.1056/NEJM199205283262204

Schiffer D, Cordera S, Cavalla P, Migheli A (1996) Reactive astrogliosis of the spinal cord in amyotrophic lateral sclerosis. J Neurol Sci 139:27–33

Scotter E, Chen H-J, Shaw C (2015) TDP-43 proteinopathy and ALS: insights into disease mechanisms and therapeutic targets. Neurotherapeutics 12(2):352–363. doi:10.1007/s13311-015-0338-x

Shaw BF, Valentine JS (2007) How do ALS-associated mutations in superoxide dismutase 1 promote aggregation of the protein? Trends Biochem Sci 32(2):78–85

Shinder GA, Lacourse M-C, Minotti S, Durham HD (2001) Mutant Cu/Zn-superoxide dismutase proteins have altered solubility and interact with heat shock/stress proteins in models of amyotrophic lateral sclerosis. J Biol Chem 276(16):12791–12796. doi:10.1074/jbc.M010759200

Shobha K, Alladi PA, Nalini A, Sathyaprabha TN, Raju TR (2010) Exposure to CSF from sporadic amyotrophic lateral sclerosis patients induces morphological transformation of astroglia and enhances GFAP and S100beta expression. Neurosci Lett 473(1):56–61. doi:10.1016/j.neulet.2010.02.022

Smethurst P, Sidle KC, Hardy J (2014) Invited review: prion-like mechanisms of transactive response DNA binding protein of 43 kDa (TDP-43) in amyotrophic lateral sclerosis (ALS). Neuropathol Appl Neurobiol. doi:10.1111/nan.12206

Smith D, Uryu K, Saatman K, Trojanowski J, McIntosh T (2003) Protein accumulation in traumatic brain injury. NeuroMol Med 4(1–2):59–72. doi:10.1385/NMM:4:1-2:59

Soriani MH, Desnuelle C (2009) Epidemiology of amyotrophic lateral sclerosis. Rev Neurol 165 (8–9):627–640. doi:10.1016/j.neurol.2009.04.004 (Paris)

Sreedharan J, Blair IP, Tripathi VB, Hu X, Vance C, Rogelj B, Ackerley S, Durnall JC, Williams KL, Buratti E, Baralle F, de Belleroche J, Mitchell JD, Leigh PN, Al-Chalabi A, Miller CC, Nicholson G, Shaw CE (2008) TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. Science 319(5870):1668–1672. doi:10.1126/science.1154584

Stamer K, Vogel R, Thies E, Mandelkow E, Mandelkow E-M (2002) Tau blocks traffic of organelles, neurofilaments, and APP vesicles in neurons and enhances oxidative stress. J Cell Biol 156(6):1051–1063. doi:10.1083/jcb.200108057

Streit WJ, Mrak RE, Griffin WST (2004) Microglia and neuroinflammation: a pathological perspective. J Neuroinflamm 1(1):14

Takahashi T, Yagishita S, Amano N, Yamaoka K, Kamei T (1997) Amyotrophic lateral sclerosis with numerous axonal spheroids in the corticospinal tract and massive degeneration of the cortex. Acta Neuropathol 94(3):294–299

Tovar YRLB, Ramirez-Jarquin UN, Lazo-Gomez R, Tapia R (2014) Trophic factors as modulators of motor neuron physiology and survival: implications for ALS therapy. Front Cell Neurosci 8:61. doi:10.3389/fncel.2014.00061

Tracey KJ (2002) The inflammatory reflex. Nature 420(6917):853–859. doi:10.1038/nature01321

Traynelis SF, Wollmuth LP, McBain CJ, Menniti FS, Vance KM, Ogden KK, Hansen KB, Yuan H, Myers SJ, Dingledine R (2010) Glutamate receptor ion channels: structure, regulation, and function. Pharmacol Rev 62(3):405–496. doi:10.1124/pr.109.002451

Troost D, Sillevis Smitt PA, de Jong JM, Swaab DF (1992) Neurofilament and glial alterations in the cerebral cortex in amyotrophic lateral sclerosis. Acta Neuropathol 84(6):664–673

Tummala H, Jung C, Tiwari A, Higgins CMJ, Hayward LJ, Xu Z (2005) Inhibition of chaperone activity is a shared property of several Cu, Zn-Superoxide dismutase mutants that cause amyotrophic lateral sclerosis. J Biol Chem 280(18):17725–17731. doi:10.1074/jbc.M501705200

Turner MR, Cagnin A, Turkheimer FE, Miller CC, Shaw CE, Brooks DJ, Leigh PN, Banati RB (2004) Evidence of widespread cerebral microglial activation in amyotrophic lateral sclerosis: an [11C](R)-PK11195 positron emission tomography study. Neurobiol Dis 15(3):601–609. doi:10.1016/j.nbd.2003.12.012

Upadhyay A, Amanullah A, Chhangani D, Mishra R, Mishra A (2015a) Selective multifaceted E3 ubiquitin ligases barricade extreme defense: Potential therapeutic targets for neurodegeneration and ageing. Ageing Res Rev. doi:10.1016/j.arr.2015.07.009

Upadhyay A, Amanullah A, Chhangani D, Mishra R, Prasad A, Mishra A (2015b) Mahogunin ring finger-1 (MGRN1), a multifaceted ubiquitin ligase: recent unraveling of neurobiological mechanisms. Mol Neurobiol. doi:10.1007/s12035-015-9379-8

Urushitani M, Kurisu J, Tsukita K, Takahashi R (2002) Proteasomal inhibition by misfolded mutant superoxide dismutase 1 induces selective motor neuron death in familial amyotrophic lateral sclerosis. J Neurochem 83(5):1030–1042

Van Damme P, Robberecht W (2014) Developments in treatments for amyotrophic lateral sclerosis via intracerebroventricular or intrathecal delivery. Expert Opin Investig Drugs 23(7):955–963. doi:10.1517/13543784.2014.912275

Van Den Bosch L, Van Damme P, Bogaert E, Robberecht W (2006) The role of excitotoxicity in the pathogenesis of amyotrophic lateral sclerosis. Biochim Biophys Acta 1762(11–12):1068–1082. doi:10.1016/j.bbadis.2006.05.002

Vance C, Rogelj B, Hortobagyi T, De Vos KJ, Nishimura AL, Sreedharan J, Hu X, Smith B, Ruddy D, Wright P, Ganesalingam J, Williams KL, Tripathi V, Al-Saraj S, Al-Chalabi A, Leigh PN, Blair IP, Nicholson G, de Belleroche J, Gallo JM, Miller CC, Shaw CE (2009) Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. Science 323(5918):1208–1211. doi:10.1126/science.1165942

Watanabe M, Dykes-Hoberg M, Cizewski Culotta V, Price DL, Wong PC, Rothstein JD (2001) Histological evidence of protein aggregation in mutant SOD1 transgenic mice and in amyotrophic lateral sclerosis neural tissues. Neurobiol Dis 8(6):933–941. doi:10.1006/nbdi.2001.0443

Weydt P, Möller T (2005) Neuroinflammation in the pathogenesis of amyotrophic lateral sclerosis. NeuroReport 16(6):527–531

Wijesekera LC, Leigh PN (2009) Amyotrophic lateral sclerosis. Orphanet J Rare Dis 4:3. doi:10.1186/1750-1172-4-3

Wong NK, He BP, Strong MJ (2000) Characterization of neuronal intermediate filament protein expression in cervical spinal motor neurons in sporadic amyotrophic lateral sclerosis (ALS). J Neuropathol Exp Neurol 59(11):972–982

Yang W, Sopper MM, Leystra-Lantz C, Strong MJ (2003) Microtubule-associated tau protein positive neuronal and glial inclusions in ALS. Neurology 61(12):1766–1773

Yang Y, Gozen O, Watkins A, Lorenzini I, Lepore A, Gao Y, Vidensky S, Brennan J, Poulsen D, Won Park J, Li Jeon N, Robinson MB, Rothstein JD (2009) Presynaptic regulation of astroglial excitatory neurotransmitter transporter GLT1. Neuron 61(6):880–894. doi:10.1016/j.neuron.2009.02.010

Yoshihara T, Ishigaki S, Yamamoto M, Liang Y, Niwa J, Takeuchi H, Doyu M, Sobue G (2002) Differential expression of inflammation- and apoptosis-related genes in spinal cords of a mutant SOD1 transgenic mouse model of familial amyotrophic lateral sclerosis. J Neurochem 80(1):158–167

Zhang Y, Schuff N, Woolley SC, Chiang GC, Boreta L, Laxamana J, Katz JS, Weiner MW (2011) Progression of white matter degeneration in amyotrophic lateral sclerosis: a diffusion tensor imaging study. Amyotroph Lateral Scler 12(6):421–429. doi:10.3109/17482968.2011.593036

Zhao W, Varghese M, Yemul S, Pan Y, Cheng A, Marano P, Hassan S, Vempati P, Chen F, Qian X, Pasinetti GM (2011) Peroxisome proliferator activator receptor gamma coactivator-1alpha (PGC-1alpha) improves motor performance and survival in a mouse model of amyotrophic lateral sclerosis. Mol Neurodegener 6(1):51. doi:10.1186/1750-1326-6-51

Zhou JY, Afjehi-Sadat L, Asress S, Duong DM, Cudkowicz M, Glass JD, Peng J (2010) Galectin-3 is a candidate biomarker for amyotrophic lateral sclerosis: discovery by a proteomics approach. J Proteome Res 9(10):5133–5141. doi:10.1021/pr100409r

Zhu S, Stavrovskaya IG, Drozda M, Kim BY, Ona V, Li M, Sarang S, Liu AS, Hartley DM, Wu DC, Gullans S, Ferrante RJ, Przedborski S, Kristal BS, Friedlander RM (2002) Minocycline inhibits cytochrome c release and delays progression of amyotrophic lateral sclerosis in mice. Nature 417(6884):74–78. doi:10.1038/417074a

Neuroinflammation in Ischaemic Stroke: Utilizing the Biphasic Niche of Neuroprotection and Neurotoxicity for Clinic

9

Mohammed Aftab Alam, V.P. Subramanyam Rallabandi and Prasun Kumar Roy

Abstract

Ischaemic stroke is a devastating disease that results in neurological disorder with maximum disease burden caused by blockade of blood vessels in the brain leading to neuronal cell death and tissue damage. Inflammatory processes have a fundamental role in the pathophysiology of ischaemic stroke, and recent studies indicate that inflammation has a temporally biphasic behaviour and acts as a double-edged sword, not only exacerbating secondary brain injury in the acute stage of stroke, but also thereafter beneficially contributing to brain recovery after the stroke. An initial event of inflammation in ischaemic stroke is activation of microglia, leading to a cascade of delicately balanced orchestration between both pro- and anti-inflammatory mediators, acting through multiple receptor signalling pathways. Understanding how microglia can actuate to both its phenotypes—such as neurotoxic M_1 type ('bad microglia') vis-à-vis neuroprotective M_2 type ('good microglia')—may be essential to implement therapeutic strategies of using differential immunomodulatory interventions in ischaemic stroke. We elucidate the role of the bimodality in inflammation in ischaemic stroke, the related signalling pathways, and the resulting immunomodulation and immunosuppression processes. A pathophysiological integration of the findings from cell culture models, animal studies, human investigations and population-based clinical trials, is undertaken. We delineate how one can utilize the manoeuvre the dynamics of inflammation and immunomodulation for enhancing therapeutic interventions on ischaemic stroke.

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9.1 Introduction

Ischaemic stroke is a leading cause of death and disability worldwide and stems from occlusion/haemorrhage of blood vessels in the brain and also the neck supplying blood to the brain. It triggers localized cell death in the region, causing inflammation and immune responses. The adult neurogenesis in mammals is a known fact, and these neural progenitor cells which may lead to neurons or glial cells that start migrating from sub-ventricular zone to the tissue adjacent to the area of neuronal cell death, the penumbra zone (Yamashita et al. 2006). This process is supplemented by ongoing angiogenesis which leads to increased vascularization to aid in recovery. The process is characterized by the following sequential stages:

I. Proliferation of endogenous neural stem cells;
II. Migration of neural stem cells to ischaemic area;
III. Maturation of neurons, and
IV. Formation of functional synapse.

Despite preeminent progress in understanding the pathophysiology of ischaemic stroke, translation of this knowledge into effective therapies have largely failed in clinics, and hence the crucial need of knowing the cause of this failure. Indeed for about 30 years, systemic thrombolysis with intravenous (*i.v*) recombinant tissue plasminogen activator (tPA) still remains the only treatment proven to improve clinical outcome of patients with acute ischaemic stroke (Brott and Bogousslavsky 2000). However, because of an increased risk of haemorrhage following the treatment beyond a few hours post-stroke, only 1–2 % of stroke patients can benefit from recombinant *i.v* tissue plasminogen activator (Wang et al. 2012a; Wechsler and Jovin 2012). Till date, trials of anti-inflammatory drugs have been limited to initial phase of stroke to promote neuroregeneration after injury, and it has shown that anti-inflammatory drugs like nimesulide and indomethacin, results in favourable outcome in experimental studies of rodents and in human stroke subjects in clinical trial setting (Candelario-Jalil 2008; Nechipurenko et al. 2001).

9.2 Bimodality in Neuroinflammatory Behaviour

Tissue damage may happen very frequently throughout the CNS. For example, it may result from small ischaemic events and localized openings of the blood–brain barrier (BBB), causing influx of plasma constituents into the brain (Hanisch and Kettenmann 2007). Microglia are well positioned to sense such disturbances (Denes et al. 2007) and can react rapidly to even small damage to the neural tissue (Nimmerjahn et al. 2005). Recent in vivo studies have shown that microglia carry out active tissue scanning, which challenges the traditional notion of 'resting' microglia in the normal brain. Transformation of microglia to reactive states in response to pathology has been known for decades as microglial activation, but seems to be more

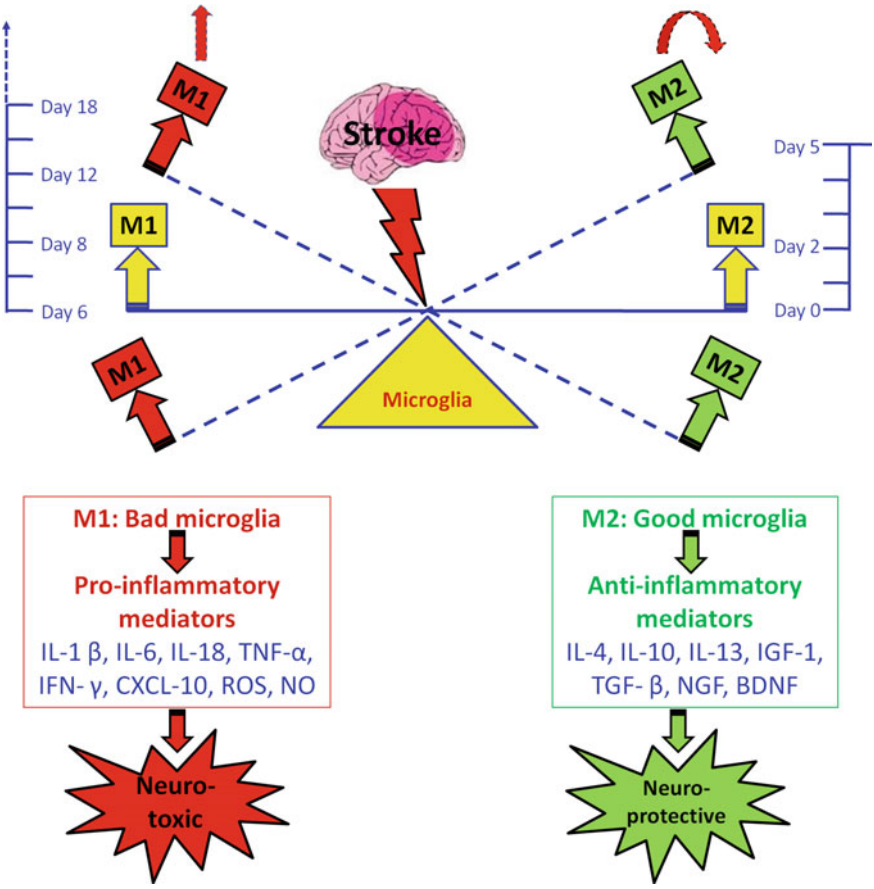

Fig. 9.1 Microglial polarization dynamics after ischaemic stroke. Soon after an ischaemic injury, microglia migrate to the infarcted areas initially assume the M2 ('good microglia') phenotype, that are healthier cells with enhanced phagocytic activity and increased production of anti-inflammatory mediators (IL-4, IL-10, IL-13, TGF-β, IGF-1, etc.), promoting the survival of neurons under ischaemic condition. Levels of these IL-10, TGF-β and CD206 mRNA increased as early as day 1 after ischaemic injury and peaks at 4–6 days. In addition, TGF-β released by microglia promotes an anti-inflammatory profile associated with increased proliferation and neuroprotection in the ischaemic brain. However, the M2 phenotype response is transient and phased out within 7 days after injury (Peri and Nüsslein-Volhard 2008). In the meantime, M1 phenotype ('Bad microglia') begins to dominate the injured area. M1 is a pro-inflammatory cellular state associated with an increase in protein synthesis of pro-inflammatory mediators (IFNγ, IL-1β, TNFα, IL-6, CXCL10, etc.), ROS and NO production, and proteolytic enzymes (MMP 9, MMP3) that act as a neurotoxic leading to increased neuronal death compared wiith alternatively activated M2 microglia

diverse and dynamic than ever anticipated—in both transcriptional and non-transcriptional features and functional consequences (Hu et al. 2012). This may help to explain why engagement of 'surveillance/hunting microglia' can be either neuroprotective (M2 microglia) or neurotoxic (M1 microglia), resulting in containment or aggravation of disease progression (Fig. 9.1).

From a translational viewpoint, for exploring the drug repurposing approach, it has been suggested that investigators working on cerebral ischaemia, should also consider off-label use of different Food and Drug Administration (FDA) approved drugs, so as to induce the sequential phases of the neural recovery. In this article, we provide an elucidation on the role of inflammation and its mediators in ischaemic stroke, and how one can modulate the process for optimal stroke recovery. We also delineate the dynamics between pro- and anti-inflammatory responses, their related pathways and the discrepancies between preclinical and clinical studies, besides evolve a corrective translational perspective.

9.3 Immunologically Significant Signalling Pathways in Stroke

We now deal with the signal transduction pathways related to the stroke phenomenology.

9.3.1 The Mechanistic Target of Immunomodulant Rapamycin (mTOR)

This target [Gene ID: 2475, updated on 30-Nov-2014], also known as mammalian target of rapamycin (mTOR), is a critical regulator of cell growth and metabolism that integrates a variety of signals under physiological and pathological conditions (Laplante and Sabatini 2009; Wiederrecht et al. 1995). Rapamycin is an FDA–approved anti-inflammatory immunosuppressant, being used to prevent rejection in organ transplantation. Recent data shows that mTOR signalling plays an important role in the modulation of both innate and adaptive immune responses (Thomson et al. 2009). In experimental stroke, rapamycin administration at an early time point, as 1 h after focal ischaemia, ameliorates the motor impairment in adult rats (Chauhan et al. 2011) and in neonatal rats (Carloni et al. 2010) and improves neuron viability in an in vitro model of stroke (Fletcher et al. 2013). However, the precise mechanisms underlying mTOR-mediated neuroprotection in stroke are unclear.

It may be underscored that rapamycin administration as late as 6 h after focal ischaemia significantly reduced infarct volume and improved motor function after stroke in rats (Xie et al. 2014). In addition, infiltration of neutrophils and γδ-type of T-lymphocytes were decreased, whilst regulatory T cell (Treg) function was increased, and pro-inflammatory activity of macrophages and microglia was reduced in the ischaemic hemispheres. Tregs from rapamycin-treated brains effectively inhibited pro-inflammatory cytokine and chemokine production by macrophages and microglia. Results from the study suggest that rapamycin attenuates secondary injury and motor deficits after focal ischaemia by modulating post-stroke neuroinflammation at later time period. One may underscore that numerous stroke patients often experience a significant delay between the onset of

ischaemia and initiation of therapy, sometimes 12 h or more. Hence it is important to determine whether rapamycin can protect from ischaemic injury when administered to stroke patients at late time points.

9.3.2 The Intranuclear Factor NF-κB Pathway in Ischaemic Stroke

Being involved in cell response to stress as inflammation and free radicals, the Nuclear Factor kappa B (NF-κB) is a key regulator of a variety of genes involved in cell survival and inflammation, and is activated after cerebral ischaemia in neurons, astrocytes, microglia and infiltrating inflammatory cells (Ridder and Schwaninger 2009). Among the 5 NF-κB subunits, one knows that p65/RelA and p50 are responsible for a detrimental effect in cerebral ischaemia (Napetschnig and Wu 2013). Previous studies showed that expression of p65 and p50, and DNA binding activity were increased in the brain after cerebral ischaemia. Increased DNA binding reflects activation of NF-κB. The NF-κB subunit p50 knockout mice have a smaller infarct size in both transient and permanent stroke models (Schneider et al. 1999). Similar observations were made by inhibiting activation of NF-κB with the treatment of S-nitrosoglutathione (Khan et al. 2005).

However, NF-κB activation is also implicated in neuroprotective mechanisms of ischaemic brain injury. For example, one study showed that rats treated with diethyl-dithio-carbamate, an NF-κB inhibitor, had enhanced neuronal DNA fragmentation and larger infarct size compared to controls, suggesting a beneficial role (Hill et al. 2001). Thus we see that NF-κB can have both a neurotoxic or neuroprotective effect, according to the situation. This can be accounted by the optimality (hormesis) effect of dose and temporality of the effect of a neuromodulator or growth factor: at a particular dose range a factor like NF-κB can be neuroprotective, whilst at higher/lower dose range the NF-κB can be neurotoxic (Kaltschmidt et al. 2005).

9.3.3 Inflammatory Response via Danger-Associated Molecular Pattern Molecules (DAMPs)

DAMPs are molecules that can initiate and perpetuate inflammatory immune response in the host, without the need for any infectious agent or microrganisms to initiate an immune response. In the ischaemic brain, the following entities can function as DAMPs: Heat shock proteins, β-amyloid (Aβ), hyaluronan, heparin sulphate, DNA or RNA immune complexes, oxidized low-density lipoproteins, and several other molecules (Shichita et al. 2012a). Among them, high mobility group box 1 (HMGB1) is a well characterized DAMP in ischaemic brain injury that increases Blood–Brain–Barrier (BBB) permeability or promote its breakdown (Hayakawa et al. 2010; Zhang et al. 2011). The HMGB1 level in the ischaemic stroke group is significantly increased compared with the control group, and has

been correlated with the severity of neurologic impairment observed in stroke patients (Yang et al. 2011).

It may also be mentioned that HMGB1, which is localized in cell nuclei in the normal brain, translocate into the cytosolic compartment and is released into the extracellular compartment in the presence of an ischaemic condition. The administration of anti-HMGB1-neutralizing antibody protects the BBB and reduces infarct volume (Qiu et al. 2008). Taken together, HMGB1 is an essential DAMP in ischaemic brain injury. Other potential DAMPs in brain homogenate lysates are peroxiredoxin (Prx) family proteins which have been identified as strong inducers of inflammatory cytokines by infiltrating macrophages (Shichita et al. 2012b). They are released into the extracellular compartment once the cells are about to die, functioning as DAMPs. Neutralization of Prx proteins, rather than HMGB1 protein, by specific antibodies has been shown to suppress inflammatory cytokine expression in the ischaemic brain (Hamanaka and Hara 2011). Hence, there is a future scope of utilizing inhibitors or antibodies against HMGB1 or Prx proteins as an approach for neuroprotection in stroke.

9.3.4 Innate Immunity-based Toll-like Receptors (TLRs) in Ischaemic Stroke

It is well known that Toll-like receptors (TLRs) are a class of receptor proteins that play a key role in the innate immune system, being expressed in as macrophages and dendritic cells. TLRs are closely implicated in cerebral ischaemia. Mice lacking either functional TLR2 or TLR4 were less susceptible to brain damage due to stroke and also had smaller infarcts than wild type controls (Cao et al. 2007; Lehnardt et al. 2008; Ziegler et al. 2007). Furthermore, TLR4−/− mice would decrease the damage due to global cerebral ischaemia and permanent focal ischaemia (Caso et al. 2007; Hua et al. 2007). TLR endogenous ligands (e.g. HSP 60, HSP70 and HMGB1) were detected in brain injury (Faraco et al. 2007; Kinouchi et al. 1993). These molecules could activate TLRs (e.g. TLR2 and TLR4) in brain, be neurotoxic, and induce pro-inflammatory mediators (e.g. TNF-α, IL-1 and IL-6) which contribute to stroke pathology. In contrast to the detrimental role of TLRs after stroke occurs, stimulation of TLRs prior to brain ischaemia could be neuroprotective. Pretreatment with TLR4 ligands (e.g. LPS) leads cells to switch their transcriptional response to TLR4 stimulation by enhancing interferon (IFN) expression and suppressing the NF-κB-induced TNF-α expression. Inhibition of NF-κB would protect the brain since mice lacking the p50 subunit of NF-κB decrease brain damage compared to the wild type mice (Schneider et al. 1999).

Increase of Interferon Regulatory Factor (IRF) signalling can also protect the brain, as IFN could downregulate the IRF3 induction and act as an acute neuroprotectant, with anti-inflammatory response (Liu et al. 2002; Veldhuis et al. 2003). This pretreatment can induce a finely controlled shift in the balance of pro-inflammatory and anti-inflammatory cytokines. Thus we see that it is also important to know that the effect of a modulating drug or agent (as TLR) can have

either detrimental or beneficial effects depending on the time of its administration: the agent can be neuroprotective if given before the ischemic induction, and neurotoxic if given after. It may be emphasized that such biphasicity can also be delineated in other modulating drugs used in stroke clinical trials (as selfotel, eliprodil), where the agent may function as neuroprotective in the initial phase, but may display neurotoxicity if given later (Ikonomidou and Turski 2002). This is an aspect of temporal hormesis principle in pharmacodynamics, where the effect of a drug temporally in a dynamical physiological system can be an inverted U function; a drug can show one type of behaviour at intermediate time period, and converse behaviours at early or later time periods.

9.3.5 Cell cycle-based Mitogen-Activated Protein Kinases (MAPKs) Pathway in Ischaemic Stroke

MAPKs are a highly conserved family of serine/threonine protein kinases involved in a variety of fundamental cellular processes such as proliferation, differentiation, motility, stress response, apoptosis, and survival. Conventional MAPKs include the extracellular signal-regulated kinase 1 and 2 (Erk1/2), the c-Jun N-terminal kinases (JNKs), the p38 MAPK, and Erk5. Signalling via the conventional MAPKs follows a classical three-tiered kinase cascade: MAPKKK → MAPKK → MAPK (Huang et al. 2010). All the four MAPK pathways are activated in cerebral ischaemia, but their roles are complicated and not yet adequately understood. Activation of JNK and p38 seems to be detrimental since injury due to stroke could be decreased after using their inhibitors (Guan et al. 2006; Kawasaki et al. 1997; Xia et al. 1995). On the other hand, ERK5 activation appears to be beneficial, whereas ERK1/2 activation could be both beneficial and detrimental (Sawe et al. 2008).

In comparison, the JNK pathway can lead to the production and activation of pro-inflammatory meditators (e.g. cytokines) in several inflammatory cells (Benakis et al. 2010; Kaminska 2005). Inhibition of JNK pathway with JNK inhibitor could decrease ischaemic injury via reducing neuroinflammation (Wang et al. 2012b). The p38 pathway is almost similar with JNK pathway. It is linked to production and activation of pro-inflammatory meditators as well. Administration of SB 239063, a p38 pathway inhibitor, could reduce p38 activity following stroke and also downregulate the stroke-induced cytokines (e.g. TNF-α and IL-1β) which contribute to stroke-induced brain injury (Barone et al. 2001). Activation of ERK1/2 in cerebral ischaemia is associated with ischaemic brain injury. Inhibition of ERK1/2 with a specific MEK1/2 inhibitor produced a neuroprotection by suppression of IL-1β expression (Wang et al. 2001). Administration of inhibitors of the MEK/ERK1/2 pathway could reduce ischaemic brain injury and improve neurological outcome (Alessandrini et al. 1999; Maddahi and Edvinsson 2008; Wang et al. 2003). ERK5 pathway was a recently identified member of MAPK family. A study showed that ERK5 activation may act in neuroprotection of ischaemic preconditioning (Wang et al. 2009). However, its effect and mechanism on inflammation in stroke is still unknown. Thus neuroprotection in stroke could be

harnessed by using agents or modulators to upregulate ERK5 pathway and/or downregulate ERK1/2 pathway.

9.4 Inflammatory Mediators in Ischaemic Stroke

The pro-inflammatory and anti-inflammatory cascades via immunosuppressing and immunostimulatory phases that occur during the short-term and long-term recovery processes in stroke are summarized below.

9.4.1 Biphasic Nature of the Effect of Immunomodulation on Stroke

Molecular and cellular mediators of neuroinflammatory responses play critical roles in the pathophysiology of ischaemic stroke, exerting either deleterious effects on the progression of tissue damage or beneficial roles during recovery and repair (Jin et al. 2010). Therefore, modulating the post-ischaemic neuroinflammation may provide a novel therapeutic approach in stroke. However, several therapeutic trials targeting neuroinflammatory response have failed to show clinical benefit (Sughrue et al. 2004). The cause for this remains unknown, which might be due to inadequate dose rate, appropriate timing duration, or poor temporal orchestration of the time windows for immunostimulant vis-à-vis immunosuppressant interventions. Moreover, targeting a single cell type or single molecule or single pathway may not be an adequate clinical strategy. In addition, immunomodulative approaches to stroke therapy may complicated by the inability to properly synchronize the therapeutic time windows with the orchestration of the biphasic nature of neuroinflammatory effects, which amplify acute short-term ischaemic injury but can contrarily contribute to long-term chronic tissue repair.

Microglia are the resident macrophages of the brain that serve both glial and immune-related functions. These include the monitoring of synapses (Wake et al. 2009), the detection and phagocytosis of infectious agents (Ribes et al. 2009, 2010), and the removal of apoptotic and necrotic cells with subsequent reciprocal behaviour of suppression, or of promotion, of neuroinflammation (Peri and Nüsslein--Volhard 2008; Magnus et al. 2001). This plethora of events consequently implicates microglia in many pathological conditions. Recent findings have revealed that under physiological central nervous system (CNS) conditions, microglia displays a constant motility and movement of their highly branched cellular processes within the intact mouse cerebral cortex and brain slices. These microglia processes are capable of ready extension (at speed of 1.25 μm/min or 2 mm/day) towards the sites of acute CNS damage (Davalos et al. 2005; Varnum and Ikezu 2012).

9.4.2 Processing of Two Distinct Types of Microglia/Macrophage: M1 and M2

Changes in microglial phenotype during their activation may be analogous to that of peripheral macrophages, as the two cell types are indistinguishable without definitive surface markers for either. Microglial responses to stimuli from a changing brain environment are characterized as (i) M1 type: classical activation with neurotoxic behaviour ('bad microglia'), or (ii) M2 type: alternative neuroprotective activation ('good microglia') (Fig. 9.1). M1 is a pro-inflammatory cellular state associated with an increase in protein synthesis of pro-inflammatory mediators or markers (IFNγ, IL-1β, TNFα, IL-6, CXCL10, iNOS, etc.), ROS and NO production, and proteolytic enzymes (MMP 9, MMP3) that act on the extracellular matrix leading to BBB breakdown (Hu et al. 2012; Varnum and Ikezu 2012). Levels of the pro-inflammatory marker such as iNOS increases from 3rd day, and goes on increasing across a longer period of over 14–15 days before arresting (Hu et al. 2012). M1 phenotype can lead to increased neuronal death compared to alternatively activated M2 microglia (Magnus et al. 2001); therefore, there is a growing interest to pharmacologically interfere with the signalling mechanisms that give rise to the classical activation phenotype of microglia, M1.

9.4.3 Fast Versus Slow Response Modalities

On the other hand, M2 microglia release anti-inflammatory mediators (IL-10, TGF-β, IL-4, IL-13, IGF-1, etc.) (Ponomarev et al. 2013), leading to enhanced expression of genes associated with inflammation reduction and resolution, scavenging and homeostasis (Hu et al. 2012; Pál et al. 2012; Shin et al. 2004; Zhou et al. 2012). The temporal pattern of the relevant markers of correlates of M2 activation is different from the M1 profile. In contrast, the levels of the anti-inflammatory factors IL-10, TGF-β and CD206 mRNA increases as early as first day after ischaemic injury and then peaked within a short period of 3–6 days, and then the levels go on decreasing. In addition, this TGF-β released by M2 microglia promotes an anti-inflammatory profile associated with increased neurogenesis (or cell proliferation) and neuroprotection in the ischaemic brain. This may be therapeutically relevant because TGF-β1 is specifically found in the salvageable peri-infarcted region of the cortex 24 h after a 60 min middle cerebral artery occlusion (MCAO) and is involved in distinct spatiotemporally regulated inflammatory and neuroprotective processes (Kanazawa et al. 2002).

9.4.4 Markers of M_1 & M_2 Phases of Microglia

During disease progression and in normal ageing, microglial activation phenotypes can switch from M2 to M1 (Penninger et al. 2001), as also from M1 to M2 (Hu et al. 2015). Hu et al. (2012) suggest that microglia are activated early after

experimental stroke induced by MCAO surgery, and thence morph into a reactive M1 phenotype by 7 days. The balance between the M1 and M2 states is dynamic in inflammatory responses and may be offset in chronic disease states such as stroke, representing a novel mechanistic target for therapy (Yenari et al. 2010). In addition, there are few molecules that are known to be expressed by macrophages in peripheral inflammation and that have been associated with different functions. These molecules include:

M_1 microglia/macrophage markers:

- CD11b,
- CD45, expressed on all nucleated hematopoietic cells (Bhatia et al. 2011),
- CD68, a marker of active phagocytosis,

M_2 microglia/macrophage markers:

- Ym1, a secretory protein that binds heparin and heparin sulphate,
- CD206, a C-type lectin carbohydrate binding protein.

Both these markers are associated with recovery and function restoration (Butovsky et al. 2006; Marín-Teva et al. 2004).

9.4.5 Immunomodulatory Milieu of Neurogenesis and Synaptic Homeostasis

Not only do microglial cells assist in CNS maturation during development—for example, by mediating the developmental death of neurons (Butovsky et al. 2006)—but they can also release factors that influence adult neurogenesis and glial development (Butovsky et al. 2007; Ekdahl et al. 2003; Kempermann and Neumann 2003; Monje et al. 2003). Microglial cells can thus exert dual effects. Inflammation-associated microglia (M1 subtype) can lessen neurogenesis, whereas microglia (M2 subtype) activated by certain T helper cell cytokines promote neurogenesis. Recent evidence indicates that microglial cells could even be a source of other brain cells. Isolated microglial cells in culture have the potential to generate neurons, astrocytes and oligodendrocytes (Cullheim and Thams 2007; Eglitis and Mezey 1997; Yokoyama et al. 2004, 2006). Besides releasing a number of neurotrophic factors, microglia also structurally remove synapses from damaged neurons (Danton and Dietrich 2003; Trapp et al. 2007). This process has been termed ‘Synaptic Stripping’ by Georg Kreutzberg in the 1960s (Kettenmann et al. 2013). New ‘evidence-based’ vis-à-vis ‘hypothesis-based’ concepts of microglial function especially deserve attention and conversion into basic and clinical research efforts, particularly with regard to macrophagic disease-relevant transformation, orchestrating between microglia’s beneficial versus detrimental contributions.

9.5 Immunomodulatory Challenges in Stroke Treatment

Despite unprecedented advances in basic neurobiology and the current knowledge on stroke, most of the clinical trials for ischaemic stroke till date have been unsuccessful, which raises the question of why most of these therapeutic interventions succeed in animal models but not in clinical application. We now explicate the possible factors that could be responsible for this the paradox in the human scenario:

9.5.1 The Heterogeneity and Complexity of Human Stroke Compared with Animal Models

In animal studies, the majority of studies induce a standardized homogenous injury, namely the MCAO model ('Middle Cerebral Artery Occlusion') administered to healthy young rodents. On the contrary, human stroke is a heterogeneous condition made up of three pathological types: Ischaemic stroke, Haemorrhagic cerebral stroke and Haemorrhagic subarachnoid strokes. Ischaemic stroke is then further divided into several locational subtypes, such as intracranial 'small vessel' disease, 'large-vessel' atherosclerotic disease, and embolism from the heart. These types and subtypes differ in terms of cause, outcome and treatment. Different types of ischaemic stroke also have distinctly different inflammatory features. In addition, the composition of emboli and the location (arteriole or venule) of occlusion may alter stroke pathophysiology (Minnerup et al. 2012). Thus, different therapeutic strategies should be considered for different types of stroke in patients.

9.5.2 Age and Comorbidities

Animal models of stroke performed on young healthy male mice/rats, do not reproduce well the condition of the heterogeneous nature of human stroke, which generally occurs on older people who may have several associated diseases (co-morbidity) (Mergenthaler and Meisel 2012). In order to model the human stroke more closely, aging animals of both sex, and with stroke-related comorbidities, such as diabetes mellitus, atherosclerosis, hyperlipidemia, hypertension or obesity, should be used in preclinical studies (Lambertsen et al. 2009).

9.5.3 Disparity of Outcome Measures

At present, most of the experimental stroke studies only report short-term outcome measures, at around 1–4 week time scale. However, the most important outcome parameters of any intervention in human stroke are long-term (3–6 months) survival and functional recovery (Lambertsen et al. 2009). For translation into clinical

application, long-term outcome, with behavioural and functional analysis, should be performed in experimental stroke studies on animal models.

9.5.4 Post-ischaemic Inflammation May Act Through Multiple Signaling Pathways

The pathways multiplicity may be another possible reason why blocking a single pathway of a cytokine or leukocyte adhesion molecule, does not succeed in clinical trials. In addition, even the same molecule produced by different cells (e.g. TNF-α derived from microglia or from leukocyte) may play different roles in the detrimental signalling cascades in stroke pathology (Kigerl et al. 2009; Zaremba and Losy 2001). Thus, identifying and blocking a common molecular signal shared by different inflammatory cells and mediators, and acting at different cascades, would be a more effective approach to stroke treatment. An encouraging instance is the cyclooxygenase-modulator drug nimuselide that has been used in stroke studies (Candelario-Jalil 2008), the agent acting on multiple pathways.

9.5.5 Use of Animal or Non-human Antibodies for Clinical Studies

The unhumanized antibodies (e.g. mouse anti-human ICAM-1) have been used in clinical trials. However, for patients, it is well known in clinical immunology of other neurological diseases as multiple sclerosis and myelitis, that human antibodies well outperform the use of rodent or porcine-derived antibodies. Hence the priority is need for using human-derived antibodies for clinical trials, instead of customary approach of using rodent ones.

9.5.6 Animal Studies: Randomized Multicentric Study and Sample Size Calculation Needed

Statistically significant sample size and rigorous quantitative power analysis should be used to perform the preclinical study. The experiments should be tested in different laboratories to negate the environmental factors. Many of the clinical trials become unsuccessful in Phase III (multicentric studies), hence it is imperative to screen animal models through a multicentric trial, and if successful, one can then consider translating the same to patients. A thorough experimental design is needed that should include randomization using rigorous statistical procedures, for eliminating experimenter bias. Many analytic software packages now include a randomization procedure. Further, blinded study in collection of the data is another consideration. If the study is not blinded, this should be indicated in the report.

9.5.7 Too-Low/Too-High Doses and Unknown Temporal Optimality

Many pharmacological compounds have hormesis effect in Dose-Response behaviour, namely the behaviour following a parabolic curve or inverted U-shaped graph. A too-low or too-high dose might miss the optimal therapeutic window having the proper response. Furthermore, as we do not have a complete understanding of the pathophysiology of brain damage in our animal models (e.g. activated microglia seem to be deleterious at early stages of lesion formation, while they could be beneficial at later stages) (Olsen et al. 2010), hence the optimal temporal schedule of administration of a given drug is difficult to determine a priori. Therefore, different temporal schedules (single vs. repeated administration, early vs. delayed administration) should be tested before discarding a promising drug.

9.5.8 Other Data Analytic Issues

Such concerns include discreet failure to take the following precautions:

- To follow intent-to-treat principles ("as randomized, so analysed" (Tobin et al. 2014) in the data analysis procedures of animal studies (quantitative and statistical),
- To adjust for multiple comparisons statistically where appropriate, and
- To account for all animals included in the experiment,
- To predefine criteria for excluding animals from analysis after randomization.

While many of these errors are generally more likely to contribute to false-positive studies, a poorly designed study can also lead to false negative results.

9.6 Neuroimmunological Exploration: An Amended Therapeutic Approach to Stroke

We now evolve some directions of therapeutic options, rectifying the approach from the cautionary experimental findings elucidated above.

9.6.1 Increasing Time Window of Thrombolytic Therapy Using Immunomodulatory Agents

Our current armamentarium (the drugs, equipment, and techniques available to a medical practitioner) to treat cerebral ischaemia relies mainly on the use of (i) curative therapy in acute cases, i.e. pharmacological thrombolytics (tissue

Plasminogen Activator, 'tPA') and (ii) preventive therapy in post-acute/chronic cases, i.e. antithrombotic therapy along with correction of the modifiable vascular risk factors for recurrent stroke prevention. The time window for initiating the treatment with tPA is limited to 3 h after stroke symptom onset. The short therapeutic window, stroke severity, concern of the occurrence of major or fatal haemorrhage, severe hypertension, and other variables, greatly limit the number of patients that can benefit from the tPA treatment (Kobayashi et al. 2013). Thus, identifying new strategies that can be used beyond the current time window, by utilization of modulatory manoeuvring of post-ischaemic inflammation by immunomodulatory agents, which can enhance neuroprotection and neuroregeneration, are likely to be a breakthrough in contemporary stroke care.

9.6.2 Neuroprotective Immunomodulation by Polyketide Antibiotics

In a mouse model of a neurodegenerative disease ALS (amyotrophic lateral sclerosis), the second-generation polyketide antibiotics, minocycline and doxycycline, attenuated microglial activation and reduced the expression of M1, but not M2, microglia/macrophage markers, suggesting that minocycline inhibits the pro-inflammatory microglia/macrophages (Weng and Kriz 2007). However, as a caution, minocycline worsens human subjects of ALS in clinical trials due to its aggravating the autoimmune component of ALS (Couzin 2007). Now coming to stroke, minocycline in mice administered two hours after transient MCAO reduced infarct volume by 25 % (Liu et al. 2007). Rats which received continual minocycline treatment for 4 weeks after ischaemia had reduced microglial activation as revealed by microscopy, which correlated with increased neurogenesis and better functional outcome (Brenneman et al. 2010). Further, minocycline in a clinical trial in the patient scenario, has been found to be beneficial for stroke recovery (Lampl et al. 2007). Now, we can paraphrase the disparity of the scope of extrapolating from animal to human situation. The rodent to human translation might not work in a neurodegenerative disease as ALS, but might work in a neurovascular disease as stroke.

9.6.3 Enhancing Therapeutic Neurogenesis by Immunomodulation

Coming to stroke therapy from neuroregenerative aspect, the transplantation of bone marrow cells (as mononuclear cells, BMMC) is also being investigated as a possible treatment for ischaemic stroke in animal models (de Vasconcelos Dos Santos et al. 2010; Keimpema et al. 2009; Sharma et al. 2010). In vitro, BMMCs reduced neuronal death due to (i) LPS and (ii) hypoxia-activated mixed culture of microglia and peritoneal macrophages. Microglial cultures in the presence of BMMCs had higher levels of neuroprotective anti-inflammatory cytokines VEGF, IGF1, SDF-1a and IL-10, (Cardoso et al. 2013). These factors can enable

neurogenesis, neuroprotection, synaptogenesis, angiogenesis and neural stem cell migration to the damaged stroke penumbra. Furthermore, the effect of regenerative intervention on stroke can be enhanced by the neurotropic polyketide antibiotic minocycline. Recent studies have investigated whether the addition of minocycline can improve functional outcome and neuroprotection after BMMC transfer post-ischaemia in vivo. Rats that received minocycline and BMMC treatment had reduced M1 macrophage marker CD68+ cells, and better functional outcome (Franco et al. 2012; Matsukawa et al. 2009).

These studies synoptically suggest that M1 microglia contribute to neuroinflammation after ischaemia, and that BMMC therapy and minocycline have additive effects in reducing post-stroke microglial activation. Nevertheless, the optimal amount and time duration of the dose of minocycline is crucial for benefit; low doses had no beneficial effect, whilst high doses induced toxicity in both neurons and astrocytes (Jiang et al. 2008). To obtain a perspective on these findings we need to have a word of caution from the clinical viewpoint. Even though neuroregenerative cell-based approaches can also be translated satisfactorily to humans in phase I stroke clinical trials (Bhasin et al. 2011), however, similar approach may not work when a study is expanded over larger human population in Phase III clinical trials, due to increasing noisy variation across multiple centres, multiple physicians and multiple patients with differing disposition (Prasad et al. 2014).

9.6.4 Blocking Cascading Effect of Neuroinflammation by Endothelial Modulators

Further studies need to be conducted to hone in on more specific targets in the inflammatory cascades that can hopefully be effective targets for future therapeutic trials. One such target is VAP-1 (vascular adhesion protein-1), which is expressed by endothelial cells and aids in neutrophil transmigration from the vasculature into the brain parenchyma. In the brain, this protein is reported to be primarily found in microvascular cells (endothelium and smooth muscle) (Unzeta et al. 2007), but absent from neurons and glia (Salmi and Jalkanen 2001). VAP-1 converts primary amines into products (e.g., H_2O_2; aldehydes) that are thought to facilitate leukocyte trafficking and promote cytotoxicity in pro-inflammatory conditions (Emsley et al. 2005). The pharmacologic agent LJP-1586, and its predecessor LJP-1207 (both amide-based inhibitors), are highly selective VAP-1 inhibitors and prevent neutrophil transmigration into the brain parenchyma. Furthermore, when these drugs are given to rodents subjected to transient forebrain ischaemia (Krams et al. 2003) or transient middle cerebral artery occlusion 6–12 h after reperfusion (Emsley et al. 2003), there is a profound anti-inflammatory action, which is linked to neuroprotection. These studies and others currently in preclinical development may identify clinical targets for anti-inflammatory therapeutic options in stroke.

9.7 Conclusion and Future Scope

To sum up, there are several issues yet to be unravelled in order to translate promising preclinical findings into clinical practice. As inflammation plays a crucial role in ischaemic stroke, we should be very careful in choosing and using a temporally orchestrating biphasic intervention of the immunomodulatory factors or drugs. Sometimes inflammation is *constructive* (chemotactic migration of the neural stem cells towards the ischaemic core), whilst at other times, it is *deleterious* as the factors released from the inflammatory cells incapacitate the neuron/neural stem cells. Furthermore, researchers working in this field should search and use agent(s) which could specifically block the neurotoxic M1 microglia/macrophage in the acute temporal phase and not the neurotropic M2 microglia/macrophage in the later sub-acute phase.

Finally, before going into further human trials on ischaemic stroke, the following key questions should be clearly and unambiguously answered in order to better understand the dynamics between M1/M2 microglia/macrophage, so as to identify the discrepancies between preclinical studies and clinical trials:

1. Different phases of experimental ischaemic stroke regarding M1 vis-à-vis M2 activation.
2. The dynamic balance in ischaemic stroke between M1/M2 pathways (and finding their modulators).
3. When and how to activate/inhibit the M1/M2 pathways as therapies?
4. Which and when the pro-/-anti-inflammatory mediators secreted by M1/M2 microglia/macrophage to be targeted, and pharmacologically ranking the blockers of each of those mediators?

Indeed, the emerging field of neuroimmunomodulation holds considerable promise for enabling a paradigm shift in understanding the temporal dynamics of neurodegeneration and neuroregeneration in stroke, as well as the relevant signal transduction cascades, and their activating and inhibiting factors or agents. Moreover, the neuroimmunomodulatotry interventions or therapies can be thus designed, taking into account the strategic aspects of dose and duration of the agent, and thereby enable the stroke clinical trial scenario to transform into a considerably more productive one.

References

Alessandrini A, Namura S, Moskowitz MA, Bonventre JV (1999) MEK1 protein kinase inhibition protects against damage resulting from focal cerebral ischemia. Proc Natl Acad Sci USA 96:12866–12869

Barone FC, Irving EA, Ray AM, Lee JC, Kassis S, Kumar S et al (2001) Inhibition of p38 mitogen-activated protein kinase provides neuroprotection in cerebral focal ischemia. Med Res Rev 21:129–145

Benakis C, Bonny C, Hirt L (2010) JNK inhibition and inflammation after cerebral ischemia. Brain Behav Immun 24:800–811

Bhasin A, Srivastava MV, Kumaran SS, Mohanty S, Bhatia R, Bose S et al (2011) Autologous mesenchymal stem cells in chronic stroke. Cerebrovasc Dis Extra 1:93–104

Bhatia S, Fei M, Yarlagadda M, Qi Z, Akira S, Saijo S et al (2011) Rapid host defense against Aspergillus fumigatus involves alveolar macrophages with a predominance of alternatively activated phenotype. PLoS One 6:e15943

Brenneman M, Sharma S, Harting M, Strong R, Cox CS, Aronowski J et al (2010) Autologous bone marrow mononuclear cells enhance recovery after acute ischemic stroke in young and middle-aged rats. J Cereb Blood Flow Metab 30:140–149

Brott T, Bogousslavsky J (2000) Treatment of acute ischemic stroke. N Engl J Med 343:710–722

Butovsky O, Ziv Y, Schwartz A, Landa G, Talpalar AE, Pluchino S et al (2006) Microglia activated by IL-4 or IFN-gamma differentially induce neurogenesis and oligodendrogenesis from adult stem/progenitor cells. Mol Cell Neurosci 31:149–160

Butovsky O, Bukshpan S, Kunis G, Jung S, Schwartz M (2007) Microglia can be induced by IFN-gamma or IL-4 to express neural or dendritic-like markers. Mol Cell Neurosci 35:490–500

Candelario-Jalil E (2008) Nimesulide as a promising neuroprotectant in brain ischemia: new experimental evidences. Pharmacol Res 57:266–273

Cao CX, Yang QW, Lv FL, Cui J, Fu HB, Wang JZ (2007) Reduced cerebral ischemia-reperfusion injury in Toll-like receptor 4 deficient mice. Biochem Biophys Res Commun 353:509–514

Cardoso MM, Franco EC, de Souza CC, da Silva MC, Gouveia A, Gomes-Leal W (2013) Minocycline treatment and bone marrow mononuclear cell transplantation after endothelin-1 induced striatal ischemia. Inflammation 36:197–205

Carloni S, Girelli S, Scopa C, Buonocore G, Longini M, Balduini W (2010) Activation of autophagy and Akt/CREB signaling play an equivalent role in the neuroprotective effect of rapamycin in neonatal hypoxia-ischemia. Autophagy 6:366–377

Caso JR, Pradillo JM, Hurtado O, Lorenzo P, Moro MA, Lizasoain I (2007) Toll-like receptor 4 is involved in brain damage and inflammation after experimental stroke. Circulation 115: 1599–1608

Chauhan A, Sharma U, Jagannathan NR, Reeta KH, Gupta YK (2011) Rapamycin protects against middle cerebral artery occlusion induced focal cerebral ischemia in rats. Behav Brain Res 225:603–609

Couzin J (2007) Clinical research. ALS trial raises questions about promising drug. Science 318:1227

Cullheim S, Thams S (2007) The microglial networks of the brain and their role in neuronal network plasticity after lesion. Brain Res Rev 55:89–96

Danton GH, Dietrich WD (2003) Inflammatory mechanisms after ischemia and stroke. J Neuropathol Exp Neurol 62:127–136

Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S et al (2005) ATP mediates rapid microglial response to local brain injury in vivo. Nat Neurosci 8:752–758

de Vasconcelos Dos Santos A, da Costa Reis J, Diaz Paredes B, Moraes L, Jasmin, Giraldi-Guimarães A, et al (2010) Therapeutic window for treatment of cortical ischemia with bone marrow-derived cells in rats. Brain Res 1306:149–158

Denes A, Vidyasagar R, Feng J, Narvainen J, McColl BW, Kauppinen RA et al (2007) Proliferating resident microglia after focal cerebral ischaemia in mice. J Cereb Blood Flow Metab 27:1941–1953

Eglitis MA, Mezey E (1997) Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. Proc Natl Acad Sci USA 94:4080–4085

Ekdahl CT, Claasen JH, Bonde S, Kokaia Z, Lindvall O (2003) Inflammation is detrimental for neurogenesis in adult brain. Proc Natl Acad Sci USA 100:13632–13637

Emsley HC, Smith CJ, Gavin CM, Georgiou RF, Vail A, Barberan EM et al (2003) An early and sustained peripheral inflammatory response in acute ischaemic stroke: relationships with infection and atherosclerosis. J Neuroimmunol 139:93–101

Emsley HC, Smith CJ, Georgiou RF, Vail A, Hopkins SJ, Rothwell NJ et al (2005) A randomised phase II study of interleukin-1 receptor antagonist in acute stroke patients. J Neurol Neurosurg Psychiatry 76:1366–1372

Faraco G, Fossati S, Bianchi ME, Patrone M, Pedrazzi M, Sparatore B et al (2007) High mobility group box 1 protein is released by neural cells upon different stresses and worsens ischemic neurodegeneration in vitro and in vivo. J Neurochem 103:590–603

Fletcher L, Evans TM, Watts LT, Jimenez DF, Digicaylioglu M (2013) Rapamycin treatment improves neuron viability in an in vitro model of stroke. PLoS One 8:e68281

Franco EC, Cardoso MM, Gouvêia A, Pereira A, Gomes-Leal W (2012) Modulation of microglial activation enhances neuroprotection and functional recovery derived from bone marrow mononuclear cell transplantation after cortical ischemia. Neurosci Res 73:122–132

Guan QH, Pei DS, Liu XM, Wang XT, Xu TL, Zhang GY (2006) Neuroprotection against ischemic brain injury by SP600125 via suppressing the extrinsic and intrinsic pathways of apoptosis. Brain Res 1092:36–46

Hamanaka J, Hara H (2011) Involvement of Toll-like receptors in ischemia-induced neuronal damage. Cent Nerv Syst Agents Med Chem 11:107–113

Hanisch UK, Kettenmann H (2007) Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat Neurosci 10:1387–1394

Hayakawa K, Qiu J, Lo EH (2010) Biphasic actions of HMGB1 signaling in inflammation and recovery after stroke. Ann N Y Acad Sci 1207:50–57

Hill WD, Hess DC, Carroll JE, Wakade CG, Howard EF, Chen Q et al (2001) The NF-kappaB inhibitor diethyldithiocarbamate (DDTC) increases brain cell death in a transient middle cerebral artery occlusion model of ischemia. Brain Res Bull 55:375–386

Hu X, Li P, Guo Y, Wang H, Leak RK, Chen S et al (2012) Microglia/macrophage polarization dynamics reveal novel mechanism of injury expansion after focal cerebral ischemia. Stroke 43:3063–3070

Hu X, Leak RK, Shi Y, Suenaga J, Gao Y, Zheng P et al (2015) Microglial and macrophage polarization—new prospects for brain repair. Nat Rev Neurol 11:56–64

Hua F, Ma J, Ha T, Xia Y, Kelley J, Williams DL et al (2007) Activation of Toll-like receptor 4 signaling contributes to hippocampal neuronal death following global cerebral ischemia/reperfusion. J Neuroimmunol 190:101–111

Huang P, Han J, Hui L (2010) MAPK signaling in inflammation-associated cancer development. Protein Cell 1:218–226

Ikonomidou C, Turski L (2002) Why did NMDA receptor antagonists fail clinical trials for stroke and traumatic brain injury? Lancet Neurol 1:383–386

Jiang ZJ, Richardson JS, Yu PH (2008) The contribution of cerebral vascular semicarbazide-sensitive amine oxidase to cerebral amyloid angiopathy in Alzheimer's disease. Neuropathol Appl Neurobiol 34:194–204

Jin R, Yang G, Li G (2010) Inflammatory mechanisms in ischemic stroke: role of inflammatory cells. J Leukoc Biol 87:779–789

Kaltschmidt B, Widera D, Kaltschmidt C (2005) Signaling via NF-kappaB in the nervous system. Biochim Biophys Acta 1745:287–299

Kaminska B (2005) MAPK signalling pathways as molecular targets for anti-inflammatory therapy–from molecular mechanisms to therapeutic benefits. Biochim Biophys Acta 1754: 253–262

Kanazawa H, Ohsawa K, Sasaki Y, Kohsaka S, Imai Y (2002) Macrophage/microglia-specific protein Iba1 enhances membrane ruffling and Rac activation via phospholipase C-gamma—dependent pathway. J Biol Chem 277:20026–20032

Kawasaki H, Morooka T, Shimohama S, Kimura J, Hirano T, Gotoh Y et al (1997) Activation and involvement of p38 mitogen-activated protein kinase in glutamate-induced apoptosis in rat cerebellar granule cells. J Biol Chem 272:18518–18521

Keimpema E, Fokkens MR, Nagy Z, Agoston V, Luiten PG, Nyakas C et al (2009) Early transient presence of implanted bone marrow stem cells reduces lesion size after cerebral ischaemia in adult rats. Neuropathol Appl Neurobiol 35:89–102

Kempermann G, Neumann H (2003) Neuroscience. Microglia: the enemy within? Science 302:1689–1690

Kettenmann H, Kirchhoff F, Verkhratsky A (2013) Microglia: new roles for the synaptic stripper. Neuron 77:10–18

Khan M, Sekhon B, Giri S, Jatana M, Gilg AG, Ayasolla K et al (2005) S-Nitrosoglutathione reduces inflammation and protects brain against focal cerebral ischemia in a rat model of experimental stroke. J Cereb Blood Flow Metab 25:177–192

Kigerl KA, Gensel JC, Ankeny DP, Alexander JK, Donnelly DJ, Popovich PG (2009) Identification of two distinct macrophage subsets with divergent effects causing either neurotoxicity or regeneration in the injured mouse spinal cord. J Neurosci 29:13435–13444

Kinouchi H, Sharp FR, Hill MP, Koistinaho J, Sagar SM, Chan PH (1993) Induction of 70-kDa heat shock protein and hsp70 mRNA following transient focal cerebral ischemia in the rat. J Cereb Blood Flow Metab 13:105–115

Kobayashi K, Imagama S, Ohgomori T, Hirano K, Uchimura K, Sakamoto K et al (2013) Minocycline selectively inhibits M1 polarization of microglia. Cell Death Dis 4:e525

Krams M, Lees KR, Hacke W, Grieve AP, Orgogozo JM, Ford GA et al (2003) Acute Stroke Therapy by Inhibition of Neutrophils (ASTIN): an adaptive dose-response study of UK-279,276 in acute ischemic stroke. Stroke 34:2543–2548

Lambertsen KL, Clausen BH, Babcock AA, Gregersen R, Fenger C, Nielsen HH et al (2009) Microglia protect neurons against ischemia by synthesis of tumor necrosis factor. J Neurosci 29:1319–1330

Lampl Y, Boaz M, Gilad R, Lorberboym M, Dabby R, Rapoport A et al (2007) Minocycline treatment in acute stroke: an open-label, evaluator-blinded study. Neurology 69:1404–1410

Laplante M, Sabatini DM (2009) mTOR signaling at a glance. J Cell Sci 122:3589–3594

Lehnardt S, Schott E, Trimbuch T, Laubisch D, Krueger C, Wulczyn G et al (2008) A vicious cycle involving release of heat shock protein 60 from injured cells and activation of toll-like receptor 4 mediates neurodegeneration in the CNS. J Neurosci 28:2320–2331

Liu H, Xin L, Chan BP, Teoh R, Tang BL, Tan YH (2002) Interferon-beta administration confers a beneficial outcome in a rabbit model of thromboembolic cerebral ischemia. Neurosci Lett 327:146–148

Liu Z, Fan Y, Won SJ, Neumann M, Hu D, Zhou L et al (2007) Chronic treatment with minocycline preserves adult new neurons and reduces functional impairment after focal cerebral ischemia. Stroke 38:146–152

Maddahi A, Edvinsson L (2008) Enhanced expressions of microvascular smooth muscle receptors after focal cerebral ischemia occur via the MAPK MEK/ERK pathway. BMC Neurosci 9:85

Magnus T, Chan A, Grauer O, Toyka KV, Gold R (2001) Microglial phagocytosis of apoptotic inflammatory T cells leads to down-regulation of microglial immune activation. J Immunol 167:5004–5010

Marín-Teva JL, Dusart I, Colin C, Gervais A, van Rooijen N, Mallat M (2004) Microglia promote the death of developing Purkinje cells. Neuron 41:535–547

Matsukawa N, Yasuhara T, Hara K, Xu L, Maki M, Yu G et al (2009) Therapeutic targets and limits of minocycline neuroprotection in experimental ischemic stroke. BMC Neurosci 10:126

Mergenthaler P, Meisel A (2012) Do stroke models model stroke? Dis Model Mech 5:718–725

Minnerup J, Sutherland BA, Buchan AM, Kleinschnitz C (2012) Neuroprotection for stroke: current status and future perspectives. Int J Mol Sci 13:11753–11772

Monje ML, Toda H, Palmer TD (2003) Inflammatory blockade restores adult hippocampal neurogenesis. Science 302:1760–1765

Napetschnig J, Wu H (2013) Molecular basis of NF-κB signaling. Annu Rev Biophys 42:443–468

Nechipurenko NI, Kotova SG, Shal'kevich VB, Vlasiuk PA, Griboedova TV (2001) Experimental basis and clinical evaluation of indomethacin efficacy in patients with ischemic stroke. Zh Nevrol Psikhiatr Im S S Korsakova 56–61

Nimmerjahn A, Kirchhoff F, Helmchen F (2005) Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308:1314–1318

Olsen J, Christensen K, Murray J, Ekbom A (2010) An introduction to epidemiology for health professionals, edn 1. Springer Science and Business Media, New York

Pál G, Vincze C, Renner É, Wappler EA, Nagy Z, Lovas G et al (2012) Time course, distribution and cell types of induction of transforming growth factor betas following middle cerebral artery occlusion in the rat brain. PLoS ONE 7:e46731

Penninger JM, Irie-Sasaki J, Sasaki T, Oliveira-dos-Santos AJ (2001) CD45: new jobs for an old acquaintance. Nat Immunol 2:389–396

Peri F, Nüsslein-Volhard C (2008) Live imaging of neuronal degradation by microglia reveals a role for v0-ATPase a1 in phagosomal fusion in vivo. Cell 133:916–927

Ponomarev ED, Veremeyko T, Weiner HL (2013) MicroRNAs are universal regulators of differentiation, activation, and polarization of microglia and macrophages in normal and diseased CNS. Glia 61:91–103

Prasad K, Sharma A, Garg A, Mohanty S, Bhatnagar S, Johri S et al (2014) Intravenous autologous bone marrow mononuclear stem cell therapy for ischemic stroke: a multicentric, randomized trial. Stroke 45:3618–3624

Qiu J, Nishimura M, Wang Y, Sims JR, Qiu S, Savitz SI et al (2008) Early release of HMGB-1 from neurons after the onset of brain ischemia. J Cereb Blood Flow Metab 28:927–938

Ribes S, Ebert S, Czesnik D, Regen T, Zeug A, Bukowski S et al (2009) Toll-like receptor prestimulation increases phagocytosis of Escherichia coli DH5alpha and Escherichia coli K1 strains by murine microglial cells. Infect Immun 77:557–564

Ribes S, Ebert S, Regen T, Agarwal A, Tauber SC, Czesnik D et al (2010) Toll-like receptor stimulation enhances phagocytosis and intracellular killing of nonencapsulated and encapsulated Streptococcus pneumoniae by murine microglia. Infect Immun 78:865–871

Ridder DA, Schwaninger M (2009) NF-kappaB signaling in cerebral ischemia. Neuroscience 158:995–1006

Salmi M, Jalkanen S (2001) VAP-1: an adhesin and an enzyme. Trends Immunol 22:211–216

Sawe N, Steinberg G, Zhao H (2008) Dual roles of the MAPK/ERK1/2 cell signaling pathway after stroke. J Neurosci Res 86:1659–1669

Schneider A, Martin-Villalba A, Weih F, Vogel J, Wirth T, Schwaninger M (1999) NF-kappaB is activated and promotes cell death in focal cerebral ischemia. Nat Med 5:554–559

Sharma S, Yang B, Strong R, Xi X, Brenneman M, Grotta JC et al (2010) Bone marrow mononuclear cells protect neurons and modulate microglia in cell culture models of ischemic stroke. J Neurosci Res 88:2869–2876

Shichita T, Ago T, Kamouchi M, Kitazono T, Yoshimura A, Ooboshi H (2012a) Novel therapeutic strategies targeting innate immune responses and early inflammation after stroke. J Neurochem 123(Suppl 2):29–38

Shichita T, Hasegawa E, Kimura A, Morita R, Sakaguchi R, Takada I et al (2012b) Peroxiredoxin family proteins are key initiators of post-ischemic inflammation in the brain. Nat Med 18:911–917

Shin WH, Lee DY, Park KW, Kim SU, Yang MS, Joe EH et al (2004) Microglia expressing interleukin-13 undergo cell death and contribute to neuronal survival in vivo. Glia 46:142–152

Sughrue ME, Mehra A, Connolly ES, D'Ambrosio AL (2004) Anti-adhesion molecule strategies as potential neuroprotective agents in cerebral ischemia: a critical review of the literature. Inflamm Res 53:497–508

Thomson AW, Turnquist HR, Raimondi G (2009) Immunoregulatory functions of mTOR inhibition. Nat Rev Immunol 9:324–337

Tobin MK, Bonds JA, Minshall RD, Pelligrino DA, Testai FD, Lazarov O (2014) Neurogenesis and inflammation after ischemic stroke: what is known and where we go from here. J Cereb Blood Flow Metab 34:1573–1584

Trapp BD, Wujek JR, Criste GA, Jalabi W, Yin X, Kidd GJ et al (2007) Evidence for synaptic stripping by cortical microglia. Glia 55:360–368

Unzeta M, Solé M, Boada M, Hernández M (2007) Semicarbazide-sensitive amine oxidase (SSAO) and its possible contribution to vascular damage in Alzheimer's disease. J Neural Transm 114:857–862

Varnum MM, Ikezu T (2012) The classification of microglial activation phenotypes on neurodegeneration and regeneration in Alzheimer's disease brain. Arch Immunol Ther Exp (Warsz) 60:251–266

Veldhuis WB, Derksen JW, Floris S, Van Der Meide PH, De Vries HE, Schepers J et al (2003) Interferon-beta blocks infiltration of inflammatory cells and reduces infarct volume after ischemic stroke in the rat. J Cereb Blood Flow Metab 23:1029–1039

Wake H, Moorhouse AJ, Jinno S, Kohsaka S, Nabekura J (2009) Resting microglia directly monitor the functional state of synapses in vivo and determine the fate of ischemic terminals. J Neurosci 29:3974–3980

Wang H, Xu L, Venkatachalam S, Trzaskos JM, Friedman SM, Feuerstein GZ et al (2001) Differential regulation of IL-1beta and TNF-alpha RNA expression by MEK1 inhibitor after focal cerebral ischemia in mice. Biochem Biophys Res Commun 286:869–874

Wang X, Wang H, Xu L, Rozanski DJ, Sugawara T, Chan PH et al (2003) Significant neuroprotection against ischemic brain injury by inhibition of the MEK1 protein kinase in mice: exploration of potential mechanism associated with apoptosis. J Pharmacol Exp Ther 304:172–178

Wang RM, Zhang QG, Li J, Yang LC, Yang F, Brann DW (2009) The ERK5-MEF2C transcription factor pathway contributes to anti-apoptotic effect of cerebral ischemia preconditioning in the hippocampal CA1 region of rats. Brain Res 1255:32–41

Wang Y, Zhang Z, Chow N, Davis TP, Griffin JH, Chopp M et al (2012a) An activated protein C analog with reduced anticoagulant activity extends the therapeutic window of tissue plasminogen activator for ischemic stroke in rodents. Stroke 43:2444–2449

Wang LW, Tu YF, Huang CC, Ho CJ (2012b) JNK signaling is the shared pathway linking neuroinflammation, blood-brain barrier disruption, and oligodendroglial apoptosis in the white matter injury of the immature brain. J Neuroinflammation 9:175

Wechsler LR, Jovin TG (2012) Intravenous recombinant tissue-type plasminogen activator in the extended time window and the US food and drug administration: confused about the time. Stroke 43:2517–2519

Weng YC, Kriz J (2007) Differential neuroprotective effects of a minocycline-based drug cocktail in transient and permanent focal cerebral ischemia. Exp Neurol 204:433–442

Wiederrecht GJ, Sabers CJ, Brunn GJ, Martin MM, Dumont FJ, Abraham RT (1995) Mechanism of action of rapamycin: new insights into the regulation of G1-phase progression in eukaryotic cells. Prog Cell Cycle Res 1:53–71

Xia Z, Dickens M, Raingeaud J, Davis RJ, Greenberg ME (1995) Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science 270:1326–1331

Xie L, Sun F, Wang J, Mao X, Yang SH, Su DM et al (2014) mTOR signaling inhibition modulates macrophage/microglia-mediated neuroinflammation and secondary injury via regulatory T cells after focal ischemia. J Immunol 192:6009–6019

Yamashita T, Ninomiya M, Hernández Acosta P, García-Verdugo JM, Sunabori T, Sakaguchi M et al (2006) Subventricular zone-derived neuroblasts migrate and differentiate into mature neurons in the post-stroke adult striatum. J Neurosci 26:6627–6636

Yang QW, Lu FL, Zhou Y, Wang L, Zhong Q, Lin S et al (2011) HMBG1 mediates ischemia-reperfusion injury by TRIF-adaptor independent Toll-like receptor 4 signaling. J Cereb Blood Flow Metab 31:593–605

Yenari MA, Kauppinen TM, Swanson RA (2010) Microglial activation in stroke: therapeutic targets. Neurotherapeutics 7:378–391

Yokoyama A, Yang L, Itoh S, Mori K, Tanaka J (2004) Microglia, a potential source of neurons, astrocytes, and oligodendrocytes. Glia 45:96–104

Yokoyama A, Sakamoto A, Kameda K, Imai Y, Tanaka J (2006) NG2 proteoglycan-expressing microglia as multipotent neural progenitors in normal and pathologic brains. Glia 53:754–768

Zaremba J, Losy J (2001) Early TNF-alpha levels correlate with ischaemic stroke severity. Acta Neurol Scand 104:288–295

Zhang J, Takahashi HK, Liu K, Wake H, Liu R, Maruo T et al (2011) Anti-high mobility group box-1 monoclonal antibody protects the blood-brain barrier from ischemia-induced disruption in rats. Stroke 42:1420–1428

Zhou X, Spittau B, Krieglstein K (2012) TGFβ signalling plays an important role in IL4-induced alternative activation of microglia. J Neuroinflammation 9:210

Ziegler G, Harhausen D, Schepers C, Hoffmann O, Röhr C, Prinz V et al (2007) TLR2 has a detrimental role in mouse transient focal cerebral ischemia. Biochem Biophys Res Commun 359:574–579

Brain Tumors and Inflammation

10

Khushboo Irshad, Evanka Madan and Kunzang Chosdol

Abstract

The heterocellular concept of tumor development where the interplay of both cancer cells as well as non-cancer cells potentiates the progression of cancer is well accepted. The relationship between cancer and inflammation derived from such heterotypic interactions has been known for long. The biology of cancer is illustrated by key features called the 'hallmarks of cancer'. These hallmarks include proliferative signaling, resistance to anti-proliferative signals, evasion of apoptosis, replicative immortality, maintenance of vascularisation; and activation of tissue invasion and metastasis. In addition, tumor-promoting inflammation has been recognized as one of the emerging hallmarks of cancer. Two main pathways have been known to link cancer and inflammation: the intrinsic pathway and the extrinsic pathway. Regardless of the stimulus, whether extrinsic (infections, non-healing wounds, irritants, etc.) or intrinsic (oncogenes, protein kinases, etc.), inflammation is responsible for augmenting tumor progression by promoting angiogenesis; cells proliferation and survival; evasion of cell death; weakening of adaptive immune responses and altering cellular response to therapy. Brain tumors can be divided into *primary tumors* that originate within the brain, and *secondary tumors* that metastasize to the brain from primary extracranial tumors. Inflammation is closely associated with primary brain tumors and facilitates tumor progression and invasiveness. This chapter focuses on the role of inflammatory mediators and the inflammatory signaling cascades in cancers; recent advances in understanding the role of inflammatory mediators in primary brain tumors; and current challenges impeding the therapeutic intervention in inflammatory pathways in brain cancer as well as the future prospects of immunotherapy in brain tumors.

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10.1 Introduction

10.1.1 Brain Tumors

Brain tumors are categorized into two main groups, namely, primary and secondary brain tumors. The primary brain tumors are a heterogenous group of malignancies that originate and reside within the brain. In contrast, secondary brain tumors originate from a primary cancer outside the central nervous system (CNS) and are metastasised to the brain.

Primary brain tumors are classified on the basis of cellular origin and histologic characteristics into different types and grades (Louis et al. 2007). The grading system of primary brain tumors published by the World Health Organization (WHO) is accepted worldwide (Pollo 2012; Louis et al. 2007). WHO grading helps in predicting the biological outcome as well as provide a universally acceptable index, which would correlate with tumor behavior, response to therapy, propensity for recurrence and overall prognosis (Louis et al. 2007).

Among the primary brain tumors, according to CBTRUS statistical report (Ostrom et al. 2013), the most common is meningioma, accounting for more than one-third of all primary brain tumors. They are graded into WHO grade I, II, and III. Meningiomas are relatively less malignant and can be easily managed clinically as compared to gliomas due to their easy accessibility for surgical resection. Gliomas which originate in the glial cells (supportive cells) are the second most common group, accounting for approximately 28 % of all tumors and 80 % of the primary malignant brain tumors (Ostrom et al. 2013). Gliomas are further classified into astrocytoma, derived from astrocytes; oligodendroglioma from oligodendrocytes and ependymoma from ependymal cells. Astrocytomas, oligodendrogliomas and ependymomas are further subdivided into different WHO grades (Kleihues et al. 1993; Longo et al. 2012). Astrocytomas are subdivided into; grade I (pilocytic astrocytoma), grade II (diffuse astrocytoma), grade III (anaplastic astrocytoma), and grade IV (glioblastoma; GBM). GBM (WHO grade IV) is the most common and most aggressive type of glioma. Glioblastoma is further classified into primary or de novo GBM and secondary GBM. Primary glioblastomas are those that arise de novo with a short clinical history (usually <3 months) without any evidence of an earlier precursor lesion whereas secondary glioblastomas progress over a period of years from a lower grade astrocytoma (Kleihues and Ohgaki 1999; Kleihues et al. 1993). Similarly, oligodendrogliomas, and ependymomas are also graded into WHO grade I, II, and III. In general, WHO grade I and II of all tumor types are regarded as less malignant as compared to grade III and IV (Table 10.1). Other less frequent tumors comprising the remaining 20–30 % of primary brain tumors include acoustic neuroma (8–10 %), pituitary tumors (10–15 %), medulloblastomas (1 %), and lymphomas (2 %) (Longo et al. 2012).

Patient survival, time to tumor progression and response to therapy in primary brain tumors are all associated with tumor grade (Louis et al. 2007). Therapy is

Table 10.1 WHO classification of gliomas (Pollo 2012; Louis et al. 2007; Vigneswaran et al. 2015; Walker et al. 2011)

Type	Grade	Histopathological features	Median survival (years)
Pilocytic astrocytoma	I	Relatively circumscribed, slow growing, cystic astrocytomas, comprise 5–6 % of all gliomas	Usually cured by surgical resection
Oligodendroglioma	II	Tumors of white matter and cortex of cerebral hemispheres, low rate of mitosis, necrosis absent	12
Oligoastrocytoma	II	Diffuse tumors, mixed glial background	3 to >10
Diffuse astrocytoma	II	Diffuse infiltration into neighboring neural tissue; moderately increased cellularity; mitotic activity generally absent; occasional atypical nuclei and some cells with enlarged cytoplasm	6–8
Anaplastic astrocytoma/ oligodendroglioma	III	Infiltrating tumors with increased cellularity, mitotic activity and nuclear atypia; no necrosis or vascular proliferation, mild infiltration by neutrophils	3
Glioblastoma (GBM)	IV	Extremely infiltrating tumors characterized by necrosis and prominent microvascular proliferation forming multilayered vessels; high rate of mitosis; pleomorphic astrocytic cells with marked nuclear atypia	1–2

decided mostly on the basis of histologic classification but since it can be subject to interobserver variation and difficult at times (Gravendeel et al. 2009), genetic alterations and gene expression profiles have recently been recognized as an adjunct to histopathological diagnosis and patient prognosis (Walker et al. 2011). Gene expression profiling has been shown to reveal intrinsic molecular subtypes of gliomas that correlate better with patient survival than histologic diagnosis (Gravendeel et al. 2009; Li et al. 2009; Shirahata et al. 2007). It is, therefore, conceivable to divide GBM into subsets based upon molecular signatures of genes that regulate glioma progression with the purpose to develop prognostic markers as well as more specifically targeted and effective treatments (Rich and Bigner 2004; Ruano et al. 2008). On the basis of molecular signatures, designing a tailor made therapy has become a reality which could prove to be cost effective, especially in a developing country like ours where the majority of patients belong to economically weaker section.

Secondary brain tumors are those which are metastasized to CNS from tumors originated in other parts of the body. The tumors of lung, breast, kidney, colon, melanoma, etc., are the most common types of cancers associated with brain metastases (Rivkin and Kanoff 2013). Secondary brain tumors are known to be three times more common than all the primary brain tumors combined (Longo et al. 2012). Since secondary tumors are detected in the late stage of the tumor originated

in the extra cranial region, therapy is not much beneficial to the patients who are terminally ill and are usually on palliative treatment.

10.1.2 Inflammation and Cancer

Inflammation is an integral part of physiological and pathological processes such as wound healing and infection (Coussens and Werb 2002; Reinke and Sorg 2012). The inflammation associated with wound healing is a controlled process which is self-limiting. The resolution of inflammation is brought about by rapid clearance of the inflammatory cells by neighboring macrophages and dendritic cells through induction of apoptosis (Savill et al. 2002; Savill and Fadok 2000). However, in case this resolution gets dysregulated, the continuous tissue damage may favor chronic inflammation and sustained cell proliferation, thereby, predisposing cells to neoplasia (Balkwill and Mantovani 2001). Hence, tumors have often been referred to as 'wounds that fail to heal' (Dvorak 1986).

The initial evidences for association between inflammation and cancer were obtained from epidemiologic and clinical studies (Balkwill and Mantovani 2001; Coussens and Werb 2002). The risk of colorectal cancer is known to be around 10-fold greater in patients with inflammatory diseases like ulcerative colitis (Itzkowitz and Yio 2004; Seril et al. 2003), chronic hepatitis caused by HBV/HCV predisposes to hepatocellular carcinoma (Hoshida et al. 2014; Zhang et al. 2014; Block et al. 2003) and in the gastrointestinal tract, *Helicobacter pylori* infection is the leading cause of adenocarcinoma and lymphoma (Kim et al. 2011; Coussens and Werb 2002; Macarthur et al. 2004). The increased incidence of lung cancer is also positively associated with the severity and duration of inflammatory diseases in the respiratory system (Valavanidis et al. 2013; Borm and Driscoll 1996; Keeley and Rees 1997).

Recent cancer research has stratified the heterocellular concept of tumor development where the interplay of both cancer as well as non-cancer cells (e.g., immune cells) is known to potentiate tumor progression. Fundamentally, the biology of cancer is illustrated by six essential features (proliferative signaling, resistance to anti-proliferative signals, evasion of apoptosis, replicative immortality, maintenance of vascularisation; and activation of tissue invasion and metastasis) of tumor cells (Hanahan and Weinberg 2000, 2011). More recently, tumor-promoting inflammation is being discussed as one of the emerging hallmarks of cancer (Colotta et al. 2009; Hanahan and Weinberg 2011). In most solid tumors, including brain cancers, apart from the inflammation-inducing genetic events in tumor cells, the cells undergoing necrosis release proinflammatory signals to recruit immune cells into the microenvironment (Hanahan and Weinberg 2011; Grivennikov et al. 2010), responsible for promoting tumorigenesis. Furthermore, the inflammatory cells, namely, macrophages and leukocytes recruited into the tumor microenvironment releases reactive oxygen species which is deleterious and acts as mutagenic for the nearby cancer cells, thereby, accelerating their malignancy (Grivennikov

et al. 2010). Hence, the process of inflammation is an enabling characteristic during the acquisition of the classical hallmark features by the tumor cells.

10.1.3 Mechanisms of Cancer-Related Inflammation

Inflammation and cancer can be connected to each other via two pathways. One is extrinsic pathway, driven by chronic inflammatory condition/disease with increased accumulation of inflammatory cells like leukocytes, releasing inflammatory mediators at the site which increases the risk of cancer development. Another is intrinsic pathway, mediated by genetic alterations of the tumor cells (e.g., activation of oncogenes by mutations, chromosomal rearrangement and amplification or inactivation of tumor suppressor genes) that result in the release of cytokines and chemokines, leading to inflammatory microenvironment and promoting tumor progression (Colotta et al. 2009; Mantovani et al. 2008). The intrinsic mechanism, thus, holds the genetic events that initiate tumorigenesis as responsible for generating an inflammatory environment (Mantovani et al. 2008). Regardless of the original pathway, whether extrinsic (non-healing wounds, irritants, infections, etc.) or intrinsic (oncogenes, protein kinases, etc.), the inflammatory cytokines and signals are responsible for triggering an inflammatory microenvironment in tumors which augment tumor progression by aiding the survival and proliferation of tumor cells, evading cell death, promoting angiogenesis, weakening adaptive immune responses and altering cellular response to therapy.

The role and significance of inflammation and its related mechanisms during cancer progression have been elaborately studied in most of the solid tumors, including brain tumors. In this chapter, the role of inflammatory cells and mediators in cancers in general is discussed first, followed by a discussion of the known as well as potential mechanisms and mediators involved in primary brain tumors.

10.2 Role of Inflammatory Cells and Mediators in Tumor Development

Although the immune system provides an early protection against cancer, the healing arm of inflammatory responses is exploited by the tumor to promote its growth and metastasis (Abad et al. 2014; Hanahan and Weinberg 2011). A wide population of leukocytes and other immune cells infiltrate into the developing tumor site and establish a proinflammatory tumor microenvironment (Yang et al. 2005a). Basically, the infiltration of these cells to tumors is meant to repress tumor growth (Brigati et al. 2002; Dunn et al. 2002; Nakano et al. 2001; Tsung et al. 2002; Zhang et al. 2003). However, these cells may act more as tumor promoters (Coussens and Werb 2002; Khong and Restifo 2002; Smyth et al. 2004). Tumor cells may also themselves produce cytokines and chemokines to attract immune cells and further facilitate cancer development (Coussens and Werb 2002; Lin and Pollard 2004; Yang et al. 2005a).

Macrophages, neutrophils, eosinophils, dendritic cells, mast cells and lymphocytes form the key components of inflammation in epithelial-originated tumors (Coussens and Werb 2001; Macarthur et al. 2004; Yang et al. 2005a). Among primary brain tumors, malignant gliomas are histologically heterogeneous and characterized by diffuse infiltration into normal brain parenchyma (Rolle et al. 2012; Lisi et al. 2014). Microglia and macrophages represent the largest population of tumor-infiltrating cells in glioma and account for one-third of the total tumor (Lisi et al. 2014).

Clinical studies have shown increased tumor-associated macrophages (TAM) density to be associated with poor prognosis in cancers (Amann et al. 1998; Leek and Harris 2002; Lin et al. 2002; Saji et al. 2001). They release IL-10 and prostaglandin E2, which suppress anti-tumor response (Elgert et al. 1998), apart from releasing angiogenic factors like vascular endothelial growth factor (VEGF), endothelin-2 and urokinase-type plasminogen activator (uPA) (Bando and Toi 2000; Foekens et al. 2000; Fox et al. 2001; Grimshaw et al. 2002; Pollard 2004). TAMs facilitate tumor cell invasion and metastasis by releasing inflammation-induced matrix metalloproteinases (MMPs), MMP-2, and MMP-9, which are responsible for degradation of extracellular matrix and basement membrane (Coussens et al. 1999; Pollard 2004). Also, TAMs release TNF-α, iNOS, epidermal growth factor and other epidermal growth factor receptor (EGFR) family ligands to promote tumor cell proliferation and migration (Leek and Harris 2002; Leek et al. 2000; Wyckoff et al. 2000). On the other hand, activated mast cells participate in tumor angiogenesis, invasion and metastasis by generating VEGF/vascular permeability factor, basic FGF, specific proteases, etc., (Hiromatsu and Toda 2003; Lin and Pollard 2004; Ribatti et al. 2001). Mast cells are also responsible for the release of cytokines and chemokines (Lin and Pollard 2004). Tumor-associated neutrophils enhance tumor development in a manner similar to TAMs and mast cells (Lin and Pollard 2004; Scapini et al. 2002; Schaider et al. 2003). They are known to participate in the genetic instability of tumors (Haqqani et al. 2000). T-lymphocytes are recruited to the tumors by a series of chemokines and the increase of CD4+ T-cells has been positively correlated with poor prognosis in several cancers (Bromwich et al. 2003; Canna et al. 2005).

Several molecules have been identified as crucial for the regulation of inflammation during cancer progression, including in brain tumors. These mediators comprise cytokines and chemokines; certain transcription factors and enzymes that link inflammation with cancer.

10.2.1 Cytokines

The immune response to tumors is constituted by cytokines which are produced by both tumor cells as well as host stromal cells (Smyth et al. 2004). ILs, TNF-α, growth factors, and differentiation factors (colony-stimulating factors) are secreted or membrane-bound cytokines that play a regulatory role in the growth, differentiation and activation of immune cells (Dranoff 2004). Alterations in the levels of

various proinflammatory as well as anti-inflammatory cytokines, facilitate tumor development by initiating a spectrum of signaling cascades at the inflammatory sites (Dranoff 2004; Philip et al. 2004; Smyth et al. 2004). Tumor derived cytokines such as Fas ligand, VEGF, and TGF-β help in suppression of immune response to the tumors (Smyth et al. 2004). TNF, produced by activated macrophages and also by tumor cells, promotes angiogenesis and tumor growth by inducing angiogenic factors, thymidine phosphorylase and MMPs (Aggarwal 2003; Balkwill 2002; Balkwill and Mantovani 2001; Leek et al. 1998). It also links inflammation to cancer by regulating of a network of chemokines (Balkwill 2002). Chemokines, the largest family of cytokines, are major soluble regulators that control the directional migration of leukocytes to the inflammatory site (Daniel et al. 2003). Chemokines, like CXCR2, are also known to promote preneoplastic cell transformation and tumor cell growth (Coussens and Werb 2002; Strieter 2001). CXCL-8 has been documented for its role in initiating tumor inflammation and angiogenesis, thus facilitating cancer progression (Sparmann and Bar-Sagi 2004). Moreover, chemokines also facilitate tumor invasion and metastasis in various cancer types (Ardestani et al. 1999; Daniel et al. 2003; Wilson and Balkwill 2002) by mediating the directional migration of tumor cells to distal organs via circulation in a similar manner to its control of leukocyte migration (Hanahan and Weinberg 2000). They induce the expression of MMPs and collagenases, which degrade basement membrane (Inoue et al. 2000; Muller et al. 2001; Scotton et al. 2001).

10.2.2 Transcription Factors

Nuclear Factor-ḰB: Targets of the transcription factor Nuclear Factor-ḰB (NF-ḰB) include immune-mediating genes, inflammatory genes and cell proliferation regulation genes (Karin and Lin 2002). Within the immune system, NF-ḰB is involved in the maturation of dendritic cells (Caamano and Hunter 2002) and development of lymphocytes (Li and Verma 2002; Mora et al. 2001; Senftleben et al. 2001). It is critical for regulating the expression of inflammatory cytokines and adhesion factors (Perkins 2000; Tak and Firestein 2001). During tumor development, NF-ḰB stimulates cell proliferation via activation of the expression of c-Myc, cyclin D1, and other growth factor genes (Guttridge et al. 1999; Hinz et al. 1999; Karin and Lin 2002). In mucosa-associated lymphoid tissue lymphoma, activation of NF-ḰB pathway is followed by inhibition of p53-mediated apoptosis (Stoffel et al. 2004). NF-ḰB may also contribute to genomic instability in two aspects. It promotes the production of reactive oxygen species, which have a potential to cause mutations (Karin and Lin 2002). On the other hand, the anti-apoptotic activity of NF-ḰB prevents mutated precancerous cells from being eliminated (Karin and Lin 2002). NF-ḰB might be involved in linking inflammation to cancer through its association with the induction of proinflammatory cytokines and chemokines such as IL-6, TNF-α, IL-8, adhesion molecules, MMPs, COX-2, and iNOS (Li and Verma 2002). NF-ḰB is also required in leukocyte adhesion and

migration, which are important in cancer associated inflammation (Chen et al. 1995).

Hypoxia-inducible factor-1α: Hypoxia-inducible factor-1α (HIF-1α) is the master regulator of tissue oxygen homeostasis. In response to hypoxia, HIF-1 activates a wide range of molecules like iNOS, VEGF, erythropoietin, glucose transporter-1, and other glycolytic enzymes which enable cell survival under hypoxic stress (Semenza 1999). Hypoxia is a common feature at the sites of inflammatory lesions mainly resulting from metabolic shifts (Kong et al. 2004). Although the role of HIF-1 in driving progression of inflammation may be tissue specific, it has been found to play an essential role in inducing leukocyte adhesion (Cramer et al. 2003) and maintaining normal functions of myeloid cells recruited to sites of inflammation (Walmsley et al. 2005). HIF-1 also promotes chronic inflammation by preventing the hypoxic apoptosis of neutrophils and T-lymphocytes (Makino et al. 2003; Walmsley et al. 2005). Induction of NF-ḰB by hypoxia also depends on HIF-1α existence (Semenza 1999). In normoxic cancer cell lines, HIF-1α can be activated by proinflammatory cytokines such as TNF-α and IL-1β, in a NF-ḰB dependent manner (Jung et al. 2003a, b; Zhou et al. 2003). COX-2 also mediates IL-1β-induced HIF-1α by its product prostaglandin E2 (Jung et al. 2003b).

STAT3: Cytokines can activate STAT family transcription factors via Janus activated kinases (JAK) signaling (Hodge et al. 2005; Yang et al. 2005b). IL-6, for instance, is a well established inducer of STAT3. As STAT3 is constitutively activated in different cancers (Hodge et al. 2005) and has been shown to contribute to immune tolerance of tumor cells (Wang et al. 2004), IL-6/JAK/STAT3 signaling axis could be a critical link between cancer development and inflammation. Notably, STAT3 activation occurs after the occurrence of primary malignant cells and plays a role in promoting the development of an inflammatory microenvironment.

10.2.3 Enzymes

Cyclooxygenase-2: Cyclooxygenase-2 (COX-2) expression may be induced by a wide range of stimuli, including proinflammatory cytokines such as IL-1 and TNF, and growth factors such as epidermal growth factor (Karin and Lin 2002; Williams et al. 1999). The products of COX-2 enzyme are prostaglandins, which are key mediators of inflammation (Nathan 2002; Steele et al. 2003). The long-term use of non-steroidal anti-inflammatory drugs has been shown to reduce the risk of several cancers in population-based studies (Buskens et al. 2002; Farrow et al. 1998; Williams et al. 1999). COX-2 is also overexpressed in various types of cancers and involved in cellular proliferation, anti-apoptotic activity, angiogenesis and increased metastasis (Hida et al. 1998; Hwang et al. 1998; Okami et al. 1999; Prescott and Fitzpatrick 2000; Tsujii et al. 1997).

Inducible Nitric Oxide Synthase: Inducible Nitric Oxide Synthase (iNOS), an enzyme-catalyzing NO production, was found to be overexpressed in various types of cancers and chronic inflammatory diseases (Kim et al. 2005). NO has also been

implicated in the regulation of both inflammation (Hussain et al. 2004) and cancer development (Hofseth et al. 2003). During chronic inflammation, continuous generation of NO may lead to DNA damage, disruption of DNA repair and cancer-prone post-translational modifications (Goodman et al. 2004; Jaiswal et al. 2000; Rao 2004). Increased NO production might result in p53 activation but also carcinogenic p53 mutations (Goodman et al. 2004; Hofseth et al. 2003; Hussain et al. 2004). Once the inflammation-associated tumors are formed, iNOS expression is persistently stimulated by cytokines and NF-ḰB that are prevalent within the tumor inflammatory microenvironment (Li and Verma 2002). In addition, NO may also regulate angiogenesis, leukocyte adhesion, and infiltration and metastasis (Rao 2004). Noticeably, studies using both in vitro and in vivo models show that iNOS/NO signaling can induce COX-2, which is a well-studied link between inflammation and cancer (Rao 2004).

10.2.4 Other Promising Links Between Inflammation and Cancer

Another transcription factor Nrf2, which regulates a wide range of detoxifying and antioxidant genes, has been identified as critical for response to cellular stresses (Motohashi and Yamamoto 2004). Nrf2 may also be induced by NO (Buckley et al. 2003) and lead to reduced susceptibility to apoptotic signals such as TNF-α (Morito et al. 2003).

Nuclear factor of activated T cells (NFAT) is another inflammatory mediator expressed by both immune and nonimmune cells and plays an essential role in inflammatory responses by regulating the expression of cytokines IL-2, IL-3, IL-4, IL-5, IL-13, granulocyte-macrophage colony-stimulating factor and TNF-α (Chen et al. 2003b; Crabtree 1999; Kiani et al. 2000). Moreover, in both T cells and colon carcinoma cells, NFAT is associated with overexpression of COX-2, which is implicated in both cancer progression and inflammation (Duque et al. 2005; Jimenez et al. 2004).

10.3 Inflammation Associated with Primary Brain Tumors

10.3.1 Glioma and Inflammation

Malignant gliomas are the most common type of brain tumors; with GBM being the most aggressive of all gliomas (Konnecke and Bechmann 2013; Holland 2000). Consistent with its role in other malignancies, the inflammatory microenvironment acts as a driving force for progression of GBM lesions into highly malignant tumors. Recently, the role of tumor microenvironment and glioma cells themselves as producers and targets of inflammatory mediators has been discussed in many

reports (Tarassishin et al. 2014a, b; Charles et al. 2011; Cooper et al. 2012; Carmi et al. 2013).

GBM are known to be surrounded by several proinflammatory cytokines, chemokines and growth factors that contribute to their pathophysiology. Cytokines play a major role in the proliferation, invasiveness and stemness of GBM cells. Upregulation of IL-1β, IL-6, IL-8, etc., has been reported in GBM tumor samples as well as cell lines (Yeung et al. 2013). Notably, a differential increase in the inflammatory response to glial tumors has been observed with the increasing grade of malignancy. For example, in comparison to low grade gliomas, the chemokine CX3CL1 shows an increased expression in grade III-IV gliomas, namely, anaplastic astrocytomas, GBM and oligodendrogliomas (Erreni et al. 2010). Similarly, expression of PTX, a component of the humoral arm of innate immunity and a candidate marker of inflammation, differs across low and high grade gliomas, with a positive correlation with tumor grade and severity (Locatelli et al. 2013). There is also a positive correlation between the density of microglia/macrophages in gliomas and the grade of gliomas (Konnecke and Bechmann 2013). Several cell culture and xenograft studies have supported the hypothesis that targeting the production and activity of inflammatory molecules could be beneficial to GBM patients (Wang et al. 2009a, 2012; de la Iglesia et al. 2008). Therefore, anti-inflammatory agents in combination with cytotoxic agents can offer an improvised strategy in GBM therapy (Yeung et al. 2013). The ongoing glioma studies are, thus, investigating specific anti-tumor immunity with the aim of providing adjuvant therapies to patients.

10.3.1.1 Role of Interleukins (ILs) in Glioma Pathophysiology

IL-1β: IL-1β is a master proinflammatory cytokine involved in the malignant process (Yeung et al. 2013). Elevated levels of IL-1β and its receptor (IL-1R) have been observed in human GBM cell lines and tumor specimens (Yeung et al. 2012; Sasaki et al. 1998; Lu et al. 2007; Sharma et al. 2011b). The binding of IL-1β to IL-1R is known to activate NF-ḰB and MAPK signaling pathways in glial cells (Griffin and Moynagh 2006; McCulloch et al. 2006). Other pathways that show IL-1β-dependent activation in GBM cells are ERK (Meini et al. 2008) and JNKs pathways which also lead to upregulation of VEGF and sphingosine kinase 1 (Yoshino et al. 2006; Paugh et al. 2009). IL-1β mediates upregulation of hypoxia-inducible factor-1 which is the main regulator of hypoxic response during GBM progression (Sharma et al. 2011b). IL-1 has been identified as a strong inducer of MMPs and miR-155 in human glioma cells (Tarassishin et al. 2014a).

IL-1 produced by GBM cells is responsible for their mesenchymal phenotype, increased migratory capacity, unique gene signature and proinflammatory signaling (Tarassishin et al. 2014a). IL-1β has been identified as crucial for maintenance of stemness properties and self-renewal capacity of GBM cells. IL-1β in combination with TGF-β has been found to induce increased expression of stemness genes, invasiveness and drug resistance in LN-229 cell line, leading to enhanced tumor growth in vivo (Wang et al. 2012).

The increase in IL-1β in GBM cells is followed by increased secretion of IL-6 and IL-8; and upregulation of COX-2, which further aggravates inflammation (Spooren et al. 2011; Sharma et al. 2011a; Yeung et al. 2012; Tanabe et al. 2011). IL-1 has been shown to suppress anti-tumor immunity and vaccine efficacy by expansion of myeloid-derived suppressor cells (MDSC) and Th17 cells through activation of STAT3 in tumor cells (Wang et al. 2009b; Bruchard et al. 2013).

IL-6: IL-6 gene amplification has been shown to correlate with GBM aggressiveness and poor patient survival (Rolhion et al. 2001; Tchirkov et al. 2007). IL-1β and TNF-α are responsible for activating signaling pathways that lead to stabilization of IL-6 mRNA and its increased biosynthesis (Spooren et al. 2011; Yeung et al. 2012). IL-6 signaling is triggered by the binding of IL-6 to heteromeric plasma membrane receptor complexes formed by IL-6 receptor (IL-6R) and glycoprotein 130 (gp130) (Yeung et al. 2013). Henceforth, the downstream signaling is propagated through the JAK/STAT pathway (JAK1-3), resulting in activation of STAT transcription factors, chiefly STAT3 (Rahaman et al. 2002). Activated STAT3 has been correlated with increased glioma-infiltrating T-cells as well as poor survival of patients with high grade glioma as compared to low grade glioma (Abou-Ghazal et al. 2008). IL-6-mediated JAK/STAT signaling is reported to promote invasion and migration in GBM cell lines (U251, T98G and U87MG) (Liu et al. 2010) and confers glioma stem cell-like features via activation of Jagged–Notch pathway (Jin et al. 2012). It also correlates with increased expression and secretion of MMP-2 which enhances GBM motility (Li et al. 2010). IL-6 derived from neighboring cells, like the microglia, has also been known to stimulate GBM cell invasion (Zhang et al. 2012).

IL-8: High expression and secretion of IL-8 has been reported in human GBM samples, cell lines and stem cells (Yeung et al. 2013). Its expression is positively regulated by IL-1β, TNF-α or macrophage infiltration (Yeung et al. 2012; Hong et al. 2009) and it has been demonstrated as a potent angiogenic factor in GBM (Brat et al. 2005). CXCR1, a G-protein coupled chemokine receptor, binds IL-8 in GBM cells (Raychaudhuri and Vogelbaum 2011) and its downstream signaling activates PI3K, Raf–MAPK/ERK kinase (MEK)–ERK, p38 MAPK and JAK2–STAT3 pathways (Waugh and Wilson 2008). IL-8 acts as an inflammatory chemoattractant for GBM cells and promotes their invasiveness (Raychaudhuri and Vogelbaum 2011; Wakabayashi et al. 2004). It is also secreted by the tumor cells, augmenting their growth in an autocrine manner (Sun et al. 2011; Wakabayashi et al. 2004).

10.3.1.2 Role of COX-2 in Glioma Pathophysiology

The significance of inflammation for glioma progression has been shown by a study that analyzed the effect of chronic IL-1β exposure on cancer stem-like cells (CSCs) derived from GBM cell line U87MG (Sharma et al. 2011a). Apart from increasing oxidative DNA damage, the IL-1β treatment increased the nuclear and cytoplasmic levels of COX-2. Celecoxib, an inhibitor of COX-2, was seen to decrease self-renewal capacity and increase apoptosis of control as well as IL-1β treated CSCs. Due to the regulation of CSCs proliferation by COX-2 independent of IL-1β

treatment COX-2 was implicated as a potential anti-glioma target. Other studies where COX-2 was detected in the astrocytes surrounding necrotic areas in glial tumors suggest the use of COX-2 inhibitors in newer therapeutic strategies combining chemotherapy and radiotherapy for treatment of glioma patients (Temel and Kahveci 2009; Deininger et al. 1999).

10.3.1.3 Role of HIF-1α in Glioma Pathophysiology

The importance of HIF-1α in glioma-related inflammation was revealed in a recent study where increased HIF-1α activity was found to be concurrent with TNF-α-induced increase in major histocompatibility complex class I (MHC-I) expression and activation (Ghosh et al. 2013). In corroboration, HIF-1α knockdown blocked TNF-α-induced MHC-I activation, thereby, demonstrating HIF-1α as a key link between inflammation, immune evasion and glioma progression via MHC-I gene regulation.

10.3.1.4 Role of NF-ḰB in Glioma Pathophysiology

A recent study has linked NF-ḰB activation with the regulation of inflammatory microenvironment in glioma cells. TNF-α, a proinflammatory cytokine, modulates inflammatory responses in GBM cells via toll-like receptor 4 (TLR4) signaling and subsequent activation of NF-ḰB (Tewari et al. 2012). TNF-α-induced TLR4 signaling was also seen to result in increased AKT activation and HIF-1α transcriptional activation in the process. Overall, the study showed that under the influence of TNF-α, TLR4-HIF-1α and NF-ḰB-TLR4 form feed-forward loops that play an important role in sustaining the inflammatory response in glioma.

10.3.1.5 Role of STAT3 in Glioma Pathophysiology

STAT3 has been implicated as a negative regulator of the anti-tumor immune response. STAT3-associated immunosuppressive mediators are either expressed by glioma cells or the immune cell populations in the tumor microenvironment (Kim et al. 2014). siRNA-mediated knockdown of STAT3 in human GBM cell lines has shown changes in the profiles of inflammatory cytokines (See et al. 2012). STAT3 expression has been correlated with poor survival in astrocytomas (Abou-Ghazal et al. 2008) and in high grade gliomas, JAK/STAT pathway activation has been found as an independent prognostic indicator of decreased survival (Tu et al. 2011).

10.3.1.6 Release of Inflammatory Mediators by the Neighboring Cells in Glioma Microenvironment

A substantial part of the tumor mass of glioblastoma is constituted by microglia cells, the immunocompetent cells of brain, which form the largest population of tumor-infiltrating cells (Graeber et al. 2002; Badie and Schartner 2001; Konnecke and Bechmann 2013). Microglia create a microenvironment that supports tumor initiation and progression (Markovic et al. 2005) by providing an immunosuppressive milieu, for instance, with release of IL-10 (Huettner et al. 1997). Under the influence of glioma cells, microglia suppress their defense properties (Hussain et al.

2006) and instead of releasing cytokines, they upregulate the inflammatory biomarkers MMPs, especially, MMP-9 and MMP-2 (Rao 2003; Hanisch and Kettenmann 2007). MMP-9 has been termed as a tuner and amplifier of immune functions since it helps in peripheralization of leukocytes in response to chemokines at the sites of inflammation and acts as a switch and catalyst between innate and adaptive immune systems (Opdenakker et al. 2001; Konnecke and Bechmann 2013). The production of MMP-9 is enhanced by TGF-β in transformed lymphocytes (Zhou et al. 1993) whereas it is negatively regulated by IL-4, IL-10, and interferon-β (Corcoran et al. 1992; Arthur et al. 1987; Malik et al. 1996; Zhou et al. 1993; Konnecke and Bechmann 2013). Furthermore, immunosuppressive inflammatory cells like regulatory T-cells (T-regs) and MDSCs are known to promote brain neoplastic growth via suppression of activity of cytotoxic T-lymphocytes (CTLs) (Abad et al. 2014; Hanahan and Weinberg 2011).

10.3.1.7 Inflammation Mediated by FAT1 Gene in Glioma

Inflammatory mediators are known to coordinate with intracellular signaling cascades and regulate hubs of transcriptional networks that are critical for glioma cell survival and growth (Sen 2011). Similarly, certain signal transduction mechanisms exercise upstream of the inflammatory pathways and exhibit cross-talks that enhance inflammatory response in the tumor cells. One such important example is the signaling axis mediated by FAT1, a novel cadherin recently implicated as an oncogene in glioma (Dikshit et al. 2013). siRNA-mediated knockdown of FAT1 gene has been shown to increase the expression of PDCD4 (programmed cell death 4, a tumor suppressor gene) in GBM cell lines. This, in turn, reduces phospho-c-Jun which diminishes AP-1 dependent transcription of downstream genes which include proinflammatory markers (COX-2, IL-1β and IL-6) and extra cellular matrix (ECM)-remodeling molecules (MMP-3, PLAU and VEGF-C). The process was seen to be reversed by the simultaneous knockdown of FAT1 and PDCD4, thereby, confirming the link between the two molecules in induction of a proinflammatory microenvironment in glioma. In GBM tumors, the mRNA expression of COX-2 and IL-6 expression was positive correlated with FAT1 expression along with inverse correlation between FAT1 and PDCD4 expression (Dikshit et al. 2013) hence, reporting an important role of FAT1 gene in promoting inflammation in GBM tumors. Apart from assisting as a possible tool for future diagnosis and prognosis, FAT1 seems to be a potential marker to enable molecular subgrouping of GBM and a prospective means for designing a targeted therapy in combating proinflammatory microenvironment in GBM and tumor invasion.

10.3.2 Oligodendroglioma and Inflammation

There exists a distinction between the expression profiles of inflammatory mediators in oligodendroglial tumors versus the low grade gliomas of astrocytic origin. As per a recent analysis done using the expression data retrieved from The Cancer

Genome Atlas (TCGA), relative to oligodendrogliomas, low grade astrocytomas displayed higher expression of inflammation-related genes (Gonda et al. 2014). In another study, overexpression of genes related to inflammation and immune response was found in oligodendroglial tumors with intact 1p-19q (Ferrer-Luna et al. 2009). These tumors also displayed p53 mutations and EGFR trisomy in some cases.

10.3.3 Ependymoma and Inflammation

Published reports on tumor-related inflammation are few in cases of ependymal tumors. Recently, Griesinger et al. have reported the importance of IL-6/STAT3 signaling in driving tumor growth and inflammatory crosstalk in high-risk ependymal tumors (Griesinger et al. 2015). Distinct immunobiologic signatures were analyzed in molecular subgrouping of ependymoma providing aid in diagnosis, recurrence and clinical outcomes (Hoffman et al. 2014). A panel inflammatory cytokines was analyzed pre- and post-radiotherapy in patients with ependymoma and IL-8 was found to be decreased during therapy (Merchant et al. 2009).

10.3.4 Meningioma and Inflammation

Meningiomas are the second most prevalent primary neoplasm of the CNS after gliomas, and arise from the CNS meninges (Doroudchi et al. 2013; Kujas 1993; Wiemels et al. 2010). Although a majority of these tumors are benign, in a few cases they may metastasize and become aggressive (Pfisterer et al. 2004). Similar to other brain tumors, a deregulated expression pattern of inflammatory mediators and cytokines is observed in meningiomas. A majority of these tumors express receptors for IL-4, an immune-regulatory cytokine known to be produced predominantly by type 2 T-helper cells and mast cells (Puri et al. 2005). In contrast to tumor cells, the normal brain tissue expresses either low or non-detectable levels of IL-4 receptor. Likewise, cyclooxygenase-2 (COX-2), a well-known inflammatory mediator, is expressed selectively in meningioma tissue, and not in the normal dura or dura adjacent to the tumors (Ragel et al. 2007). Selective COX-2 inhibition by the use of celecoxib in meningioma tumors grown in mice has shown reduction in blood vessel density through direct inhibition of COX-2 and downregulation of VEGF-mediated angiogenesis. In another study, increased co-expression of macrophage migration inhibitory factor (MIF; a multifunctional cytokine associated with inflammation and tumorigenesis) and MMP-9, together with the histological grade of the tumor, were found to be an important predictor for recurrence of benign meningiomas (Huang et al. 2013). Elevated expression of both MIF and MMP-9 were associated with increased microvessel density in meningioma. A research group has also reported TGF-β1 overexpression in the microenvironment of different pathological types of meningiomas (Gogineni et al. 2012). TGF-β1

induced cell invasion in malignant meningioma along with upregulation of uPA, uPAR, cathepsin B, and MMP-9. The increase in cell proliferation was associated with the expression of anti-apoptotic and pro-survival signaling molecules.

10.3.5 Medulloblastoma and Inflammation

Medulloblastoma is a common primary brain tumor in children with a male preponderance and represents a heterogeneous disease (Maslinska et al. 2012). The major biological factors and mechanisms underlying the pathogenesis of the tumor subtypes are unclear. Both genetic and epigenetic factors are known to influence the host response which contributes to growth of the tumors. Local inflammation-induced extracellular matrix structural changes are a characteristic phenomenon during neoplastic invasion in intracranial tumors including medulloblastoma. It aids the tumor cells to infiltrate adjacent tissues or enter peripheral circulation. This process is facilitated by the increased expression of MMP-2 and MMP-9 that are responsible for degradation of extracellular matrix (Annabi et al. 2013). Toll-like receptors (TLRs), which are receptors of innate immunity, may play a role in immune mechanisms of medulloblastoma patients. The TLR downstream signaling involves activation of transcription factors that induce genes encoding various proinflammatory cytokines, enzymes and mediators. TLR 2 and TLR 9 have been found to be associated with the ligands released by the necrotic tissue in medulloblastoma tumors and provide a key link between innate immunity and inflammation in medulloblastoma tumors (Maslinska et al. 2012).

10.3.6 Acoustic Neuroma (Schwannoma) and Inflammation

Acoustic neuromas or vestibular schwannomas are benign tumors originating from the myelin-producing Schwann cells of the vestibular branch of the eighth cranial nerve in the internal auditory canal. These tumors grow slowly and progressively; and eventually cause brain stem compression. They account for about 8 in 100 primary brain tumors (Taurone et al. 2015). They behave as WHO grade I tumors and only rarely undergo malignant transformation (Hilton and Hanemann 2014). Inflammatory infiltrates representing the host's humoral and cellular response to schwannomas have been documented (Rossi et al. 1990; Stevens et al. 1988). This infiltrate induces increased vessel permeability and results in adjacent intratumoral edema (Mahadewa et al. 2005). Additionally, corticosteroids, the drugs that are known to repress cytokine gene transcription, have been found to be effective in cases of sensorineural hearing loss associated with vestibular schwannoma, pointing to the important function of inflammatory response in the process (Barnes and Adcock 1993; Chen et al. 2003a; Lebel et al. 1988).

10.3.7 Pituitary Tumors and Inflammation

Almost all pituitary tumors or pituitary adenomas are benign tumors and cause significant health problems mainly because of their location near the brain and secretion of excess hormones (Ezzat et al. 2004). Among cytokines, increased levels of IL-6 and IL-8 have been detected in human pituitary tumors (as compared to normal pituitary tissue) and pituitary adenoma cell cultures. There is also a possible role of inflammation-modulated angiogenesis via induction of NF-ḰB and HIF-1α (Arzt et al. 1999). gp130 cytokines have a regulatory action on pituitary physiology for secretion of ACTH and PRL which represent two models of neuroendocrine and immune interaction (Gerez et al. 2007). Their disruption might lead to abnormal growth of pituitary cells as well as immune disorders, for which, targeting these cytokines could be a potential therapeutic approach.

Very few or no studies have been done on tumor-related inflammation in other very rare intracranial cancers and hence, they have not been discussed in this chapter.

10.4 Current Challenges and Future Prospects of Immunotherapy in Brain Tumors

Until recently, most efforts to treat cancer have been directed towards the eradication of tumor cells. But newer studies have revealed molecular pathways that interconnect cancer and inflammation, thereby, offering a fresh perspective to modulate the tumor microenvironment (Colotta et al. 2009). Cancer-related inflammation is indeed a chief component of malignant brain tumor microenvironment and represents a target for innovative therapeutic strategies. Proinflammatory cytokines have been the prime targets in most cancers and continual efforts in the direction of targeting them are well justified (Harrison et al. 2007; Loberg et al. 2007).

The current challenge in cancer immunotherapy lies in identifying the mechanisms that accentuate the benefits of inflammation and result in tumor inhibition while neutralizing its tumor-promoting effects (Colotta et al. 2009). The same approach needs to be applied in case of brain tumors by utilizing the innate arm of the immune system to recognize and destroy malignant cells. Immunotherapy for malignant gliomas is an emerging field that ensures highly specific and less toxic treatment as compared to conventional chemotherapy. In addition, it has the advantage of sustained efficacy by way of immunologic memory (Bloch 2015). Anti-tumor immunity is achievable through vaccination. Till date, the immunotherapeutic strategies have focused upon active vaccination against tumor-specific antigens in gliomas. Many such early phase clinical trials have also shown promising results for vaccine therapy. But no therapy has yet improved survival in a randomized, controlled trial as such. The major obstacle to immunotherapy and a pressing issue in malignant gliomas is the tumor-induced immunosuppression. The mechanisms of immunosuppression involve a

combination of factors like the activity of T-reg cells, signaling mediated by TGF-β, etc., that paralyze the functions of CTLs and NK cells (Bloch 2015; Platten et al. 2014). As has been demonstrated in other cancers, the efficacy of immunomodulatory agents may be considerably improved only when they are developed to combat the immunosuppressive factors (Bloch 2015). A positive example comes from immunotherapy clinical trials in other cancers targeting to inhibit T-cell suppressive pathways mediated by PD1 (protein encoded by human PDCD1 gene) receptor and ligand using humanized antibodies. It underscores the potential option of using agents that can obliterate the immunosuppressive microenvironment in glioma (Platten et al. 2014). Such approaches would ensure greater efficacy of the peripheral anti-tumor immune response induced by vaccination.

Bibliography

Abad C, Nobuta H, Li J, Kasai A, Yong WH, Waschek JA (2014) Targeted STAT3 disruption in myeloid cells alters immunosuppressor cell abundance in a murine model of spontaneous medulloblastoma. J Leukoc Biol 95(2):357–367

Abou-Ghazal M, Yang DS, Qiao W, Reina-Ortiz C, Wei J, Kong LY, Fuller GN, Hiraoka N, Priebe W, Sawaya R, Heimberger AB (2008) The incidence, correlation with tumor-infiltrating inflammation, and prognosis of phosphorylated STAT3 expression in human gliomas. Clin Cancer Res 14(24):8228–8235

Aggarwal BB (2003) Signalling pathways of the TNF superfamily: a double-edged sword. Nat Rev Immunol 3(9):745–756

Amann B, Perabo FG, Wirger A, Hugenschmidt H, Schultze-Seemann W (1998) Urinary levels of monocyte chemo-attractant protein-1 correlate with tumour stage and grade in patients with bladder cancer. Br J Urol 82(1):118–121

Annabi B, Vaillancourt-Jean E, Beliveau R (2013) MT1-MMP expression level status dictates the in vitro action of lupeol on inflammatory biomarkers MMP-9 and COX-2 in medulloblastoma cells. Inflammopharmacology 21(1):91–99

Ardestani SK, Inserra P, Solkoff D, Watson RR (1999) The role of cytokines and chemokines on tumor progression: a review. Cancer Detect Prev 23(3):215–225

Arthur FE, Shivers RR, Bowman PD (1987) Astrocyte-mediated induction of tight junctions in brain capillary endothelium: an efficient in vitro model. Brain Res 433(1):155–159

Arzt E, Pereda MP, Castro CP, Pagotto U, Renner U, Stalla GK (1999) Pathophysiological role of the cytokine network in the anterior pituitary gland. Front Neuroendocrinol 20(1):71–95

Badie B, Schartner J (2001) Role of microglia in glioma biology. Microsc Res Tech 54(2):106–113.

Balkwill F (2002) Tumor necrosis factor or tumor promoting factor? Cytokine Growth Factor Rev 13(2):135–141

Balkwill F, Mantovani A (2001) Inflammation and cancer: back to Virchow? Lancet 357 (9255):539–545

Bando H, Toi M (2000) Tumor angiogenesis, macrophages, and cytokines. Adv Exp Med Biol 476:267–284

Barnes PJ, Adcock I (1993) Anti-inflammatory actions of steroids: molecular mechanisms. Trends Pharmacol Sci 14(12):436–441

Bloch O (2015) Immunotherapy for malignant gliomas. Cancer Treat Res 163:143–158

Block TM, Mehta AS, Fimmel CJ, Jordan R (2003) Molecular viral oncology of hepatocellular carcinoma. Oncogene 22(33):5093–5107

Borm PJ, Driscoll K (1996) Particles, inflammation and respiratory tract carcinogenesis. Toxicol Lett 88(1–3):109–113

Brat DJ, Bellail AC, Van Meir EG (2005) The role of interleukin-8 and its receptors in gliomagenesis and tumoral angiogenesis. Neuro Oncol 7(2):122–133

Brigati C, Noonan DM, Albini A, Benelli R (2002) Tumors and inflammatory infiltrates: friends or foes? Clin Exp Metastasis 19(3):247–258

Bromwich EJ, McArdle PA, Canna K, McMillan DC, McNicol AM, Brown M, Aitchison M (2003) The relationship between T-lymphocyte infiltration, stage, tumour grade and survival in patients undergoing curative surgery for renal cell cancer. Br J Cancer 89(10):1906–1908

Bruchard M, Mignot G, Derangere V, Chalmin F, Chevriaux A, Vegran F, Boireau W, Simon B, Ryffel B, Connat JL, Kanellopoulos J, Martin F, Rebe C, Apetoh L, Ghiringhelli F (2013) Chemotherapy-triggered cathepsin B release in myeloid-derived suppressor cells activates the Nlrp3 inflammasome and promotes tumor growth. Nat Med 19(1):57–64

Buckley BJ, Marshall ZM, Whorton AR (2003) Nitric oxide stimulates Nrf2 nuclear translocation in vascular endothelium. Biochem Biophys Res Commun 307(4):973–979

Buskens CJ, Van Rees BP, Sivula A, Reitsma JB, Haglund C, Bosma PJ, Offerhaus GJ, Van Lanschot JJ, Ristimaki A (2002) Prognostic significance of elevated cyclooxygenase 2 expression in patients with adenocarcinoma of the esophagus. Gastroenterology 122(7):1800–1807

Caamano J, Hunter CA (2002) NF-kappaB family of transcription factors: central regulators of innate and adaptive immune functions. Clin Microbiol Rev 15(3):414–429

Canna K, McArdle PA, McMillan DC, McNicol AM, Smith GW, McKee RF, McArdle CS (2005) The relationship between tumour T-lymphocyte infiltration, the systemic inflammatory response and survival in patients undergoing curative resection for colorectal cancer. Br J Cancer 92(4):651–654

Carmi Y, Dotan S, Rider P, Kaplanov I, White MR, Baron R, Abutbul S, Huszar M, Dinarello CA, Apte RN, Voronov E (2013) The role of IL-1beta in the early tumor cell-induced angiogenic response. J Immunol 190(7):3500–3509

Charles NA, Holland EC, Gilbertson R, Glass R, Kettenmann H (2011) The brain tumor microenvironment. Glia 59(8):1169–1180

Chen CC, Rosenbloom CL, Anderson DC, Manning AM (1995) Selective inhibition of E-selectin, vascular cell adhesion molecule-1, and intercellular adhesion molecule-1 expression by inhibitors of I kappa B-alpha phosphorylation. J Immunol 155(7):3538–3545

Chen CY, Halpin C, Rauch SD (2003a) Oral steroid treatment of sudden sensorineural hearing loss: a ten year retrospective analysis. Otol Neurotol 24(5):728–733

Chen J, Amasaki Y, Kamogawa Y, Nagoya M, Arai N, Arai K, Miyatake S (2003b) Role of NFATx (NFAT4/NFATc3) in expression of immunoregulatory genes in murine peripheral CD4+ T cells. J Immunol 170(6):3109–3117

Colotta F, Allavena P, Sica A, Garlanda C, Mantovani A (2009) Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. Carcinogenesis 30(7):1073–1081

Cooper LA, Gutman DA, Chisolm C, Appin C, Kong J, Rong Y, Kurc T, Van Meir EG, Saltz JH, Moreno CS, Brat DJ (2012) The tumor microenvironment strongly impacts master transcriptional regulators and gene expression class of glioblastoma. Am J Pathol 180 (5):2108–2119

Corcoran ML, Stetler-Stevenson WG, Brown PD, Wahl LM (1992) Interleukin 4 inhibition of prostaglandin E2 synthesis blocks interstitial collagenase and 92-kDa type IV collagenase/gelatinase production by human monocytes. J Biol Chem 267(1):515–519

Coussens LM, Raymond WW, Bergers G, Laig-Webster M, Behrendtsen O, Werb Z, Caughey GH, Hanahan D (1999) Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis. Genes Dev 13(11):1382–1397

Coussens LM, Werb Z (2001) Inflammatory cells and cancer: think different! J Exp Med 193(6): F23–F26

Coussens LM, Werb Z (2002) Inflammation and cancer. Nature 420(6917):860–867

Crabtree GR (1999) Generic signals and specific outcomes: signaling through Ca2+, calcineurin, and NF-AT. Cell 96(5):611–614

Cramer T, Yamanishi Y, Clausen BE, Forster I, Pawlinski R, Mackman N, Haase VH, Jaenisch R, Corr M, Nizet V, Firestein GS, Gerber HP, Ferrara N, Johnson RS (2003) HIF-1alpha is essential for myeloid cell-mediated inflammation. Cell 112(5):645–657

Daniel D, Meyer-Morse N, Bergsland EK, Dehne K, Coussens LM, Hanahan D (2003) Immune enhancement of skin carcinogenesis by CD4+ T cells. J Exp Med 197(8):1017–1028

de la Iglesia N, Konopka G, Lim KL, Nutt CL, Bromberg JF, Frank DA, Mischel PS, Louis DN, Bonni A (2008) Deregulation of a STAT3-interleukin 8 signaling pathway promotes human glioblastoma cell proliferation and invasiveness. J Neurosci 28(23):5870–5878

Deininger MH, Weller M, Streffer J, Mittelbronn M, Meyermann R (1999) Patterns of cyclooxygenase-1 and -2 expression in human gliomas in vivo. Acta Neuropathol 98 (3):240–244

Dikshit B, Irshad K, Madan E, Aggarwal N, Sarkar C, Chandra PS, Gupta DK, Chattopadhyay P, Sinha S, Chosdol K (2013) FAT1 acts as an upstream regulator of oncogenic and inflammatory pathways, via PDCD4, in glioma cells. Oncogene 32(33):3798–3808

Doroudchi M, Pishe ZG, Malekzadeh M, Golmoghaddam H, Taghipour M, Ghaderi A (2013) Elevated serum IL-17A but not IL-6 in glioma versus meningioma and schwannoma. Asian Pac J Cancer Prev 14(9):5225–5230

Dranoff G (2004) Cytokines in cancer pathogenesis and cancer therapy. Nat Rev Cancer 4 (1):11–22

Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD (2002) Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol 3(11):991–998

Duque J, Fresno M, Iniguez MA (2005) Expression and function of the nuclear factor of activated T cells in colon carcinoma cells: involvement in the regulation of cyclooxygenase-2. J Biol Chem 280(10):8686–8693

Dvorak HF (1986) Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. N Engl J Med 315(26):1650–1659

Elgert KD, Alleva DG, Mullins DW (1998) Tumor-induced immune dysfunction: the macrophage connection. J Leukoc Biol 64(3):275–290

Erreni M, Solinas G, Brescia P, Osti D, Zunino F, Colombo P, Destro A, Roncalli M, Mantovani A, Draghi R, Levi D, Rodriguez YBR, Gaetani P, Pelicci G, Allavena P (2010) Human glioblastoma tumours and neural cancer stem cells express the chemokine CX3CL1 and its receptor CX3CR1. Eur J Cancer 46(18):3383–3392

Ezzat S, Asa SL, Couldwell WT, Barr CE, Dodge WE, Vance ML, McCutcheon IE (2004) The prevalence of pituitary adenomas: a systematic review. Cancer 101(3):613–619

Farrow DC, Vaughan TL, Hansten PD, Stanford JL, Risch HA, Gammon MD, Chow WH, Dubrow R, Ahsan H, Mayne ST, Schoenberg JB, West AB, Rotterdam H, Fraumeni JF Jr, Blot WJ (1998) Use of aspirin and other nonsteroidal anti-inflammatory drugs and risk of esophageal and gastric cancer. Cancer Epidemiol Biomarkers Prev 7(2):97–102

Ferrer-Luna R, Mata M, Nunez L, Calvar J, Dasi F, Arias E, Piquer J, Cerda-Nicolas M, Taratuto AL, Sevlever G, Celda B, Martinetto H (2009) Loss of heterozygosity at 1p-19q induces a global change in oligodendroglial tumor gene expression. J Neurooncol 95(3): 343–354

Foekens JA, Peters HA, Look MP, Portengen H, Schmitt M, Kramer MD, Brunner N, Janicke F, Meijer-van Gelder ME, Henzen-Logmans SC, van Putten WL, Klijn JG (2000) The urokinase system of plasminogen activation and prognosis in 2780 breast cancer patients. Cancer Res 60 (3):636–643

Fox SB, Taylor M, Grondahl-Hansen J, Kakolyris S, Gatter KC, Harris AL (2001) Plasminogen activator inhibitor-1 as a measure of vascular remodelling in breast cancer. J Pathol 195 (2):236–243

Gerez J, Bonfiglio J, Sosa S, Giacomini D, Acuna M, Nagashima AC, Perone MJ, Silberstein S, Renner U, Stalla GK, Arzt E (2007) Molecular transduction mechanisms of cytokine-hormone interactions: role of gp130 cytokines. Exp Physiol 92(5):801–806

Ghosh S, Paul A, Sen E (2013) Tumor necrosis factor alpha-induced hypoxia-inducible factor 1alpha-beta-catenin axis regulates major histocompatibility complex class I gene activation through chromatin remodeling. Mol Cell Biol 33(14):2718–2731

Gogineni VR, Gupta R, Nalla AK, Velpula KK, Rao JS (2012) uPAR and cathepsin B shRNA impedes TGF-beta1-driven proliferation and invasion of meningioma cells in a XIAP-dependent pathway. Cell Death Dis 3(12):e439

Gonda DD, Cheung VJ, Muller KA, Goyal A, Carter BS, Chen CC (2014) The Cancer Genome Atlas expression profiles of low-grade gliomas. Neurosurg Focus 36(4):E23

Goodman JE, Hofseth LJ, Hussain SP, Harris CC (2004) Nitric oxide and p53 in cancer-prone chronic inflammation and oxyradical overload disease. Environ Mol Mutagen 44(1):3–9

Graeber MB, Scheithauer BW, Kreutzberg GW (2002) Microglia in brain tumors. Glia 40(2): 252–259

Gravendeel LA, Kouwenhoven MC, Gevaert O, de Rooi JJ, Stubbs AP, Duijm JE, Daemen A, Bleeker FE, Bralten LB, Kloosterhof NK, De Moor B, Eilers PH, van der Spek PJ, Kros JM, Sillevis Smitt PA, van den Bent MJ, French PJ (2009) Intrinsic gene expression profiles of gliomas are a better predictor of survival than histology. Cancer Res 69(23):9065–9072

Griesinger AM, Josephson RJ, Donson AM, Mulcahy Levy JM, Amani V, Birks DK, Hoffman LM, Furtek SL, Reigan P, Handler MH, Vibhakar R, Foreman NK (2015) Interleukin-6/STAT3 pathway signaling drives an inflammatory phenotype in Group A ependymoma. Cancer Immunol Res. 3(10):1165–1174

Griffin BD, Moynagh PN (2006) Persistent interleukin-1beta signaling causes long term activation of NFkappaB in a promoter-specific manner in human glial cells. J Biol Chem 281(15):10316–10326

Grimshaw MJ, Wilson JL, Balkwill FR (2002) Endothelin-2 is a macrophage chemoattractant: implications for macrophage distribution in tumors. Eur J Immunol 32(9):2393–2400

Grivennikov SI, Greten FR, Karin M (2010) Immunity, inflammation, and cancer. Cell 140 (6):883–899

Guttridge DC, Albanese C, Reuther JY, Pestell RG, Baldwin AS Jr (1999) NF-kappaB controls cell growth and differentiation through transcriptional regulation of cyclin D1. Mol Cell Biol 19(8):5785–5799

Hanahan D, Weinberg RA (2000) The hallmarks of cancer. Cell 100(1):57–70

Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144(5):646–674

Hanisch UK, Kettenmann H (2007) Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat Neurosci 10(11):1387–1394

Haqqani AS, Sandhu JK, Birnboim HC (2000) Expression of interleukin-8 promotes neutrophil infiltration and genetic instability in mutatect tumors. Neoplasia 2(6):561–568

Harrison ML, Obermueller E, Maisey NR, Hoare S, Edmonds K, Li NF, Chao D, Hall K, Lee C, Timotheadou E, Charles K, Ahern R, King DM, Eisen T, Corringham R, DeWitte M, Balkwill F, Gore M (2007) Tumor necrosis factor alpha as a new target for renal cell carcinoma: two sequential phase II trials of infliximab at standard and high dose. J Clin Oncol 25(29):4542–4549

Hida T, Yatabe Y, Achiwa H, Muramatsu H, Kozaki K, Nakamura S, Ogawa M, Mitsudomi T, Sugiura T, Takahashi T (1998) Increased expression of cyclooxygenase 2 occurs frequently in human lung cancers, specifically in adenocarcinomas. Cancer Res 58(17):3761–3764

Hilton DA, Hanemann CO (2014) Schwannomas and their pathogenesis. Brain Pathol 24(3): 205–220

Hinz M, Krappmann D, Eichten A, Heder A, Scheidereit C, Strauss M (1999) NF-kappaB function in growth control: regulation of cyclin D1 expression and G0/G1-to-S-phase transition. Mol Cell Biol 19(4):2690–2698

Hiromatsu Y, Toda S (2003) Mast cells and angiogenesis. Microsc Res Tech 60(1):64–69

Hodge DR, Hurt EM, Farrar WL (2005) The role of IL-6 and STAT3 in inflammation and cancer. Eur J Cancer 41(16):2502–2512

Hoffman LM, Donson AM, Nakachi I, Griesinger AM, Birks DK, Amani V, Hemenway MS, Liu AK, Wang M, Hankinson TC, Handler MH, Foreman NK (2014) Molecular sub-group-specific immunophenotypic changes are associated with outcome in recurrent posterior fossa ependymoma. Acta Neuropathol 127(5):731–745

Hofseth LJ, Hussain SP, Wogan GN, Harris CC (2003) Nitric oxide in cancer and chemoprevention. Free Radic Biol Med 34(8):955–968

Holland EC (2000) Glioblastoma multiforme: the terminator. Proc Natl Acad Sci USA 97 (12):6242–6244

Hong TM, Teng LJ, Shun CT, Peng MC, Tsai JC (2009) Induced interleukin-8 expression in gliomas by tumor-associated macrophages. J Neurooncol 93(3):289–301

Hoshida Y, Fuchs BC, Bardeesy N, Baumert TF, Chung RT (2014) Pathogenesis and prevention of hepatitis C virus-induced hepatocellular carcinoma. J Hepatol 61(1S):S79–S90

Huang Q, Zhao SL, Tian XY, Li B, Li Z (2013) Increased co-expression of macrophage migration inhibitory factor and matrix metalloproteinase 9 is associated with tumor recurrence of meningioma. Int J Med Sci 10(3):276–285

Huettner C, Czub S, Kerkau S, Roggendorf W, Tonn JC (1997) Interleukin 10 is expressed in human gliomas in vivo and increases glioma cell proliferation and motility in vitro. Anticancer Res 17(5A):3217–3224

Hussain SF, Yang D, Suki D, Aldape K, Grimm E, Heimberger AB (2006) The role of human glioma-infiltrating microglia/macrophages in mediating antitumor immune responses. Neuro Oncol 8(3):261–279

Hussain SP, Trivers GE, Hofseth LJ, He P, Shaikh I, Mechanic LE, Doja S, Jiang W, Subleski J, Shorts L, Haines D, Laubach VE, Wiltrout RH, Djurickovic D, Harris CC (2004) Nitric oxide, a mediator of inflammation, suppresses tumorigenesis. Cancer Res 64(19):6849–6853

Hwang D, Scollard D, Byrne J, Levine E (1998) Expression of cyclooxygenase-1 and cyclooxygenase-2 in human breast cancer. J Natl Cancer Inst 90(6):455–460

Inoue K, Slaton JW, Eve BY, Kim SJ, Perrotte P, Balbay MD, Yano S, Bar-Eli M, Radinsky R, Pettaway CA, Dinney CP (2000) Interleukin 8 expression regulates tumorigenicity and metastases in androgen-independent prostate cancer. Clin Cancer Res 6(5):2104–2119

Itzkowitz SH, Yio X (2004) Inflammation and cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. Am J Physiol Gastrointest Liver Physiol 287(1):G7–17

Jaiswal M, LaRusso NF, Burgart LJ, Gores GJ (2000) Inflammatory cytokines induce DNA damage and inhibit DNA repair in cholangiocarcinoma cells by a nitric oxide-dependent mechanism. Cancer Res 60(1):184–190

Jimenez JL, Iniguez MA, Munoz-Fernandez MA, Fresno M (2004) Effect of phosphodiesterase 4 inhibitors on NFAT-dependent cyclooxygenase-2 expression in human T lymphocytes. Cell Signal 16(12):1363–1373

Jin X, Kim SH, Jeon HM, Beck S, Sohn YW, Yin J, Kim JK, Lim YC, Lee JH, Kang SH, Pian X, Song MS, Park JB, Chae YS, Chung YG, Lee SH, Choi YJ, Nam DH, Choi YK, Kim H (2012) Interferon regulatory factor 7 regulates glioma stem cells via interleukin-6 and Notch signalling. Brain 135(Pt 4):1055–1069

Jung Y, Isaacs JS, Lee S, Trepel J, Liu ZG, Neckers L (2003a) Hypoxia-inducible factor induction by tumour necrosis factor in normoxic cells requires receptor-interacting protein-dependent nuclear factor kappa B activation. Biochem J 370(Pt 3):1011–1017

Jung YJ, Isaacs JS, Lee S, Trepel J, Neckers L (2003b) IL-1beta-mediated up-regulation of HIF-1alpha via an NFkappaB/COX-2 pathway identifies HIF-1 as a critical link between inflammation and oncogenesis. FASEB J 17(14):2115–2117

Karin M, Lin A (2002) NF-kappaB at the crossroads of life and death. Nat Immunol 3(3):221–227

Keeley D, Rees J (1997) New guidelines on asthma management. BMJ 314(7077):315–316

Khong HT, Restifo NP (2002) Natural selection of tumor variants in the generation of "tumor escape" phenotypes. Nat Immunol 3(11):999–1005

Kiani A, Rao A, Aramburu J (2000) Manipulating immune responses with immunosuppressive agents that target NFAT. Immunity 12(4):359–372

Kim JE, Patel M, Ruzevick J, Jackson CM, Lim M (2014) STAT3 activation in glioblastoma: biochemical and therapeutic implications. Cancers (Basel) 6(1):376–395

Kim SS, Ruiz VE, Carroll JD, Moss SF (2011) Helicobacter pylori in the pathogenesis of gastric cancer and gastric lymphoma. Cancer Lett 305(2):228–238

Kim YH, Woo KJ, Lim JH, Kim S, Lee TJ, Jung EM, Lee JM, Park JW, Kwon TK (2005) 8-Hydroxyquinoline inhibits iNOS expression and nitric oxide production by down-regulating LPS-induced activity of NF-kappaB and C/EBPbeta in Raw 264.7 cells. Biochem Biophys Res Commun 329(2):591–597

Kleihues P, Burger PC, Scheithauer BW (1993) The new WHO classification of brain tumours. Brain Pathol 3(3):255–268

Kleihues P, Ohgaki H (1999) Primary and secondary glioblastomas: from concept to clinical diagnosis. Neuro Oncol 1(1):44–51

Kong T, Eltzschig HK, Karhausen J, Colgan SP, Shelley CS (2004) Leukocyte adhesion during hypoxia is mediated by HIF-1-dependent induction of beta2 integrin gene expression. Proc Natl Acad Sci USA 101(28):10440–10445

Konnecke H, Bechmann I (2013) The role of microglia and matrix metalloproteinases involvement in neuroinflammation and gliomas. Clin Dev Immunol 2013:914104

Kujas M (1993) Meningioma. Curr Opin Neurol 6(6):882–887

Lebel MH, Freij BJ, Syrogiannopoulos GA, Chrane DF, Hoyt MJ, Stewart SM, Kennard BD, Olsen KD, McCracken GH Jr (1988) Dexamethasone therapy for bacterial meningitis. Results of two double-blind, placebo-controlled trials. N Engl J Med 319(15):964–971

Leek RD, Harris AL (2002) Tumor-associated macrophages in breast cancer. J Mammary Gland Biol Neoplasia 7(2):177–189

Leek RD, Hunt NC, Landers RJ, Lewis CE, Royds JA, Harris AL (2000) Macrophage infiltration is associated with VEGF and EGFR expression in breast cancer. J Pathol 190(4):430–436

Leek RD, Landers R, Fox SB, Ng F, Harris AL, Lewis CE (1998) Association of tumour necrosis factor alpha and its receptors with thymidine phosphorylase expression in invasive breast carcinoma. Br J Cancer 77(12):2246–2251

Li A, Walling J, Ahn S, Kotliarov Y, Su Q, Quezado M, Oberholtzer JC, Park J, Zenklusen JC, Fine HA (2009) Unsupervised analysis of transcriptomic profiles reveals six glioma subtypes. Cancer Res 69(5):2091–2099

Li Q, Verma IM (2002) NF-kappaB regulation in the immune system. Nat Rev Immunol 2 (10):725–734

Li R, Li G, Deng L, Liu Q, Dai J, Shen J, Zhang J (2010) IL-6 augments the invasiveness of U87MG human glioblastoma multiforme cells via up-regulation of MMP-2 and fascin-1. Oncol Rep 23(6):1553–1559

Lin EY, Gouon-Evans V, Nguyen AV, Pollard JW (2002) The macrophage growth factor CSF-1 in mammary gland development and tumor progression. J Mammary Gland Biol Neoplasia 7 (2):147–162

Lin EY, Pollard JW (2004) Role of infiltrated leucocytes in tumour growth and spread. Br J Cancer 90(11):2053–2058

Lisi L, Stigliano E, Lauriola L, Navarra P, Dello Russo C (2014) Proinflammatory-activated glioma cells induce a switch in microglial polarization and activation status, from a predominant M2b phenotype to a mixture of M1 and M2a/B polarized cells. ASN Neuro 6 (3):171–183

Liu Q, Li G, Li R, Shen J, He Q, Deng L, Zhang C, Zhang J (2010) IL-6 promotion of glioblastoma cell invasion and angiogenesis in U251 and T98G cell lines. J Neurooncol 100 (2):165–176

Loberg RD, Ying C, Craig M, Day LL, Sargent E, Neeley C, Wojno K, Snyder LA, Yan L, Pienta KJ (2007) Targeting CCL2 with systemic delivery of neutralizing antibodies induces prostate cancer tumor regression in vivo. Cancer Res 67(19):9417–9424

Locatelli M, Ferrero S, Martinelli Boneschi F, Boiocchi L, Zavanone M, Maria Gaini S, Bello L, Valentino S, Barbati E, Nebuloni M, Mantovani A, Garlanda C (2013) The long pentraxin

PTX3 as a correlate of cancer-related inflammation and prognosis of malignancy in gliomas. J Neuroimmunol 260(1–2):99–106
Longo DL, Harrison TR, Fauci AS (2012) Harrison's principles of internal medicine, 18th edn. McGraw-Hill Medical, New York, London
Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, Scheithauer BW, Kleihues P (2007) The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol 114(2):97–109
Lu T, Tian L, Han Y, Vogelbaum M, Stark GR (2007) Dose-dependent cross-talk between the transforming growth factor-beta and interleukin-1 signaling pathways. Proc Natl Acad Sci USA 104(11):4365–4370
Macarthur M, Hold GL, El-Omar EM (2004) Inflammation and Cancer II. Role of chronic inflammation and cytokine gene polymorphisms in the pathogenesis of gastrointestinal malignancy. Am J Physiol Gastrointest Liver Physiol 286(4):G515–G520
Mahadewa T, Harsan H, Nugroho S, Bernstein M (2005) Postoperative recovery of complete sudden paraplegia due to lumbar schwannoma. Case report. J Neurosurg Spine 2(5):601–603
Makino Y, Nakamura H, Ikeda E, Ohnuma K, Yamauchi K, Yabe Y, Poellinger L, Okada Y, Morimoto C, Tanaka H (2003) Hypoxia-inducible factor regulates survival of antigen receptor-driven T cells. J Immunol 171(12):6534–6540
Malik N, Greenfield BW, Wahl AF, Kiener PA (1996) Activation of human monocytes through CD40 induces matrix metalloproteinases. J Immunol 156(10):3952–3960
Mantovani A, Allavena P, Sica A, Balkwill F (2008) Cancer-related inflammation. Nature 454 (7203):436–444
Markovic DS, Glass R, Synowitz M, Rooijen N, Kettenmann H (2005) Microglia stimulate the invasiveness of glioma cells by increasing the activity of metalloprotease-2. J Neuropathol Exp Neurol 64(9):754–762
Maslinska D, Laure-Kamionowska M, Maslinska S (2012) Toll-like receptors as an innate immunity bridge to neuroinflammation in medulloblastoma. Folia Neuropathol 50(4):375–381
McCulloch CA, Downey GP, El-Gabalawy H (2006) Signalling platforms that modulate the inflammatory response: new targets for drug development. Nat Rev Drug Discov 5(10): 864–876
Meini A, Sticozzi C, Massai L, Palmi M (2008) A nitric oxide/Ca(2+)/calmodulin/ERK1/2 mitogen-activated protein kinase pathway is involved in the mitogenic effect of IL-1beta in human astrocytoma cells. Br J Pharmacol 153(8):1706–1717
Merchant TE, Li C, Xiong X, Gaber MW (2009) Cytokine and growth factor responses after radiotherapy for localized ependymoma. Int J Radiat Oncol Biol Phys 74(1):159–167
Mora A, Youn J, Keegan A, Boothby M (2001) NF-kappa B/Rel participation in the lymphokine-dependent proliferation of T lymphoid cells. J Immunol 166(4):2218–2227
Morito N, Yoh K, Itoh K, Hirayama A, Koyama A, Yamamoto M, Takahashi S (2003) Nrf2 regulates the sensitivity of death receptor signals by affecting intracellular glutathione levels. Oncogene 22(58):9275–9281
Motohashi H, Yamamoto M (2004) Nrf2-Keap1 defines a physiologically important stress response mechanism. Trends Mol Med 10(11):549–557
Muller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verastegui E, Zlotnik A (2001) Involvement of chemokine receptors in breast cancer metastasis. Nature 410(6824):50–56
Nakano O, Sato M, Naito Y, Suzuki K, Orikasa S, Aizawa M, Suzuki Y, Shintaku I, Nagura H, Ohtani H (2001) Proliferative activity of intratumoral CD8(+) T-lymphocytes as a prognostic factor in human renal cell carcinoma: clinicopathologic demonstration of antitumor immunity. Cancer Res 61(13):5132–5136
Nathan C (2002) Points of control in inflammation. Nature 420(6917):846–852
Okami J, Yamamoto H, Fujiwara Y, Tsujie M, Kondo M, Noura S, Oshima S, Nagano H, Dono K, Umeshita K, Ishikawa O, Sakon M, Matsuura N, Nakamori S, Monden M (1999)

Overexpression of cyclooxygenase-2 in carcinoma of the pancreas. Clin Cancer Res 5 (8):2018–2024
Opdenakker G, Van den Steen PE, Van Damme J (2001) Gelatinase B: a tuner and amplifier of immune functions. Trends Immunol 22(10):571–579
Ostrom QT, Gittleman H, Farah P, Ondracek A, Chen Y, Wolinsky Y, Stroup NE, Kruchko C, Barnholtz-Sloan JS (2013) CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2006–2010. Neuro Oncol 15(Suppl 2):ii1-56
Paugh BS, Bryan L, Paugh SW, Wilczynska KM, Alvarez SM, Singh SK, Kapitonov D, Rokita H, Wright S, Griswold-Prenner I, Milstien S, Spiegel S, Kordula T (2009) Interleukin-1 regulates the expression of sphingosine kinase 1 in glioblastoma cells. J Biol Chem 284(6):3408–3417
Perkins ND (2000) The Rel/NF-kappa B family: friend and foe. Trends Biochem Sci 25(9): 434–440
Pfisterer WK, Hank NC, Preul MC, Hendricks WP, Pueschel J, Coons SW, Scheck AC (2004) Diagnostic and prognostic significance of genetic regional heterogeneity in meningiomas. Neuro Oncol 6(4):290–299
Philip M, Rowley DA, Schreiber H (2004) Inflammation as a tumor promoter in cancer induction. Semin Cancer Biol 14(6):433–439
Platten M, Ochs K, Lemke D, Opitz C, Wick W (2014) Microenvironmental clues for glioma immunotherapy. Curr Neurol Neurosci Rep 14(4):440
Pollard JW (2004) Tumour-educated macrophages promote tumour progression and metastasis. Nat Rev Cancer 4(1):71–78
Pollo B (2012) Pathological classification of brain tumors. Q J Nucl Med Mol Imaging 56(2): 103–111
Prescott SM, Fitzpatrick FA (2000) Cyclooxygenase-2 and carcinogenesis. Biochim Biophys Acta 1470(2):M69–M78
Puri S, Joshi BH, Sarkar C, Mahapatra AK, Hussain E, Sinha S (2005) Expression and structure of interleukin 4 receptors in primary meningeal tumors. Cancer 103(10):2132–2142
Ragel BT, Jensen RL, Couldwell WT (2007) Inflammatory response and meningioma tumorigenesis and the effect of cyclooxygenase-2 inhibitors. Neurosurg Focus 23(4):E7
Rahaman SO, Harbor PC, Chernova O, Barnett GH, Vogelbaum MA, Haque SJ (2002) Inhibition of constitutively active Stat3 suppresses proliferation and induces apoptosis in glioblastoma multiforme cells. Oncogene 21(55):8404–8413
Rao CV (2004) Nitric oxide signaling in colon cancer chemoprevention. Mutat Res 555(1–2): 107–119
Rao JS (2003) Molecular mechanisms of glioma invasiveness: the role of proteases. Nat Rev Cancer 3(7):489–501
Raychaudhuri B, Vogelbaum MA (2011) IL-8 is a mediator of NF-kappaB induced invasion by gliomas. J Neurooncol 101(2):227–235
Reinke JM, Sorg H (2012) Wound repair and regeneration. Eur Surg Res 49(1):35–43
Ribatti D, Vacca A, Nico B, Crivellato E, Roncali L, Dammacco F (2001) The role of mast cells in tumour angiogenesis. Br J Haematol 115(3):514–521
Rich JN, Bigner DD (2004) Development of novel targeted therapies in the treatment of malignant glioma. Nat Rev Drug Discov 3(5):430–446
Rivkin M, Kanoff RB (2013) Metastatic brain tumors: current therapeutic options and historical perspective. J Am Osteopath Assoc 113(5):418–423
Rolhion C, Penault-Llorca F, Kemeny JL, Lemaire JJ, Jullien C, Labit-Bouvier C, Finat-Duclos F, Verrelle P (2001) Interleukin-6 overexpression as a marker of malignancy in human gliomas. J Neurosurg 94(1):97–101
Rolle CE, Sengupta S, Lesniak MS (2012) Mechanisms of immune evasion by gliomas. Adv Exp Med Biol 746:53–76
Rossi ML, Jones NR, Esiri MM, Havas L, Nakamura N, Coakham HB (1990) Mononuclear cell infiltrate, HLA-Dr expression and proliferation in 37 acoustic schwannomas. Histol Histopathol 5(4):427–432

Ruano Y, Mollejo M, Camacho FI, Rodriguez de Lope A, Fiano C, Ribalta T, Martinez P, Hernandez-Moneo JL, Melendez B (2008) Identification of survival-related genes of the phosphatidylinositol 3'-kinase signaling pathway in glioblastoma multiforme. Cancer 112 (7):1575–1584

Saji H, Koike M, Yamori T, Saji S, Seiki M, Matsushima K, Toi M (2001) Significant correlation of monocyte chemoattractant protein-1 expression with neovascularization and progression of breast carcinoma. Cancer 92(5):1085–1091

Sasaki A, Tamura M, Hasegawa M, Ishiuchi S, Hirato J, Nakazato Y (1998) Expression of interleukin-1beta mRNA and protein in human gliomas assessed by RT-PCR and immunohistochemistry. J Neuropathol Exp Neurol 57(7):653–663

Savill J, Dransfield I, Gregory C, Haslett C (2002) A blast from the past: clearance of apoptotic cells regulates immune responses. Nat Rev Immunol 2(12):965–975

Savill J, Fadok V (2000) Corpse clearance defines the meaning of cell death. Nature 407 (6805):784–788

Scapini P, Nesi L, Morini M, Tanghetti E, Belleri M, Noonan D, Presta M, Albini A, Cassatella MA (2002) Generation of biologically active angiostatin kringle 1-3 by activated human neutrophils. J Immunol 168(11):5798–5804

Schaider H, Oka M, Bogenrieder T, Nesbit M, Satyamoorthy K, Berking C, Matsushima K, Herlyn M (2003) Differential response of primary and metastatic melanomas to neutrophils attracted by IL-8. Int J Cancer 103(3):335–343

Scotton CJ, Wilson JL, Milliken D, Stamp G, Balkwill FR (2001) Epithelial cancer cell migration: a role for chemokine receptors? Cancer Res 61(13):4961–4965

See AP, Han JE, Phallen J, Binder Z, Gallia G, Pan F, Jinasena D, Jackson C, Belcaid Z, Jeong SJ, Gottschalk C, Zeng J, Ruzevick J, Nicholas S, Kim Y, Albesiano E, Pardoll DM, Lim M (2012) The role of STAT3 activation in modulating the immune microenvironment of GBM. J Neurooncol 110(3):359–368

Semenza GL (1999) Regulation of mammalian O2 homeostasis by hypoxia-inducible factor 1. Annu Rev Cell Dev Biol 15:551–578

Sen E (2011) Targeting inflammation-induced transcription factor activation: an open frontier for glioma therapy. Drug Discov Today 16(23–24):1044–1051

Senftleben U, Li ZW, Baud V, Karin M (2001) IKKbeta is essential for protecting T cells from TNFalpha-induced apoptosis. Immunity 14(3):217–230

Seril DN, Liao J, Yang GY, Yang CS (2003) Oxidative stress and ulcerative colitis-associated carcinogenesis: studies in humans and animal models. Carcinogenesis 24(3):353–362

Sharma V, Dixit D, Ghosh S, Sen E (2011a) COX-2 regulates the proliferation of glioma stem like cells. Neurochem Int 59(5):567–571

Sharma V, Dixit D, Koul N, Mehta VS, Sen E (2011b) Ras regulates interleukin-1beta-induced HIF-1alpha transcriptional activity in glioblastoma. J Mol Med (Berl) 89(2):123–136

Shirahata M, Iwao-Koizumi K, Saito S, Ueno N, Oda M, Hashimoto N, Takahashi JA, Kato K (2007) Gene expression-based molecular diagnostic system for malignant gliomas is superior to histological diagnosis. Clin Cancer Res 13(24):7341–7356

Smyth MJ, Cretney E, Kershaw MH, Hayakawa Y (2004) Cytokines in cancer immunity and immunotherapy. Immunol Rev 202:275–293

Sparmann A, Bar-Sagi D (2004) Ras-induced interleukin-8 expression plays a critical role in tumor growth and angiogenesis. Cancer Cell 6(5):447–458

Spooren A, Mestdagh P, Rondou P, Kolmus K, Haegeman G, Gerlo S (2011) IL-1beta potently stabilizes IL-6 mRNA in human astrocytes. Biochem Pharmacol 81(8):1004–1015

Steele VE, Hawk ET, Viner JL, Lubet RA (2003) Mechanisms and applications of non-steroidal anti-inflammatory drugs in the chemoprevention of cancer. Mutat Res 523–524:137–144

Stevens A, Kloter I, Roggendorf W (1988) Inflammatory infiltrates and natural killer cell presence in human brain tumors. Cancer 61(4):738–743

Stoffel A, Chaurushiya M, Singh B, Levine AJ (2004) Activation of NF-kappaB and inhibition of p53-mediated apoptosis by API2/mucosa-associated lymphoid tissue 1 fusions promote oncogenesis. Proc Natl Acad Sci USA 101(24):9079–9084

Strieter RM (2001) Chemokines: not just leukocyte chemoattractants in the promotion of cancer. Nat Immunol 2(4):285–286

Sun S, Wang Q, Giang A, Cheng C, Soo C, Wang CY, Liau LM, Chiu R (2011) Knockdown of CypA inhibits interleukin-8 (IL-8) and IL-8-mediated proliferation and tumor growth of glioblastoma cells through down-regulated NF-kappaB. J Neurooncol 101(1):1–14

Tak PP, Firestein GS (2001) NF-kappaB: a key role in inflammatory diseases. J Clin Invest 107 (1):7–11

Tanabe K, Kozawa O, Iida H (2011) Midazolam suppresses interleukin-1beta-induced interleukin-6 release from rat glial cells. J Neuroinflammation 8:68

Tarassishin L, Casper D, Lee SC (2014a) Aberrant expression of interleukin-1beta and inflammasome activation in human malignant gliomas. PLoS ONE 9(7):e103432

Tarassishin L, Lim J, Weatherly DB, Angeletti RH, Lee SC (2014b) Interleukin-1-induced changes in the glioblastoma secretome suggest its role in tumor progression. J Proteomics 99:152–168

Taurone S, Bianchi E, Attanasio G, Di Gioia C, Ierino R, Carubbi C, Galli D, Pastore FS, Giangaspero F, Filipo R, Zanza C, Artico M (2015) Immunohistochemical profile of cytokines and growth factors expressed in vestibular schwannoma and in normal vestibular nerve tissue. Mol Med Rep 12(1):737–745

Tchirkov A, Khalil T, Chautard E, Mokhtari K, Veronese L, Irthum B, Vago P, Kemeny JL, Verrelle P (2007) Interleukin-6 gene amplification and shortened survival in glioblastoma patients. Br J Cancer 96(3):474–476

Temel SG, Kahveci Z (2009) Cyclooxygenase-2 expression in astrocytes and microglia in human oligodendroglioma and astrocytoma. J Mol Histol 40(5–6):369–377

Tewari R, Choudhury SR, Ghosh S, Mehta VS, Sen E (2012) Involvement of TNFalpha-induced TLR4-NF-kappaB and TLR4-HIF-1alpha feed-forward loops in the regulation of inflammatory responses in glioma. J Mol Med (Berl) 90(1):67–80

Tsujii M, Kawano S, DuBois RN (1997) Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. Proc Natl Acad Sci USA 94(7):3336–3340

Tsung K, Dolan JP, Tsung YL, Norton JA (2002) Macrophages as effector cells in interleukin 12-induced T cell-dependent tumor rejection. Cancer Res 62(17):5069–5075

Tu Y, Zhong Y, Fu J, Cao Y, Fu G, Tian X, Wang B (2011) Activation of JAK/STAT signal pathway predicts poor prognosis of patients with gliomas. Med Oncol 28(1):15–23

Valavanidis A, Vlachogianni T, Fiotakis K, Loridas S (2013) Pulmonary oxidative stress, inflammation and cancer: respirable particulate matter, fibrous dusts and ozone as major causes of lung carcinogenesis through reactive oxygen species mechanisms. Int J Environ Res Public Health 10(9):3886–3907

Vigneswaran K, Neill S, Hadjipanayis CG (2015) Beyond the World Health Organization grading of infiltrating gliomas: advances in the molecular genetics of glioma classification. Ann Transl Med 3(7):95

Wakabayashi K, Kambe F, Cao X, Murakami R, Mitsuyama H, Nagaya T, Saito K, Yoshida J, Seo H (2004) Inhibitory effects of cyclosporin A on calcium mobilization-dependent interleukin-8 expression and invasive potential of human glioblastoma U251MG cells. Oncogene 23(41):6924–6932

Walker C, Baborie A, Crooks D, Wilkins S, Jenkinson MD (2011) Biology, genetics and imaging of glial cell tumours. Br J Radiol 84(2):S90-106

Walmsley SR, Print C, Farahi N, Peyssonnaux C, Johnson RS, Cramer T, Sobolewski A, Condliffe AM, Cowburn AS, Johnson N, Chilvers ER (2005) Hypoxia-induced neutrophil survival is mediated by HIF-1alpha-dependent NF-kappaB activity. J Exp Med 201(1):105–115

Wang H, Lathia JD, Wu Q, Wang J, Li Z, Heddleston JM, Eyler CE, Elderbroom J, Gallagher J, Schuschu J, MacSwords J, Cao Y, McLendon RE, Wang XF, Hjelmeland AB, Rich JN (2009a) Targeting interleukin 6 signaling suppresses glioma stem cell survival and tumor growth. Stem Cells 27(10):2393–2404

Wang L, Liu Z, Balivada S, Shrestha T, Bossmann S, Pyle M, Pappan L, Shi J, Troyer D (2012) Interleukin-1beta and transforming growth factor-beta cooperate to induce neurosphere formation and increase tumorigenicity of adherent LN-229 glioma cells. Stem Cell Res Ther 3(1):5

Wang L, Yi T, Kortylewski M, Pardoll DM, Zeng D, Yu H (2009b) IL-17 can promote tumor growth through an IL-6-Stat3 signaling pathway. J Exp Med 206(7):1457–1464

Wang T, Niu G, Kortylewski M, Burdelya L, Shain K, Zhang S, Bhattacharya R, Gabrilovich D, Heller R, Coppola D, Dalton W, Jove R, Pardoll D, Yu H (2004) Regulation of the innate and adaptive immune responses by Stat-3 signaling in tumor cells. Nat Med 10(1):48–54

Waugh DJ, Wilson C (2008) The interleukin-8 pathway in cancer. Clin Cancer Res 14(21): 6735–6741

Wiemels J, Wrensch M, Claus EB (2010) Epidemiology and etiology of meningioma. J Neurooncol 99(3):307–314

Williams CS, Mann M, DuBois RN (1999) The role of cyclooxygenases in inflammation, cancer, and development. Oncogene 18(55):7908–7916

Wilson J, Balkwill F (2002) The role of cytokines in the epithelial cancer microenvironment. Semin Cancer Biol 12(2):113–120

Wyckoff JB, Segall JE, Condeelis JS (2000) The collection of the motile population of cells from a living tumor. Cancer Res 60(19):5401–5404

Yang CR, Hsieh SL, Ho FM, Lin WW (2005a) Decoy receptor 3 increases monocyte adhesion to endothelial cells via NF-kappa B-dependent up-regulation of intercellular adhesion molecule-1, VCAM-1, and IL-8 expression. J Immunol 174(3):1647–1656

Yang J, Chatterjee-Kishore M, Staugaitis SM, Nguyen H, Schlessinger K, Levy DE, Stark GR (2005b) Novel roles of unphosphorylated STAT3 in oncogenesis and transcriptional regulation. Cancer Res 65(3):939–947

Yeung YT, Bryce NS, Adams S, Braidy N, Konayagi M, McDonald KL, Teo C, Guillemin GJ, Grewal T, Munoz L (2012) p38 MAPK inhibitors attenuate pro-inflammatory cytokine production and the invasiveness of human U251 glioblastoma cells. J Neurooncol 109 (1):35–44

Yeung YT, McDonald KL, Grewal T, Munoz L (2013) Interleukins in glioblastoma pathophysiology: implications for therapy. Br J Pharmacol 168(3):591–606

Yoshino Y, Aoyagi M, Tamaki M, Duan L, Morimoto T, Ohno K (2006) Activation of p38 MAPK and/or JNK contributes to increased levels of VEGF secretion in human malignant glioma cells. Int J Oncol 29(4):981–987

Zhang J, Sarkar S, Cua R, Zhou Y, Hader W, Yong VW (2012) A dialog between glioma and microglia that promotes tumor invasiveness through the CCL2/CCR2/interleukin-6 axis. Carcinogenesis 33(2):312–319

Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, Makrigiannakis A, Gray H, Schlienger K, Liebman MN, Rubin SC, Coukos G (2003) Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med 348(3):203–213

Zhang XD, Wang Y, Ye LH (2014) Hepatitis B virus X protein accelerates the development of hepatoma. Cancer Biol Med 11(3):182–190

Zhou H, Bernhard EJ, Fox FE, Billings PC (1993) Induction of metalloproteinase activity in human T-lymphocytes. Biochim Biophys Acta 1177(2):174–178

Zhou J, Schmid T, Brune B (2003) Tumor necrosis factor-alpha causes accumulation of a ubiquitinated form of hypoxia inducible factor-1alpha through a nuclear factor-kappaB-dependent pathway. Mol Biol Cell 14(6):2216–2225

Glia in the Retina: Pluri-Functional Mediators in Diverse Ocular Disorders

11

Nivedita Chatterjee

Abstract

Dysfunction of glia is emerging as a cause for many retinal disorders. Originally identified as supporting cells in the brain, it is increasingly becoming clear that they play major roles in maintaining homeostasis and regulating many aspects of disease progression in the retina. A complex signalling system exists which allows neuron–glia and neurovascular interaction. Besides releasing glial and neuronal signalling molecules directed to cellular homeostasis, glia respond also to infectious and non-infectious external stressors, to create a milieu which heavily decides survival of surrounding cells. This review article describes some of the latest advances in our knowledge on the role of the glia and their involvement in immune responses. Understanding glial contribution will significantly improve comprehension of disease susceptibility and progression. Targeting glial-specific pathways might ultimately impact the development of therapies for clinical management of retinal neurodegeneration.

11.1 Introduction

Neurodegenerative processes adversely affect vision in a significant portion of the human population. Almost all of them have an immune-pathology, even those not initiated by infections. These include glaucoma, age-related macular degeneration (AMD), diabetic retinopathy, ischemia, retinopathy of prematurity and traumatic injuries. As an immune-privileged organ, the retina is designed to attenuate

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inflammatory response to safeguard vision. Retinal glia play an important part in maintaining homeostasis, aiding in synaptic activity by transporting neurotransmitters and preserving neuronal health. Novel techniques and models have increased our understanding of the mechanisms underlying ocular inflammation and innate immunity that are operative in the abrogation of immune privilege. This review encapsulates some of the latest literature on the molecular events at glial cells of the retina during some common ocular diseases.

Much of the functions performed by immune-competent cells elsewhere in the body are taken up by glial cells in the retina. Glial cells consisting of oligodendrocytes, astrocytes, microglia, and NG2 positive cells make a large part of the central nervous system (CNS), numberwise. They function as effectors and modulators of neurodevelopment through diverse neuron–glial interactions. Glia can be affected by both genetic and environmental factors, leading to their dysfunction in supporting neuronal development and functions. These in turn can affect neuronal cells, causing alterations at the circuitry level that manifest as behavioural characteristics. Glial anomalies can be either structural or cellular changes. Changes in glial functions are pervasive in most neurodegenerative disease making them important targets for therapy. Glial cells share many of the signalling pathways observed in neurons. However, differences in form and function make them clinically feasible and potentially applicable targets for treatment. In the mammalian retina, astrocytes, Muller cells and possibly microglia are coupled to each other to form a glial syncytium. Curiously, this syncytium is conditional allowing for greater control over activation of contacting groups of cells (Robinson et al. 1993; Newman and Zahs 1998). For example, it is suggested that the syncytium may be controlling not just the retinal milieu but can also affect retinal pigmented epithelial cells through microglia during AMD (Ma et al. 2009). Much of the immune response induced in retinal glia can be classified as innate immune response. This is not specific to particular pathogens, but initiated by diverse proteins.

The eye has evolved to limit intraocular inflammation so as to protect visual acuity. The normal brain and retina are protected by vascular endothelium at the blood brain barrier (BBB) and blood retinal barrier (BRB), while epithelial cells of the choroid plexus form one more barrier. Additionally, astrocytic end feet and the parenchymal basement membrane form a further barrier, the glia limitans. A range of other mechanisms exist to limit immune responses in the retina. An active anti-inflammatory milieu is maintained as well as suppressing systemic induction of immunosuppressive regulatory T cells by eye-specific mechanisms. This protection is relative rather than absolute, and is partial. Apart from the lack of antigen presenting cells (APC) and a lymphatic drainage system, a further blockage is in place by production of FAS and TGF-β, implicating soluble factors released either in paracrine or autocrine manner as contributors to the ocular immune privilege. Traditionally, innate immunity (Fig. 11.1) has been viewed as the first line of defence discriminating benign from dangerous molecules. Emerging literature suggests that innate immunity actually serves as a system for sensing signals of ‘danger’, such as pathogenic microbes or host-derived signals of cellular stress, while remaining unresponsive to “self” motifs, such as normal host molecules and

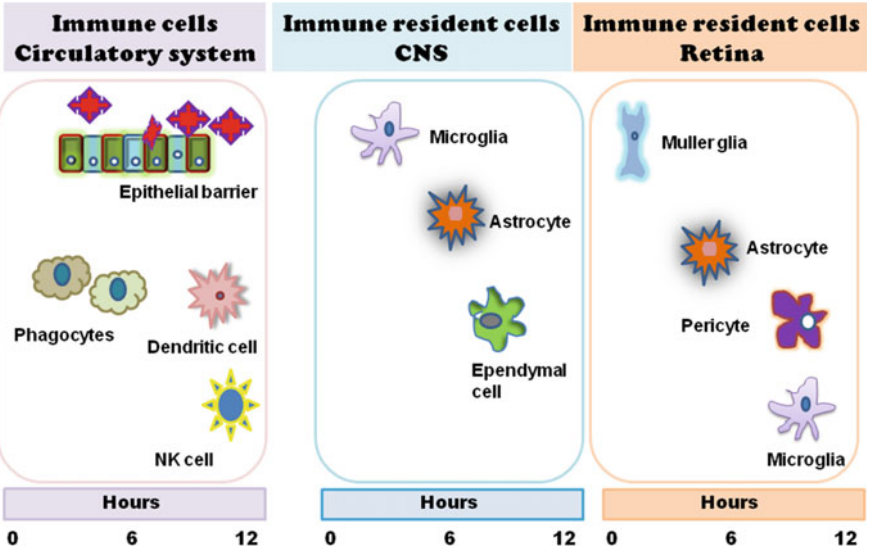

Fig. 11.1 Cells which contribute to innate immune response. Many cells in the body can contribute to innate immune response. However, only a subset can respond by pattern recognition of foreign bodies. Blood contains the largest number of circulating cells which contribute to innate immunity. In the central nervous system, the glial cells are dominant players in innate immunity. Retina possess specialized cells undertaking innate immune functions

dietary antigens. Infectious agents may cause neurological disease through (i) a direct lytic effect, (ii) by inducing immunopathology directed against CNS tissue, (iii) by induction of immune responses that damage CNS tissue in a bystander fashion, or (iv) through induction of molecular mimicry. There is still some debate whether primary neurodegenerative changes can occur in the retina independent of optic nerve inflammation and compromise of the blood–retinal barrier. However, in many diseases with wider systemic manifestations, it has been found that glial cells show subtle inflammatory changes long before preclinical signs are obvious. Promoting the healing functions of retinal glia may act as adjunct therapy during retinal neurodegeneration (Fig. 11.2).

11.2 Adaptive Immune Response: Who Are the Players?

Antigen presenting cells (APC) are limited in the central nervous system and the retina. $CD11c^{+}$ cells are the most common ones. These cells are likely to be microglial precursors stimulated with GM-CSF or even blood derived (Prodinger et al. 2011). Apart from these, conventional dendritic cells (DC) derived from bone marrow pre-DC progenitors, have been observed in meninges and choroid plexus (Anandasabapathy et al. 2011). In most instances, however, adaptive immune response in the CNS is quickly clamped down and resolved. Several animal models

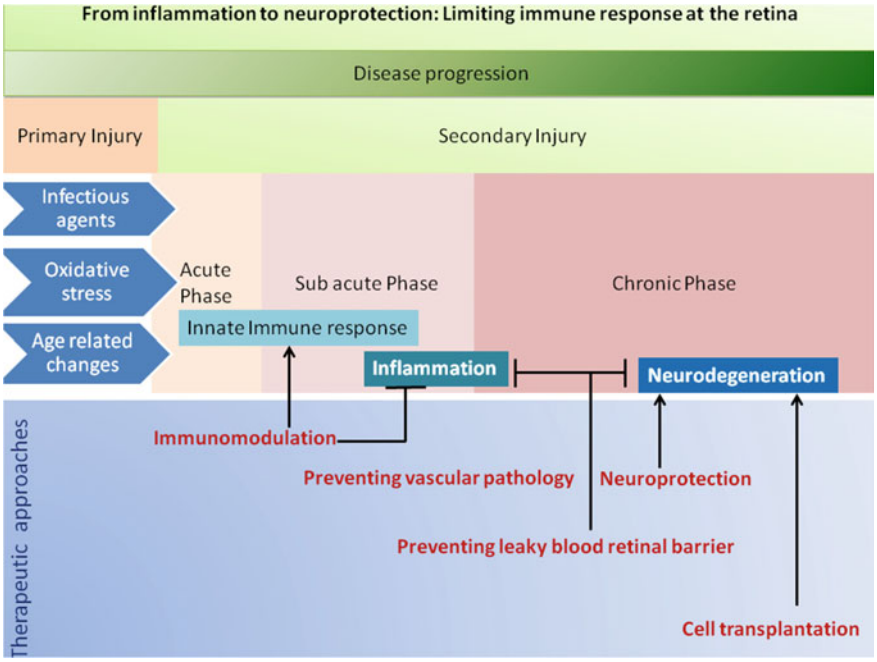

Fig. 11.2 Disease progression and therapeutic potential at the retina. Potential therapeutic strategies to alleviate or prevent the progression of retinal degeneration

with viral infection suggest that the CNS can support antibody-secreting cells after resolution of virus infection, with possibly protective but non-lytic functions (Verjans et al. 2007; Wakim et al. 2010). At the retina, such phenomena may manifest as multiple evanescent white dot syndrome and putative immune granulomas (Ben and Forrester 1995). Adaptive immunity at the retina is a relatively new research area. It is likely though that like the CNS there is a modified immune-surveillance as befits its immune-privileged nature. BBB disruption is necessary before antibodies cause pathology. Immune mediated pathology involves glia heavily. While retinal microglia are designed to replicate many of the functions seen at the CNS, it still remains to be seen what adaptive immune roles they take up.

11.3 Innate Immunity at the Retina

Retinal glia comprise of Muller glia, astrocytes and microglia. These cells have evolved to permit effective immune-surveillance while limiting immune-pathology. Injury and infection of glial cells inflict excitotoxic and inflammatory response. Innate response encompasses production of inflammatory and anti-inflammatory milieu as well as an increase in excitatory neurotransmitters such as glutamate and

oxidative stress. The cumulative effect of overexpressed proinflammatory factors can result in collateral damage. In the retina the number of astrocytes and microglia are few compared to the predominant glia, the Muller cells.

11.3.1 Major Glial Cells of the Retina

Muller glial cells make up the largest cell population of glial cells in the retina. These cells originate from progenitor cells at the apical margin of the neuroepithelium, after the generation of retinal ganglion cells, horizontal cells and cones (Rasmussen 1972). They migrate proximally and as they extend their processes act as guides to subsequently migrating neurons. Basal Muller cell end-feet participate at both inner limiting membrane and blood vessels in the superficial retina (Raviola 1977). They are crucial for metabolic functions with their numerous glycogen granules, mitochondria and a host of transporters and ion channels (Schnitzer 1988). Normally, they function to provide a stable environment in the retina. By mediating transcellular ion, water, and bicarbonate transport, Muller cells also control the composition of the extracellular space fluid. Additionally, they provide trophic and anti-oxidative support of photoreceptors and neurons and regulate the tightness of the blood–retinal barrier. Muller glia being one of the most robust cells in the retina, are involved in practically all types of injury that occur. Recent work has also identified a Muller glial role in innate immune response. It has been established that retinal Muller glia sense pathogens through the TLRs and contribute directly to retinal innate defence via production of inflammatory mediators and antimicrobial peptides (Shamsuddin and Kumar 2011). They are also a major source of inflammatory factors during infection (Krishnan and Chatterjee 2012, 2014).

Astrocytes do not arise from the retinal neuroepithelium but migrate into the retina along the optic nerve (Watanabe and Raff 1988). As a retina develops astrocytes space themselves across the retina, while maintaining contact among each other through their processes. Astrocyte processes sometimes follow blood vessels into the RGC layer but are mostly confined to the plane parallel to the retinal surface. They are known to be important in regulating blood flow to the retina (Schnitzer 1987). In normal retina, astrocytes are the only cells expressing Glial acidic fibrillary protein (GFAP). When injured, Muller glia also express GFAP suggesting that the protein acts as an activation marker (Krishnan and Chatterjee 2012).

Much like astrocytes, microglia move into the retina. They are thought to be of mesodermal origin and appear in the retina as blood vessels develop (Dräger 1983). In the normal retina they are sparse and are present in a dormant state in the nerve fibre layer (NFL), inner and outer plexiform layers (IPL, OPL) (Schnitzer 1989). In their dormant state, they are characterized by their slender, hair-like processes. As one of the most motile cells in the CNS microglia respond by moving into injured areas. They also proliferate in response to retinal injury and proceed to differentiate into macrophage-like cells expressing many markers common to macrophages (Herbomel et al. 2001). Prolonged microglial activation may lead to chronic inflammation leading to severe pathological side effects and worsening retinal dystrophies.

11.3.2 Microglial Cells in Ocular Pathologies

Current reports propose a vital role for innate immunity and complement overactivation for age related macular degeneration (AMD) pathogenesis. It also is a prime example that inflammation in the retina need not be a by-product of infection. In AMD persistent inflammation may be a reason for predisposition. AMD primarily affect the photoreceptors, RPE, Bruch's membrane, and choriocapillaries. A study of age-related changes of the retina, demonstrate recruitment of leukocytes and activation of the complement cascade in mouse RPE and choroid during AMD (Chen et al. 2008). RPE dysfunction (Kaarniranta et al. 2013) in complement system had been one of the earliest causes identified in AMD; recent reports however also suggest the role of microglia. This involves the complement system and the innate immune response embodied by cytokines. Chemokines are cytokines with chemoattractive properties, playing a central role in recruitment of immune cells to inflamed tissues. Such chemokines bind to chemokine receptors on inflammatory cells like macrophages to promote the mobilization of the cells into tissues from the circulation. One chemokine receptor that has generated much interest in AMD is CX3CR1. The CX3CR1 chemokine receptor is a G-coupled receptor found on a variety of inflammatory cells, including microglia, macrophages, T cells, and astrocytes. When bound by its ligand CX3CL1 (also known as fractalkine), CX3CR1 moves leukocytes to inflamed tissues and subsequently causes activation of these inflammatory cells (Fong et al. 1998). CX3CR1 and CX3CL1 are present in the retina and brain (Combadiere et al. 1998). Histological analysis of AMD retina show microglia to be the only cells to express the receptor. Macular lesions are also positive for many activated microglia expressing the receptor (Gupta et al. 2003). Activated microglia can speed up their proliferation, migration to damaged tissue, phagocytize debris, secrete pro-inflammatory cytokines, and neurotoxins (Langmann 2007). Photoreceptor injury noticeably increases after administration of activated microglia to healthy photoreceptors. In the retina, an investigation in experimental retinal detachment using mice deficient for production of Ccl2 and Ccl2-specific antibody neutralization showed substantial decrease in the recruitment of parenchymal microglia to the outer nuclear layer (ONL). This lead to lesser retinal detachment, in conjunction with reduced photoreceptor death (Nakazawa et al. 2007).

Drusen formation is a notable feature in AMD. Microglia contribute to drusen-like deposits. Increased fundus autofluorescence seen in ageing wild-type mice consists of perivascular and subretinal microglia and lipofuscin granules (Xu et al. 2008). Accumulation of macrophages has been linked to that of drusen in the Ccl2−/− and Ccr2−/− murine models. Key features in these models also include (Ambati et al. 2003) lipofuscin accumulation, thickening of Bruch membrane, and increased melanosomes in the RPE. Furthermore, identification of inflammation-associated SNPs emphasize the inflammatory underpinning that modulate AMD

risk. These SNPs encode complement factors, chemokines, chemokine receptors, and toll-like receptors.

Germ line encoded pattern recognition receptors which occur on retinal microglia may also be important in playing a key role during uveitis. A variety of symptoms seen in patients can be classified as being causally related to uveitis. While some symptoms may be part of a generalized systemic syndrome in which the eye is one of several organs affected, others are confined to the posterior eye, such as sympathetic ophthalmia and birdshot retinochoroidopathy. It has also been suggested that an individual whose T cell repertoire contains retinal antigen–specific T cells with higher affinity may have a greater likelihood of developing uveitis (Kerr et al. 2008). T cells capable of recognizing retinal antigens are primed in the periphery on microbial stimuli by antigenic mimicry. While APCs in the retina are not known, the retina does possess DC and microglia which have MHC class II molecules belonging to the same class of proteins that initiate pathogen-associated molecular patterns. Immune responses in the CNS act through CD11+ cells found in the juxtavascular parenchyma, which have cell processes extending into the glia limitans. Even though infiltrating T cells are considered as playing critical role in driving the inflammation, it is increasingly thought that retinal glia can also be part of the supporting cast. Glial support to the retina may be achieved in several ways, including inherent provision of neurotrophic support factors and by immune-modulatory mechanisms.

Glia has also been implicated in diabetic retinopathy. Diabetic retinopathy (DR) occurs on chronic exposure to high glucose (Ibrahim et al. 2011). DR is associated with microglial activation and increased levels of inflammatory cytokines. In rodent models, mRNA as well as protein levels for tumour necrosis factor α (TNF-α), a robust marker of inflammation, increased in the microglial cells of the retina, early in the course of diabetes. Moreover, diabetes resulted in elevation of ionized calcium binding adaptor molecule-1 (Iba1) mRNA, known to be upregulated in activated microglia (Ibrahim et al. 2010).

In glaucoma patients, retinal ganglion cell loss was associated with inflammatory components and strong retinal IgG auto-antibody accumulations, with levels twice as high as in patients without ocular complications. This suggests that IgG antibody depositions may be contributed by immune-competent cells such as microglia (Gramlich et al. 2013). Experimental glaucoma models in rat show that pro-inflammatory microglial activation may contribute to neurodegeneration through the release of inflammatory mediators (Narayan et al. 2014). As specialized cells for immune functions, microglia can secrete local paracrine mediators and communicate with other potential immune effector cells such as Muller glia. In the DBA/2 J mice model of glaucoma, where progressive detrimental changes similar to human hereditary glaucoma occur, complement factor C1q and microglial engulfment is upregulated. Retinal sections also show greater severity at retinal ganglion cell (RGC) synapses.

11.3.3 Astrocytes in Ocular Pathologies

In several diseases where the retina is affected, targeting astrocytes may prove to be beneficial. While RPE and microglia are the main contenders for the progressive damage in AMD, astrocytes may also act to destabilize homeostasis. Exosomes are one of the newest modes of neuron-glia signalling (Frühbeis et al. 2012). Astrocytes function as neuroprotective agent by paracrine secretion of exosomes. Exclusively exosomes secreted from astrocytes have anti-angiogenic components such as endostatin which can suppress retinal vessel leakage and inhibit choroidal neovascularization (CNV) in a laser-induced CNV model (Hajrasouliha et al. 2013). Thus, targeting retinal astrocytes to release larger amount of exosomes, may aid in the anti-angiogenic therapy for CNV in age-related macular degeneration and diabetic retinopathy.

Glial activation and astrocytic proliferation is likely to be circumstantial for several ocular disorders with immune-pathology. Loss of astrocyte however, can be equally crucial. The autoimmune disorder, Neuromyelitis optica (NMO) is one such example. Lesions in NMO are frequently seen along with astrocytopathy. Aquaporin 4 (AQP4), which localizes predominantly at the astrocytic foot process are lost along with GFAP in astrocytes in NMO lesions. Extensive astrocyte loss may be responsible for subsequent demyelination followed by axonal degeneration. Thus, primary assault of astrocytes in lesions could in a subset of patients be the reason for progressive development of NMO (Misu et al. 2013).

Overexpression of GFAP is the hallmark of activation in both Muller cells and more particularly for astrocytes. Coupled with extracellular matrix remodelling and morphological changes, astrocytes undergo transformation into mature reactive forms from their quiescent state in glaucoma (Lye-Barthel et al. 2013). Even more intriguing, GFAP-negative fine astrocytic processes projecting into the retinal ganglion cell axon bundle are suggested to have phagocytic function since they express phagocytosis-related gene *Mac-2* (Nguyen et al. 2011). Astrocytic dysregulation of vascular permeability and endothelial cell activation is also increasingly thought to be a prominent feature of glaucoma. Increased fluorescein angiography and compromised blood–retinal barrier in diseased eyes of dogs with experimental glaucoma, implicate tight junctions made by astrocytic end-feet (Plange et al. 2012; Mangan et al. 2007). Endothelin-1 is a vasoconstrictive molecule. Endothelin and its receptors are produced by astrocytes in response to stretch and increase in experimental glaucoma in rat and mice (Howell et al. 2011; Minton et al. 2012). The importance of endothelin-1 in humans cannot be disregarded since patients of glaucoma show it in plasma and aqueous humour (Sugiyama et al. 1995).

11.3.4 Muller Glia in Ocular Pathologies

In the retina, the glial cells confer trophic support of injured neurons. Among many other roles, Muller glia can show repair and progenitor potential under certain

conditions (Bhattacharya et al. 2008). Emerging literature strongly suggest that regulation of microglial and astrocytes through Muller cell crosstalk occur in many diseases. Such enhanced intercellular communication is observed in optic nerve axotomy or excitotoxic injury to the retina. Modulation of microglial activity also promotes a return to baseline quiescence of photoreceptors (Wang et al. 2014). Intravitreal siRNA administration to silence the chemokine Ccl2 by suppressed expression of Ccl2 primarily at Müller cells result in inhibition of microglia/monocyte recruitment and reduced photoreceptor death (Rutar et al. 2012).

AMD may also be affected by Muller cells. Disruption of the cytokine receptor gp130 gene in Müller glia particularly, reduces CNTF-dependent photoreceptor survival and prevents phosphorylation in retinal degeneration animal models of AMD (Rhee et al. 2013). In the long term therapies targeting such aspects might by alleviating exaggerated chemokine response reduce inflammation-mediated cell death in retinal degenerative diseases such as AMD.

The immune nature of uveitis may be dissected in experimental autoimmune uveitis (EAU). Retinal antigens like CFA injected into animals can cause and replicate many of the features seen in patients. Future therapeutic approaches which might take advantage of tolerogenic administration of retinal antigen to correct defects in peripheral tolerance and to regulate T cell numbers and/or functions are also likely to take into cognisance the glial cells.

Many ophthalmic disorders occur at a higher frequency in immune-suppressed individuals. Such an example is AIDS, where much of the ocular manifestations were known beforehand but seen rarely. A genuinely new ophthalmic disorder however occurs in immune recovery uveitis (IRU), once immune system reconstitution has been achieved (Sudharshan et al. 2013). Uncontrolled inflammation is a hallmark of disorders such as immune recovery uveitis. Paradoxically, this is caused in the retina and uvea as the HIV-1 infected patient recovers on responding to successful anti-retroviral therapy (ART). Adjunct therapies include treatment with corticosteroids to inhibit inflammation. Unfortunately, many patients do not achieve a functional benefit, despite objective evidence of improvement (Holland 2008).

Because no cell culture model can reproduce the full complexity of a human disease, it is necessary to develop and use a variety of models to represent the different aspects and diverse clinical/immunological manifestations of any disease. In a HIV-1 Tat-transgenic mice model which is known to represent many aspects of HIV-associated neurodegeneration, glial cell staining typically increases (Fig. 11.3). In the same model with constitutive Tat protein expression, massive disruption of retinal structure and loss of neurons occurred (Chatterjee et al. 2011). This may partially be due to activated Muller glia without necessarily increasing their numbers. Establishment of chronic inflammation can take the form of GFAP stress fibre production in Muller glia, or suppression of markers such as HAM56 (Fig. 11.3c), which can identify macrophages and astrocytes (Leenstra et al. 1995; Nishihira 2000). Noticeably, HAM56, also known as Macrophage migration inhibitory factor (MIF) is completely absent in transgenic mice expressing the HIV-1

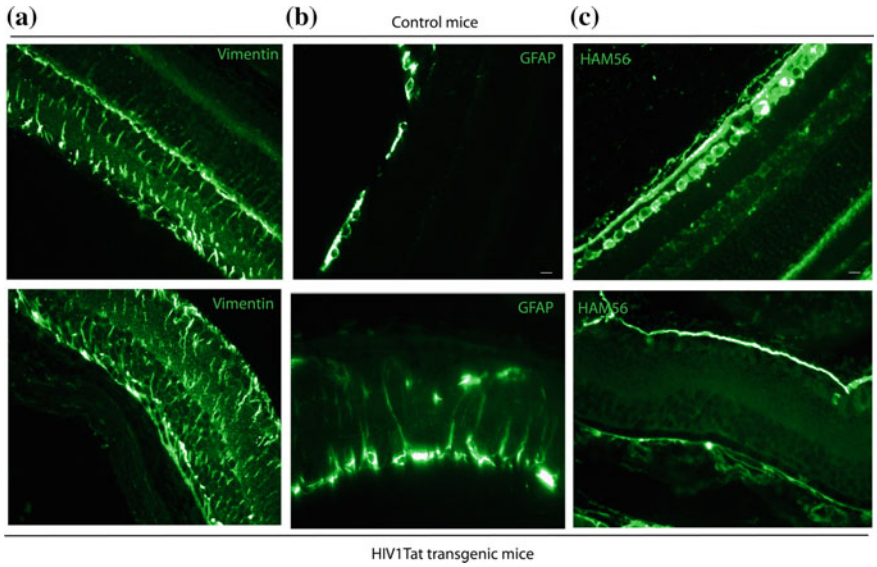

Fig. 11.3 Expression of glial and macrophage markers in retina. Immunocytochemistry of control and HIV-1 Tat-transgenic mice (n = 6) retinal sections. Glial cells stained with Vimentin (**a**) and GFAP (**b**) rise dramatically in retina of Tat-transgenic animals. Greater staining could be for several reasons including proliferative gliosis, migrating astrocytes or activation. Macrophage marker HAM56 is suppressed in retina of transgenic mice expressing HIV-1 Tat (**c**). Scale bar is 10 μm. Images were captured at ×40 magnification

Tat. This may suggest either changes in the adhesive properties or weakening of the blood–retinal barrier. Indeed slight modifications in the HIV-1 Tat structure can affect differing aspects of the barrier apparatus (Gandhi et al. 2010). In the retina, Muller glia is the largest producer of cytokines and likely communicates by both diffusible signals and contact-mediated options. Dissection of basic cellular and molecular mechanisms underlying progression of the disorder suggests that immune-modulation of Muller glia may be able to alleviate unrestrained cytokine production. In vitro primary human Muller glia cultures stimulated by the HIV-1 coat protein Tat B, simulating overwhelming inflammation, produce copious amount of pro-inflammatory factors, including several chemokines known to attract macrophage and monocytes (Krishnan and Chatterjee 2014). Multiple points in the machinery for cytokine production can be influenced by HIV-1 coat proteins to cause inflammation. HIV-1 Tat exposed Muller cells show elevated production of pro-inflammatory factors, potential neurotoxins that can cause retinal degeneration. Dissection of the signalling pathways showed the critical roles of Mitogen-activated protein kinases (MAPKs), pI3 Akt, STAT and the canonical NF-κB signalling in this process (Krishnan and Chatterjee 2014). Muller glia is so exquisitely sensitive to antigenic stimulation that clade specific HIV-1 Tat B and C can activate different pathways of cytokine production and consequently pathogenesis (Krishnan and Chatterjee 2015).

Limiting inflammation is central to immune response. A failure to regulate amplitude and duration of innate immune response can lead to chronic inflammatory state and subsequently increased loss of retinal neurons. Several studies (Matteucci et al. 2014; Patel et al. 2015) including from our group (Kaarniranta et al. 2013, 2014) have shown that Muller glia can be immune-modulated to switch production from pro-inflammatory mediators to anti-inflammatory factors. This may be harnessed for therapeutic potential.

11.3.5 Oligodendrocytes in Ocular Pathologies

Oligodendrocytes, the myelinating cells in the CNS are limited in the retina to the RGC axons. Optic neuritis and neuropathy show damage in oligodendrocytes not just in disparate ocular disorders such as NMO and glaucoma but also chronic inflammatory CNS diseases like Multiple Sclerosis. Progressive visual impairment is caused by degeneration of optic nerve axons and apoptosis of RGCs.

Glaucoma is a common condition with a neurodegenerative component and pathophysiology in glia where current therapies are often insufficient. Persistently high intraocular pressure leads to retinal ganglion cell death and visual impairment. Intraocular increase in pressure is the major diagnostic indicator of glaucoma, and is the only treatment that has been shown to reduce progressive visual loss. Strikingly, intraocular pressure reduction fails to alleviate RGC degeneration in a subset of patients with glaucoma. It is thus critical to think of adjunctive therapy along with reduction of intraocular pressure. In animal models of glaucoma it has been shown that oligodendrocyte precursor cells (OPCs), a type of neural stem cell, can protect retinal ganglion cells from damage in vivo. As RGCs are the only neuron type in the retina to possess the myelin sheath, it is crucial for their survival to have intact myelin. The success of OPCs differentiating into myelinating oligodendrocytes depended on activation of the OPCs with inflammatory stimuli. Such cells were also more successful in providing long-term protection. Intravitreal transplantation of oligodendrocyte progenitor cell in glaucoma show differentiated OPCs into myelinating oligodendrocytes that expressed myelin basic protein and other markers of mature oligodendrocytes. It has been suggested that a battery of factors secreted by microglia and likely astrocytes, may be responsible for OPC activation, communicated by diffusible signals. Indeed, multiple studies (Bull et al. 2009) support the assumption that OPCs themselves can improve retinal neuronal survival, by secretion of diffusible trophic factors, such as IGF-1 and GDNF. While OPC role in rescuing glaucomatous RGC neurons has been clearly shown, it is suggested that they can also alleviate neurodegeneration in an assortment of neuropathologies in vivo.

11.4 Conclusion

Additional studies looking at specific glial cell types in the retina would aid our understanding of disease processes, refine treatments, and help in devising long-term strategies for the management of ocular disorders. Increasing evidence emphasizes that inflammatory mediators either synthesized by the immune resident cells in the retina or invading macrophages, contribute to pathophysiological functions, including an impact on synaptic transmission and neuronal health. Targeting the inflammatory source of factors such as cytokines, and related signalling molecules, may be considered as an option during neurodegeneration at the retina.

References

Ambati J, Anand A, Fernandez S, Sakurai E, Lynn BC, Kuziel WA, Rollins BJ, Ambati BK (2003) An animal model of age-related macular degeneration in senescent Ccl-2- or Ccr-2-deficient mice. Nat Med 9(11):1390–1397

Anandasabapathy N, Victora GD, Meredith M, Feder R, Dong B, Kluger C, Yao K, Dustin ML, Nussenzweig MC, Steinman RM, Liu K (2011) Flt3L controls the development of radiosensitive dendritic cells in the meninges and choroid plexus of the steady-state mouse brain. J Exp Med 208(8):1695–1705

Ben Ezra D, Forrester JV (1995) Fundal white dots: the spectrum of a similar pathological process. Br J Ophthalmol 79(9):856–860

Bhattacharya S, Das AV, Mallya KB, Ahmad I (2008) Ciliary neurotrophic factor-mediated signaling regulates neuronal versus glial differentiation of retinal stem cells/progenitors by concentration-dependent recruitment of mitogen-activated protein kinase and Janus kinase-signal transducer and activator of transcription pathways in conjunction with Notch signaling. Stem Cells 26(10):2611–2624

Bull ND, Irvine KA, Franklin RJ, Martin KR (2009) Transplanted oligodendrocyte precursor cells reduce neurodegeneration in a model of glaucoma. Invest Ophthalmol Vis Sci 50(9): 4244–4253

Chatterjee N, Callen S, Seigel GM, Buch SJ (2011) HIV-1 Tat-mediated neurotoxicity in retinal cells. J Neuroimmune Pharmacol 6(3):399–408

Chen H, Liu B, Lukas TJ, Neufeld AH (2008) The aged retinal pigment epithelium/choroid: a potential substratum for the pathogenesis of age-related macular degeneration. PLoS One 3(6): e2339

Combadiere C, Gao J, Tiffany HL, Murphy PM (1998) Gene cloning, RNA distribution, and functional expression of mCX3CR1, a mouse chemotactic receptor for the CX3C chemokine fractalkine. Biochem Biophys Res Commun 253(3):728–732

Dräger UC (1983) Coexistence of neurofilaments and vimentin in a neurone of adult mouse retina. Nature 303(5913):169–172

Fong AM, Robinson LA, Steeber DA, Tedder TF, Yoshie O, Imai T, Patel DD (1998) Fractalkine and CX3CR1 mediate a novel mechanism of leukocyte capture, firm adhesion, and activation under physiologic flow. J Exp Med 188(8):1413–1419

Frühbeis C, Fröhlich D, Krämer-Albers EM (2012) Emerging roles of exosomes in neuron-glia communication. Front Physiol 3:119. doi:10.3389/fphys.2012.00119

Gandhi N, Saiyed ZM, Napuri J, Samikkannu T, Reddy PV, Agudelo M, Khatavkar P, Saxena SK, Nair MP (2010) Interactive role of human immunodeficiency virus type 1 (HIV-1) clade-specific Tat protein and cocaine in blood-brain barrier dysfunction: implications for HIV-1-associated neurocognitive disorder. J Neurovirol 16(4):294–305

Gramlich OW, Beck S, von Thun Und Hohenstein-Blaul N, Boehm N, Ziegler A, Vetter JM, Pfeiffer N, Grus FH (2013) Enhanced insight into the autoimmune component of glaucoma: IgG autoantibody accumulation and pro-inflammatory conditions in human glaucomatous retina. PLoS One 8(2):e57557

Gupta N, Brown KE, Milam AH (2003) Activated microglia in human retinitis pigmentosa, late-onset retinal degeneration, and age-related macular degeneration. Exp Eye Res 76(4): 463–471

Hajrasouliha AR, Jiang G, Lu Q, Lu H, Kaplan HJ, Zhang HG, Shao H (2013) Exosomes from retinal astrocytes contain antiangiogenic components that inhibit laser-induced choroidal neovascularization. J Biol Chem 288(39):28058–28067

Herbomel P, Thisse B, Thisse C (2001) Zebrafish early macrophages colonize cephalic mesenchyme and developing brain, retina, and epidermis through a M-CSF receptor-dependent invasive process. Dev Biol 238(2):274–288

Holland GN (2008) AIDS and ophthalmology: the first quarter century. Am J Ophthalmol 145(3):397–408

Howell GR, Macalinao DG, Sousa GL, Walden M, Soto I, Kneeland SC, Barbay JM, King BL, Marchant JK, Hibbs M, Stevens B, Barres BA, Clark AF, Libby RT, John SW (2011) Molecular clustering identifies complement and endothelin induction as early events in a mouse model of glaucoma. J Clin Invest 121(4):1429–1444

Ibrahim AS, El-Shishtawy MM, Peña A Jr, Liou GI (2010) Genistein attenuates retinal inflammation associated with diabetes by targeting of microglial activation. Mol Vis 16: 2033–2042

Ibrahim AS, El-Remessy AB, Matragoon S, Zhang W, Patel Y, Khan S, Al-Gayyar MM, El-Shishtawy MM, Liou GI (2011) Retinal microglial activation and inflammation induced by amadori-glycated albumin in a rat model of diabetes. Diabetes 60(4):1122–1133

Kaarniranta K, Sinha D, Blasiak J, Kauppinen A, Veréb Z, Salminen A, Boulton ME, Petrovski G (2013) Autophagy and heterophagy dysregulation leads to retinal pigment epithelium dysfunction and development of age-related macular degeneration. Autophagy 9(7):973–984

Kerr EC, Raveney BJ, Copland DA, Dick AD, Nicholson LB (2008) Analysis of retinal cellular infiltrate in experimental autoimmune uveoretinitis reveals multiple regulatory cell populations. J Autoimmun 31(4):354–361

Krishnan G, Chatterjee N (2012) Endocannabinoids alleviate proinflammatory conditions by modulating innate immune response in Muller glia during inflammation. Glia 60(11): 1629–1645

Krishnan G, Chatterjee N (2014) Endocannabinoids affect innate immunity of Muller glia during HIV-1 Tat cytotoxicity. Mol Cell Neurosci 59:10–23

Krishnan G, Chatterjee N (2015) Differential immune mechanism to HIV-1 Tat variants and its regulation by AEA. Sci Rep 5:9887

Langmann T (2007) Microglia activation in retinal degeneration. J Leukoc Biol 81(6):1345–1351

Leenstra S, Das PK, Troost D, de Boer OJ, Bosch DA (1995) Human malignant astrocytes express macrophage phenotype. J Neuroimmunol 56(1):17–25

Lye-Barthel M, Sun D, Jakobs TC (2013) Morphology of astrocytes in a glaucomatous optic nerve. Invest Ophthalmol Vis Sci 54(2):909–917

Ma W, Zhao L, Fontainhas AM, Fariss RN, Wong WT (2009) Microglia in the mouse retina alter the structure and function of retinal pigmented epithelial cells: a potential cellular interaction relevant to AMD. PLoS One 4(11):e7945

Mangan BG, Al-Yahya K, Chen CT, Gionfriddo JR, Powell CC, Dubielzig RR, Ehrhart EJ, Madl JE (2007) Retinal pigment epithelial damage, breakdown of the blood-retinal barrier, and retinal inflammation in dogs with primary glaucoma. Vet Ophthalmol 10(Suppl 1):117–124

Matteucci A, Gaddini L, Villa M, Varano M, Parravano M, Monteleone V, Cavallo F, Leo L, Mallozzi C, Malchiodi-Albedi F, Pricci F (2014) Neuroprotection by rat Müller glia against high glucose-induced neurodegeneration through a mechanism involving ERK1/2 activation. Exp Eye Res 125:20–29

Minton AZ, Phatak NR, Stankowska DL, He S, Ma HY, Mueller BH, Jiang M, Luedtke R, Yang S, Brownlee C, Krishnamoorthy RR (2012) Endothelin B receptors contribute to retinal ganglion cell loss in a rat model of glaucoma. PLoS One 7(8):e43199

Misu T, Höftberger R, Fujihara K, Wimmer I, Takai Y, Nishiyama S, Nakashima I, Konno H, Bradl M, Garzuly F, Itoyama Y, Aoki M, Lassmann H (2013) Presence of six different lesion types suggests diverse mechanisms of tissue injury inneuromyelitis optica. Acta Neuropathol 125(6):815–827

Nakazawa T, Hisatomi T, Nakazawa C, Noda K, Maruyama K, She H, Matsubara A, Miyahara S, Nakao S, Yin Y, Benowitz L, Hafezi-Moghadam A, Miller JW (2007) Monocyte chemoattractant protein 1 mediates retinal detachment-induced photoreceptor apoptosis. Proc Natl Acad Sci USA 104(7):2425–2430

Narayan DS, Casson RJ, Ebneter A, Chidlow G, Grace PM, Hutchinson MR, Wood JP (2014) Immune priming and experimental glaucoma: the effect of prior systemic lipopolysaccharide challenge on tissue outcomes after optic nerve injury. Clin Exp Ophthalmol 42(6):539–554

Newman EA, Zahs KR (1998) Modulation of neuronal activity by glial cells in the retina. J Neurosci 18(11):4022–4028

Nguyen JV, Soto I, Kim KY, Bushong EA, Oglesby E, Valiente-Soriano FJ, Yang Z, Davis CH, Bedont JL, Son JL, Wei JO, Buchman VL, Zack DJ, Vidal-Sanz M, Ellisman MH, Marsh-Armstrong N (2011) Myelination transition zone astrocytes are constitutively phagocytic and have synuclein dependent reactivity in glaucoma. Proc Natl Acad Sci USA 108 (3):1176–1181

Nishihira JJ (2000) Macrophage migration inhibitory factor (MIF): its essential role in the immune system and cell growth. Interferon Cytokin Res 20(9):751–762

Patel AK, Surapaneni K, Yi H, Nakamura RE, Karli SZ, Syeda S, Lee T, Hackam AS (2015) Activation of Wnt/β-catenin signaling in Muller glia protects photoreceptors in a mouse model of inherited retinal degeneration. Neuropharmacology 91:1–12

Plange N, Bienert M, Remky A, Arend KO (2012) Optic disc fluorescein leakage and intraocular pressure in primary open-angle glaucoma. Curr Eye Res 37(6):508–512

Prodinger C, Bunse J, Krüger M, Schiefenhövel F, Brandt C, Laman JD, Greter M, Immig K, Heppner F, Becher B, Bechmann I (2011) CD11c-expressing cells reside in the juxtavascular parenchyma and extend processes into the glia limitans of the mouse nervous system. Acta Neuropathol 121(4):445–458

Rasmussen KE (1972) A morphometric study of the Müller cell cytoplasm in the rat retina. J Ultrastruct Res 39(5):413–429

Raviola G (1977) The structural basis of the blood-ocular barriers. Exp Eye Res 25(Suppl 1):27–63

Rhee KD, Nusinowitz S, Chao K, Yu F, Bok D, Yang XJ (2013) CNTF-mediated protection of photoreceptors requires initial activation of the cytokine receptor gp130 in Müller glial cells. Proc Natl Acad Sci USA 110(47):E4520–E4529

Robinson SR, Hampson EC, Munro MN, Vaney DI (1993) Unidirectional coupling of gap junctions between neuroglia. Science 262(5136):1072–1074

Rutar M, Natoli R, Provis JM (2012) Small interfering RNA-mediated suppression of Ccl2 in Müller cells attenuates microglial recruitment and photoreceptor death following retinal degeneration. J Neuroinflammation. 9:221

Schnitzer J (1987) Retinal astrocytes: their restriction to vascularized parts of the mammalian retina. Neurosci Lett 78(1):29–34

Schnitzer J (1988) Immunocytochemical studies on the development of astrocytes, Müller (glial) cells, and oligodendrocytes in the rabbit retina. Brain Res Dev Brain Res 44(1):59–72

Schnitzer J (1989) Enzyme-histochemical demonstration of microglial cells in the adult and postnatal rabbit retina. J Comp Neurol 282(2):249–263

Shamsuddin N, Kumar A (2011) TLR2 mediates the innate response of retinal Muller glia to Staphylococcus aureus. J Immunol 186(12):7089–7097

Sudharshan S, Kaleemunnisha S, Banu AA, Shrikrishna S, George AE, Babu BR, Devaleenal B, Kumarasamy N, Biswas J (2013) Ocular lesions in 1000 consecutive HIV-positive patients in India: a long-term study. Ophthalmic Inflamm Infect 3(1):2

Sugiyama T, Moriya S, Oku H, Azuma I (1995) Association of endothelin-1 with normal tension glaucoma: clinical and fundamental studies. Surv Ophthalmol 39(Suppl 1):S49–S56

Verjans GM, Hintzen RQ, van Dun JM, Poot A, Milikan JC, Laman JD, Langerak AW, Kinchington PR, Osterhaus AD (2007) Selective retention of herpes simplex virus-specific T cells in latently infected human trigeminal ganglia. Proc Natl Acad Sci USA 104(9):3496–3501

Wakim LM, Woodward-Davis A, Bevan MJ (2010) Memory T cells persisting within the brain after local infection show functional adaptations to their tissue of residence. Proc Natl Acad Sci USA 107(42):17872–17879. doi:10.1073/pnas.1010201107

Wang JS, Nymark S, Frederiksen R, Estevez ME, Shen SQ, Corbo JC, Cornwall MC, Kefalov VJ (2014) Chromophore supply rate-limits mammalian photoreceptor dark adaptation. J Neurosci 34(34):11212–11221

Watanabe T, Raff MC (1988) Retinal astrocytes are immigrants from the optic nerve. Nature 332(6167):834–837

Xu Y, Guan N, Xu J, Yang X, Ma K, Zhou H, Zhang F, Snellingen T, Jiao Y, Liu X, Wang N, Liu N (2008) Association of CFH, LOC387715, and HTRA1 polymorphisms with exudative age-related macular degeneration in a northern Chinese population. Mol Vis 14:1373–1381

Obesity and Neuroinflammation

12

Gurcharan Kaur, Anuradha Sharma, Muskan Gupta and Taranjeet Kaur

Abstract

Obesity is the core and baseline component of metabolic syndrome and is a major risk factor for many diseases like Type II diabetes, cardiovascular diseases, hypertension, stroke and neurodegenerative diseases. Sedentary lifestyle, unhealthy eating habits and genetic predisposition are responsible for the increasing prevalence of obesity worldwide. Chronic overnutrition causes low-grade inflammation in several peripheral tissues as well as central nervous system, particularly hypothalamus. Activation of various proinflammatory pathways such as IKKβ/NF-κB, JNK and PKR are thought to be the major players in the induction of systemic and central inflammation. Further, neuroinflammation causes intracellular disturbances and exacerbates various stresses such as oxidative stress, ER stress and autophagic defects leading to impaired neurohormonal signalling as well as autonomic regulation of nutrient metabolism and energy balance. As obesity poses major health threat, effective therapies to minimize obesity-related comorbidities are surely needed. By targeting the inflammatory component, the progression of obesity can be slowed down. In vivo studies from our lab suggest that *Withania somnifera* helps to

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reduce hypothalamic inflammation triggered by high-fat-diet-induced obesity. Various lifestyle interventions along with herbal supplementation may effectively help to prevent obesity and its associated pathologies.

12.1 Introduction

Maintenance of metabolic homeostasis is a key issue in the modern society which often leads to disease consequences such as obesity, diabetes, hypertension, cardiovascular disease, etc. Mammalian body has evolved with mechanisms of excess energy storage in terms of fats and triglycerides in adipose tissue during the periods of plenty which help them to survive in critical conditions of drought and famine (Shoelson et al. 2007). Along with beneficial effects of energy storage, in longer lifespan, sustained overnutrition and reduced physical activity have negative effects which lead to overweight and obesity (Cai et al. 2013; Shoelson et al. 2007). Obesity (also known as overnutrition-induced disease) is a stage of accumulation of fatty acids and increased adipose tissue mass compared to lean mass. Incidence of obesity, characterized by body mass index (BMI > 30) is increasing alarmingly, reaching epidemic proportions worldwide (Nguyen et al. 2014). This has resulted in increased healthcare burden and reduced life expectancy (Wang et al. 2013). According to Obesity report of Organization for Economic Co-operation and Development for the year 2014 (http//www.oecd.org/health/Obesity-update-2014.pdf), 18 % adult population of the world is obese (Naguyan et al. 2014). World Health Organization (WHO) reports also present such facts and figures, according to which over 200 million men and around 300 million women are obese (Miller and Spencer 2014). Obesity is linked with comorbid conditions like high triglyceride content, glucose intolerance, a large waist circumference, etc. These conditions increase the risk of metabolic disorders such as Type 2 Diabetes Mellitus (T2DM), hypertension, cardiovascular diseases and other health problems like fatty liver disease, airway disease and some cancers. In addition to metabolic disorders, obesity also leads to dementias like Alzheimer's disease (AD), Parkinson's disease (PD), memory impairment and cognitive decline.

Interestingly, WHO has estimated that by the year 2020 psychiatric disorders such as depression will take over HIV and cardiovascular diseases significantly affecting more patients at the global level. The link between metabolic status such as obesity, diabetes and neurological and neuropsychiatric disorders has been appreciated but the underlying mechanisms are not well defined. On the other hand, mounting evidence indicates that depression and anxiety disorders are risk factors for morbidity and mortality due to T2DM, CVDs, stroke, etc., thus suggesting a bidirectional link between metabolic homeostasis and neurological and neuropsychiatric disorders.

The rising epidemic of obesity is considered as one of the major risk factors for brain ageing and related neuropathologies. Gaps in our understanding of obesity-related neuropathogenesis limits progress towards finding novel therapeutic

strategies to combat the obesity-associated brain pathologies. While it is not currently known how obesity disrupts brain homeostasis, numerous clinical and rodent studies strongly link diet-induced metabolic disturbances to the development of cognitive decline and neurodegenerative diseases. The progressive nature by which diet-induced obesity (DIO) promotes brain function impairments raises the possibility that the detailed study of this model may unravel the complex relationship between obesity and measurable aspects of brain pathology.

Research in neuroendocrinology and immunology over the recent past revealed that neuroinflammation induced due to overnutrition is a major pathologic component, leading to a range of dysfunctions in CNS in obesity and related metabolic disorders (Cai and Liu 2011, 2012; Cai 2012). In addition to negative impacts on neurohormonal signalling of hypothalamic neurons, overnutrition-induced inflammation contributes to neurodegeneration (McNay et al. 2012) and proinflammatory molecules which are mechanistically accountable for obesity-related neurodegenerative diseases. Evidences derived from epidemiology, experimental research and clinical medicine demonstrate that obesity and related disorders are associated with chronic low-grade inflammation in peripheral tissues and in circulation (Cai 2011; Gregor et al. 2011). Low-grade, chronic inflammation associated with obesity is characterized by increased circulating free fatty acids and chemoattraction of immune cells such as macrophages that also produce inflammatory mediators into the local milieu (reviewed in Hursting and Dunlap 2012) These effects are further complicated by the release of inflammatory cytokines such as interleukin (IL)-1β, IL-6, TNF-α and monocyte chemoattractant protein (MCP)-1.

Critical evaluation and understanding of the molecular mechanism(s) of crosstalk between obesity and mental health, neuroplasticity and cognitive abilities is of great concern for global healthcare system keeping in view the fact that obesity is now becoming pandemic in both developed and developing world. In India, prosperous states like Punjab are facing the challenge of overnutrition and that too of high fat content and a steep rise in the prevalence of overweight and obesity. Many recent reports from our University and others estimate obesity prevalence to about 25–30 % (BMI $\geq$ 30.0) in Punjabi population and the figures are comparable to the prevalence reported in developed nations like USA. With urban population showing significantly higher prevalence compared to rural masses correlates well with their lifestyles due to large scale socio-economic development in the cities. Obese people are at a greater risk of psychiatric and neurological disorders as well as comorbidities such as diabetes, hypertension, cancer, cardiovascular diseases, etc. The clinical significance of this possibility is further amplified by observations that the prevalence of obesity amongst the elderly (aged 60 and over) is significantly higher as compared to the younger group which also emphasizes the fact that our aging population is carrying the additional physiological burden of obesity. Moreover, women especially in the post-menopausal phase of their life have higher prevalence of overweight and obesity and are at higher risk than men of developing depression and anxiety.

12.2 Obesity and Cognitive Dysfunction

12.2.1 Clinical Studies

Negative effects of obesity on cardiovascular and metabolic functioning are well reported and apparently brain is also adversely affected by obesity. Clinical studies from literature provide link between obesity and risk of dementia associated with AD, but cognitive dysfunction is evidenced to be linked with obesity prior to discovered onset (Miller and Spencer 2014). Independent of cardiovascular and cerebrovascular disorders, high BMI is related to memory, learning and executive functioning deficits in adults (Elias et al. 2003) and midlife obesity is a predictor of mild cognitive impairment in old age. A similar study on healthy adult individuals reported inverse relation of BMI to cognition including executive functioning and memory (Gunstad et al. 2007). Another study examined the association of BMI with cognition at early adulthood (25 years), early midlife (44 years) and late midlife (61 years) by assessing multiple cognitive domains (Sabia et al. 2009). Outcomes of the study were that being obese at 2–3 times points of life lead to poor executive functioning and reduced memory. So it may be predicted that impact of obesity on cognition accumulates over the adulthood. High BMI and obesity is associated with reduced cognitive performance in elderly individuals as well (Cattin et al. 1997; Elias et al. 2003). In addition to cognition, higher BMI is also associated with brain atrophies and reduced brain volumes. According to a recent report, higher waist circumference and BMI are associated with reduced total volume of brain in elderly patients and studies on cohort of young individuals also provided the same observations (Raji et al. 2010; Taki et al. 2008). A negative relationship between obesity and regional brain atrophy has been described and particularly temporal and frontal lobes of brain are reported to be the most vulnerable regions affected by obesity (Raji et al. 2010). Greater BMI is also reported to be inversely linked with grey matter volumes, metabolic abnormalities in neurons/myelin and neuronal viability in grey and white matters (Mueller et al. 2011). Large hippocampal size is well associated with better cognition and frontal lobes are linked with executive functioning, so reduction in regional brain volumes due to obesity results in neuronal loss and impaired memory and cognition.

Taking clues from the negative relationship of obesity with brain function impairment in adults and elderly individuals, evaluation of impact of obesity on developing brains in childhood and adolescence also became an active area of obesity research. Studies carried out on younger groups revealed that executive functioning mainly develops during childhood (3–5 years) and attains maturity during adolescence and is vulnerable to stress factors such as obesity (Barkin 2013). Further obese children and adolescents have poor domains of executive functioning as compared to their healthy counterparts (Maayan et al. 2011). It has been found that obese children have reduced regional and global brain volumes, and obese adolescents have compromised white matter and reduced hippocampal volumes than healthy individuals (Yau et al. 2012; Yokum et al. 2012).

12.2.2 Experimental Studies

Several reports in literature have provided evidence against worse effects of obesity on brain health, memory and cognition using experimental animal models. Obesity is generally induced in animals by high-fat diet feeding. These studies provide the evidence that high-fat diet fed animals show compromised memory and learning skills and highlight the underlying molecular mechanisms for the memory decline. Reduced synaptic plasticity (Molteni et al. 2002) and increased neuronal apoptosis (Moraes et al. 2009; Rivera et al. 2013) in cerebral cortex and hippocampal regions of brain of mouse models is reported in high-fat diet fed (HFD) animals as compared to control lean animals. Further HFD feeding also results in disrupted cerebral vascular functioning, disrupted blood–brain barrier (BBB) and arteries functioning upstream to BBB at certain locations in hypothalamus (Pepping et al. 2013). These vascular mechanisms may underlie the pathological processes involved in dementias associated with AD and PD.

12.3 High Fat Diet-Induced Obesity

One of the major factors contributing to the high prevalence of obesity is the choice of diet. Unhealthy diet consumed by a large number of westernized populations and termed as 'Western diet', contains large amounts of red meat, refined sugars, high fat foods and refined grains. In contrast to the western diet, the 'healthy diet' (prudent) contains a large number of fruits, vegetables, lean protein and fibre (Fung et al. 2001). Western diet contains large amounts of saturated fatty acids and trans-fatty acids, whereas, healthy diet contains more n-3 polyunsaturated fatty acids. Major sources of saturated fatty acids are fatty meats, baked goods, cheese, milk, margarine and butter. The dietary intake of saturated fats is associated with a greater body mass index (BMI) (van Dam et al. 2002) which ultimately is a driving force for the development of metabolic syndrome in an individual.

Addition of more fat to the diet does not increase the rate of fat oxidation in the body (Horton et al. 1995). So, the consumption of fat from high-fat diet must be reduced in order to maintain the energy balance of the body to avoid obesity. The feeling of satiety after the consumption of fat-rich diet occurs much later as compared to the carbohydrate rich diet, so individuals consuming high fat diet tend to overeat at short intervals subsequent to the consumption of high fat diet. In case of overfeeding, first the glycogen stores are filled, followed by protein stores and ultimately, any excess left is converted to body fat. It has been suggested that consistent consumption of high fat diet results in gradual accumulation of fat until fat stores have expanded enough to bring the fat and carbohydrate intakes back to balance. Obesity is caused by an energy imbalance and is characterized by excess accumulation of body fat. The body has the ability to achieve protein and carbohydrate balance, but poor autoregulatory system for fat. Also, body has unlimited ability to store fat. High-fat diets produce obesity by enhancing passive

overconsumption of energy and increasing the energy density of the diet (Bray and Popkin 1998). In humans, continuous passive overconsumption occurs more readily with sweet and high-fat foods.

12.4 Obesity and Neuroinflammation

Elevated BMI has been reported to be associated with decreased brain volume (Ward et al. 2005) indicative of higher brain atrophy caused due to obesity. High-fat diet causes significant reduction in cerebral blood flow, presumably by impaired K^+—induced vasodilation (Li et al. 2013). In human subjects, obese women (mean BMI 32.7) have been shown to have reduced cerebral blood flow as compared to normal weight subjects (mean BMI 22.2) (Karhunen et al. 1997). Dietary saturated fatty acids have been shown to have direct influence on neuroinflammation in p38 dependent manner (Linker et al. 2014). Saturated fatty acids activate microglia and stimulate TLR4/NF-κB pathway that further triggers the proinflammatory cytokines, NO and ROS (Wang et al. 2012a, b).

High dietary fat intake has been reported to enhance ROS generation as well as prostaglandin E_2 (marker of inflammation) production in the cerebral cortex of high fat diet fed Sprague-Dawley rats (Zhang et al. 2005). This elevation is also accompanied by higher NADPH oxidase subunits and cyclooxygenases (COX). Higher levels of ROS can induce cellular damage by oxidation of critical components inside the cell such as membrane lipids, DNA and proteins (Halliwell 2001). NADPH oxidase is one of the important factors for ROS generation implicated in oxidative stress. ROS generation also leads to the activation of NF-κB, a marker for oxidative stress and inflammation (Tanaka et al. 2002). The expression of COX-2 has also been reported to be mediated through the activation of TLR4 (Lee et al. 2001). TLR4/NF-κB pathway further triggers proinflammatory cytokines, thus inducing an inflammatory state in the CNS.

It has also been reported that high fat diet-induced obesity enhances the recruitment of bone marrow derived monocytes to the CNS (Buckman et al. 2014) along with the activation of resident microglial cells. The number of infiltrating monocytes to the CNS has been found to be proportional to body weight, fat mass and CD68 and CCL2 (markers of inflammation) in the adipose tissue. Bone marrow derived monocytes, after entering CNS act like the resident macrophages. Various stimuli contribute to the recruitment of peripheral monocytes to the CNS during obesity, such as obesity-associated changes in CNS vascularity (Yi et al. 2012), permeability of blood–brain barrier (Nerurkar et al. 2011), elevated levels of chemokines such as MCP-1 in the CNS. The recruitment of monocytes into the CNS is one of the implications of neuroinflammation caused due to high-fat diet feeding.

Autoregulatory behaviour of cerebral vessels and functional hyperemia upon increased neuronal activity are main mechanisms which regulate cerebral blood perfusion. Vascular function is negatively affected by HFD as it leads to increased

myogenic tone and endothelial dysfunction in diet-induced models of obesity (Li et al. 2013). HFD-induced obesity induces central, i.e. hypothalamic inflammation in brain, oxidative stress and reduced expression of BDNF (Timmermans et al. 2014) that leads to reduced neuronal plasticity, cognitive function and learning.

12.4.1 Genetic Model of Obesity and Neuroinflammation

According to the root cause of obesity, it can be classified as genetically induced and diet-induced obesity. The idea that obesity can be inherited came from mouse models having mutated genes for leptin adipokine and its receptor (Blakemore et al. 2010). Leptin is an adipocyte derived satiety hormone, which signals brain about levels of stored fat (Sorenson et al. 1996). Family, twin and adoption studies provide enough evidence about moderate to high heritability of BMI (Hinney et al. 2010; Maes et al. 1997). Parental obesity is the major risk factor for childhood and adolescent obesity (Reilly et al. 2005) and if both the parents are obese, level of risk is elevated.

Single gene polymorphisms are known to result in monogenic obesity characterized by an extremely severe obesity (Herrera and Lindgren 2010). Most of the currently known monogenic forms of obesity in humans have been identified from studies of mutations in diverse rodent models. Majority of single gene mutations responsible for monogenic obesity are involved in leptin and melanocortin pathway (Lalouel et al. 1983). As previously mentioned leptin is a hormone produced by adipocytes and signals brain for storage of fats and energy level. Increased levels of leptin are linked to leptin receptor which forwards the signal to Melanocortin 4 receptor (MC4R). Further the satiety effect is induced by an endogenous agonist alpha melanocyte stimulating hormone (α-MSH) (Bjorbaek and Hollenberg 2002). BDNF is another gene responsible for monogenic obesity which regulates weight downstream of MC4R. MC4R mutations have been reported to induce monogenic obesity and knockout mice with obese phenotype lead to screening of mutations of human MC4R (Yeo et al. 1998). Initially studies for human mutation carriers were carried out on small pedigrees and further studies revealed that not necessarily mutation carriers have obese phenotype (Hinney et al. 1999; Vaisse et al. 2000).

In addition to MC4R and BDNF, mutations in several other genes coding for leptin receptor (LEPR) (Clement et al. 1998), leptin (LEP) (Strobel et al. 1998), prohormone convertase 1(PC1) and pro-opiomelanocortin (POMC) (Challis et al. 2002) have been reported to be associated with autosomal recessive form of obesity. Mutations in these genes are rare which lead to additional pleiotropic effects like impaired fertility (LEP, LEPR and PC1), impaired immunity (LEP1), adrenal insufficiency (POMC) and red hair (POMC) (Farooqi and Rahilly 2004; Farooqi 2006). These pleiotropic effects linked with recessive disorders are known as syndromic forms of obesity, e.g. Bardet–Biedl, Prader Willi syndrome and could help to find novel genes for idiopathic obesity (Farooqi and Rahilly 2004; Kousta et al. 2009).

Chances of presence of trait due to single gene modification are rare. There may be traits governed by simultaneous variation/modification in several genes (Hinney et al. 2010). Group of alleles at different gene locations that control collectively either modification of expression of qualitative trait or inheritance of quantitative phenotype are known as polygenic variants and traits controlled by such variants are known as polygenic traits (Hinney et al. 2010). For quantitative traits, different alleles may have different effects which may be synergistic or additive. Polygenic form of obesity is found to be controlled by more than hundred genes with each gene having small effect and specific set of these polygenic alleles may be different for different obese individuals. Variants of MC4R, FTO, INSIG2 and other novel loci genes govern polygenic obesity.

MC4R gene in addition to single gene mutation harbours two polygenic variants for weight regulation in its coding region: polymorphic variation at position 103 coding for isoleucine instead of valine (103I) and at position 251 coding for leucine instead of isoleucine (251L) of receptor protein and negatively affect obesity (Stutzmann et al. 2007). 2–9 % subjects from different populations exhibit heterozygosity for 103I (Geller et al. 2004) and MC4R gene having this allele reduces twofold potency of antagonist hAGRP, which is related to obesity protective effect of this variant. A study conducted on 16,797 European individuals provided the evidence for other polymorphic variant 251L and constant negative effect of this variant was observed with both adult and childhood obesity. This polymorphic variant, i.e. MC4R 251L also has obesity protective effect (Stutzmann et al. 2007). This form of obesity affects the general population, resulting from long-term positive energy balance; the energy excess is stored in adipose tissue and, if this process is prolonged, obesity develops.

Fat mass and obesity-associated gene (**FTO**) is one of the candidate genes highlighted by genome wide association studies (GWAS) for T2DM and it has been found that association with T2DM is due to higher BMI in diabetic subjects as compared to non-diabetic subject controls (Frayling et al. 2007). FTO is essential for normal development of cardiovascular and CNS in humans (Hinney et al. 2010). There are six SNPs in FTO which are strongly associated with development of obesity. In a study on 7 years aged children, FTO was not found associated with physical activity and energy intake but an association is reported between energy intakes, decreased satiety and FTO risk variants in adult individuals (Speakman et al. 2008). A homozygous minor allele of common SNP in vicinity of Insulin induced gene 2 (**INSIG2**) was found to be involved with obesity (Herbert et al. 2006). Future progress in this field will show how and to what extent BMI variance at population level can be explained at molecular level and products of polygenes responsible may be treated as drug targets to control obesity (Hinney et al. 2010).

12.4.2 Obesity and Systemic Inflammation

Obesity is associated with chronic low-grade inflammation in peripheral organs like adipose tissue, liver, muscles, etc., responsible for various obesity-associated

comorbidities like T2DM. Imbalances in homeostatic and proinflammatory immune responses are linked to multisystem effects of obesity. With time, ectopic accumulation of lipids in liver, muscle and blood vessels activate tissue leukocytes, contribute to organ-specific diseases, and exacerbate systemic insulin resistance. Like LPS, dietary factors such as fatty acids lead to the stimulation of toll like receptors (TLR4), lipopolysaccharide receptors and results in the initiation of the various inflammatory cascades and release of various inflammatory mediators such as proinflammatory cytokines TNF-α, IL-1β, IL-6, reactive oxygen and nitrogen species.

People with mental illness are often overweight (BMI > 25) and obese (BMI > 30), and have poorer eating habits than the general population (Davidson et al. 2000). As a result, people with psychiatric illness are at a greater risk of lifestyle diseases such as diabetes, cancer, cardiovascular and respiratory diseases (Sokal et al. 2004). Factors affecting patients with mental illness which contribute to these outcomes include more frequent physical comorbidities as compared to the general population (Sokal et al. 2004), genetic predisposition to certain pathologies (Popkin and Gordon-Larsen 2004), eating habits and sedentary lifestyles (Atlantis and Baker 2008), high levels of cigarette smoking and drug abuse (Gariepy et al. 2010), limited access to regular health care services (Marwaha et al. 2008), and potential adverse events arising during pharmacological treatment (Haddad and Sharma 2007).

In a recent study from our lab, we have used LPS activated murine BV-2 microglial cell line as a model system for endotoxin induced neuroinflammation which is a well-established model system to study neuroinflammation. The water extract from the leaves of Ashwagandha (ASH-WEX) and one of its active fractions FIV pretreated BV-2 microglia were exposed to LPS for 48 h and then the media supernatant was collected and analysed for the presence of inflammatory cytokines using sandwich ELISA-based assay. For in vivo analysis, 30 % high fat diet fed rat model was used for studying obesity induced neuroinflammation. Animals were divided into four groups: I, control animals which were fed the normal chow diet. II, HFD group: in which the animals were fed with 30 % (by weight) high fat diet. III, C + E group: in which the animals were fed with the normal chow diet mixed with the Ashwagandha leaf powder. IV, HFD + E group: in this group, the animals were fed with high-fat diet mixed with Ashwagandha leaf powder. After 3 months, the rats were scarified and serum was isolated and analysed for the presence of the proinflammatory cytokines. Both LPS-induced microglial activation (Fig. 12.1 Panel A) and high-fat feeding (Fig. 12.1 Panel B) leads to elevated levels of TNF-α, IL-1β, IL-6 which gets alleviated by Ashwagandha treatment. These results suggest that like LPS, high-fat diet also causes the surge in the levels of proinflammatory cytokines through various inflammatory cascades and results in obesity-mediated inflammation and natural products like Ashwagandha have the potential to attenuate this HFD-induced inflammation.

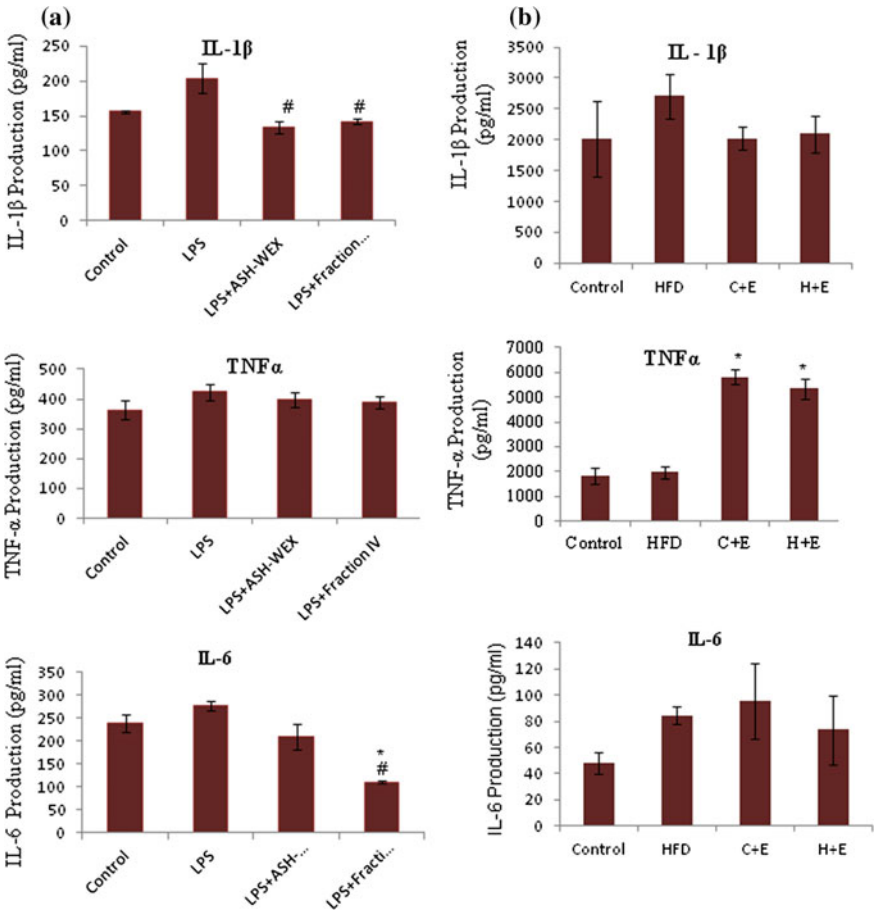

Fig. 12.1 LPS and HFD-induced proinflammatory cytokines profile. *Panel a* represents the expression of the proinflammatory cytokines TNF-α, IL-1β, IL-6 in the media from ASH-WEX and FIV pretreated BV-2 microglial cells with or without activation with the LPS. *Panel b* represents the serum profile of these proinflammatory cytokines in in vivo HFD model system. Like the LPS (model for inducing inflammation), high-fat diet also induced the expression of the inflammatory cytokines, main mediators of the inflammation. Their elevated levels have been attenuated by the treatment with ASH-WEX (Ashwagandha leaf water extract)

12.4.2.1 Adipose Tissue and Obesity Linked Inflammation

In terms of glucose homeostasis, liver, adipose tissue and muscles are the major players, but adipose tissue also regulates glucose homeostasis indirectly by regulating the lipid homeostasis. It also acts as the endocrine organ that regulates the production of various hormones and cytokines. The hormones and cytokines produced by adipose tissue in obesity are leptin, adipoleptin, resistin and cytokines such as TNF-α, IL-1β, IL-6. High-fat diet feeding is associated with the infiltration of macrophages, apoptosis of adipocytes and reduced adipose tissue vascularity

which results in macrophages proliferation in white adipose tissue when chemokine or lipid release (lipolysis) is triggered (Xu et al. 2003; Weisberg et al. 2003; Shu et al. 2012). The macrophages ÊÉ coupled with increased TLR and other pattern recognition factors results in enhanced production of inflammatory cytokines. Thus, adipose tissue releases high levels of inflammatory mediators in obesity, therefore, it is regarded as the main inflammatory organ that mediates obesity induced inflammation. Regulation of the production of inflammatory mediators in adipose tissue is mediated by stromal vascular cell (SVC) fraction that contains immune cells.

12.4.2.2 Link to Insulin Resistance and Type II Diabetes

The increased pro-inflammatory cytokines, adipokines and fatty acids cause downstream effects on muscle and liver which further contribute to systemic insulin resistance. Normally when insulin binds to insulin receptor on these cells, the insulin receptor is autophosphorylated at its Tyr residues and tyrosine kinase is activated (White 2003; Pilch et al. 2004). The insulin receptor then phosphorylates tyrosine residues on the insulin receptor substrates (IRSs), which serve as the docking proteins for SH2-containing enzymes such as p85 subunit of PI-3 kinase or SHP2. This leads to linear signalling cascades that result in Akt activation. The activation of Akt induces the translocation of Glut 4 and glycogen synthesis and thus plays an important role in metabolic signalling. Proinflammatory cytokines produced as a result of obesity mediated inflammation activate serine kinases which directly and indirectly phosphorylate insulin receptor substrate (IRS) 1 and 2. IRS1 and 2 phosphorylation further results in reduced ability of insulin to stimulate PI-3 K-dependent pathways, which normally leads to glucose uptake and its metabolism. Hence disruption of this insulin cascade induces insulin resistance and is associated with the development of T2DM.

Proinflammatory IKKβ/NF-κB pathway is also responsible for obesity-associated low-grade chronic inflammation in peripheral tissues. At the basal level, NF-κB is sequestered in the cytoplasm by its binding to IKBα, masking its nuclear translocation. In response to various stimuli like LPS, fatty acids, ceramide, etc., IKK enzyme complex is activated and IKKβ phoshorylates Ser 32 and 36 of IKBα, which exposes the nuclear localisation sequence of the NF-κB and causes the NF-κB to translocate into nucleus, where it initiates the gene expression of various inflammatory mediators like inflammatory cytokines, implicated in the development of obesity-induced insulin resistance (Oeckinghaus et al. 2011; Ghosh et al. 2012). The Jun N terminal kinase/stress activated protein kinases (JNK/SAPKs) belonging to MAP kinase family also play important role in endoplasmic reticulum stress regulating the development of obesity-induced insulin resistance (Bogoyevitch et al. 2010; Solinas et al. 2010).

12.4.2.3 Hypertension

Obesity is also one of the leading risk factors for chronic arterial hypertension. Various mechanisms that explain the development of high arterial pressure in the body during obesity have been elucidated which include increased sympathetic

nervous system activity, activation of renin–angiotensin system, endothelial dysfunction and renal functional abnormalities (Rahmouni et al. 2005). As proposed by Paton and Waki (2009), increased circulating inflammatory cells and cytokines in the brain can impair central blood pressure regulation and promote hypertension.

Components of RAS, including renin, angiotensinogen and angiotensin type 1 (AT1a) receptors are present in various brain regions and cell types. In addition to brain derived angiotensin II, blood borne angiotensin peptides may enter the brain and modulate blood pressure and fluid homeostasis. Besides having important role in the regulation of blood pressure, angiotensin II also contributes to the key events in inflammation. Angiotensin II is a potent regulator of the immune system centrally and peripherally; and induces brain inflammatory responses critical for the development and progression of hypertension. Local activation of RAS and Angiotensin II synthesis increase vascular permeability by promoting the expression and secretion of vascular endothelial growth factor (VEGF). They also enhance the expression of endothelial adhesive molecules including selectins (P- and L-selectin), vascular cell adhesion molecules-1 (VCAM-1), intercellular adhesion molecules-1 (ICAM-1) and their ligands, the integrins. Angiotensin II also promotes endothelial dysfunction via COX-2 activation, which generates vasoactive prostaglandins and ROS. Moreover, Angiotensin II promotes the infiltration of inflammatory cells into tissues by the stimulation of production of specific cytokines/chemokines. The proinflammatory activity of Angiotensin II is also mediated by activation of dendritic cells, highly specialized antigen-presenting cells, which are responsible for inflammation defence and immune response (reviewed by Benigni et al. 2010).

The proinflammatory effects of Angiotensin II can also involve T cells. T cells possess an endogenous RAS that modulates T cell proliferation and migration, NADPH activity and ROS production. During inflammation, Angiotensin II acts via its AT1 receptor to stimulate cytoskeletal rearrangements in T cells and to trigger the release of specific cytokines and chemokines that favor T cell recruitment to the sites of inflammation. Tissue infiltration of T cells contributes to the genesis of hypertension (Benigni et al. 2010). Angiotensin II dependent hypertension increases the production of proinflammatory cytokines within specific brain regions involved in blood pressure control.

Increase in the levels of proinflammatory cytokines in the plasma and other inflammatory markers are associated with the progression of hypertension, whereas immune suppression proves beneficial (Schillaci et al. 2003; Stumpf et al. 2005). Angiotensin II-induced hypertension involves activation of TNFα and NF-κB and production of reactive oxygen species in the brain (Kang et al. 2009). The paraventricular nucleus (PVN) in hypothalamus integrates signals from circumventricular organs and other cardiovascular-relevant brain areas and transmits them to the rostroventrolateral medulla (RVLM) and other downstream areas to influence sympathetic nerve activity (Guyenet 2006). Angiotensin II-induced hypertension involves activation of microglia and increases in proinflammatory cytokines (TNFα, IL-1β and IL-6) along with the decrease in IL-10 (anti-inflammatory cytokine) expression within the PVN. Inhibition of microglial activation

(by minocycline, an anti-inflammatory drug) or overexpression of IL-10 (by recombinant adenoassociated virus-mediated gene transfer) in the PVN attenuates Angiotensin II-induced hypertension (Shi et al. 2010). Chronic immune activation, increases in SNS activity and enhanced RAS activity are common features of hypertension and reciprocal communication between these systems contributes to the increased blood pressure.

12.4.2.4 Vital Organs Affected by Obesity Induced Peripheral Inflammation

Liver: Production of TNF and IL-6 is boosted in obese subjects due to low-grade inflammatory response caused due to lipid accumulation. Liver fat accumulation is called as 'Hepatosteatosis'. Lipid storage in liver occurs due to high energy consumption and less energy combustion. Energy combustion in liver is controlled by PPAR-α regulated mitochondrial and peroxisomal fatty acid β-oxidation systems and microsomal ω-oxidation system. PPAR-α acts as a sensor for fatty acids (Hashimoto et al. 2000; Rao and Reddy 2004). In case of an inflammatory response, IL-6 is also released by Kupffer cells in the liver, where its production is induced by the death of hepatocytes, which further results in the release of IL-1 (Sakurai et al. 2008). Higher levels of TNF also stimulate the production of IL-6. Production of IL-6 is highly instrumental in the propagation of inflammation (Kamimura et al. 2003). Obese individuals also have higher accumulation of ROS.

Obesity also promotes hepatocarcinogenesis. Hepatic inflammation predisposes an individual to the risk of cancer. The most common consequence is hepatocellular carcinoma (HCC) (Calle et al. 2003). Tumor promoting effect of obesity depends on the induced low-grade inflammatory response (Park et al. 2010), which involves elevation in the production of TNF and IL-6, both of which have been reported to be tumor promoting cytokines (Lin and Karin 2007). A higher level of IL-6 leads to the activation of STAT3 in hepatocytes (Naugler et al. 2007), which stimulates the proliferation and progression of hepatocytes (He et al. 2010). Thus, STAT3 may be a mediator of tumor promotion. Another mechanism proposed for tumor promotion triggered by obesity is enhanced activation of AKT caused due to high concentrations of insulin and IGF-1 in obese subjects (Calle and Kaaks 2004). Also, the activity of mTOR is elevated in obese liver (Park et al. 2010), which is an important regulator of cell and tumor growth.

Muscle: Skeletal muscle is the principal site for glucose and fatty acid utilization and composes 40–50 % of total body mass. It is a metabolically active tissue critical in the maintenance of homeostasis. It also plays an important role in fatty acid oxidation. Low rate of lipid oxidation in skeletal muscle predisposes an individual towards weight gain (Slentz et al. 2009). The levels of intramyocellular lipids are elevated in case of obese subjects. Skeletal muscle has high levels of triglycerides in obesity (Pan et al. 1997). Amount of triglycerides in skeletal muscles is closely associated to insulin resistance (Russell et al. 1998; Simoneau et al. 1995). The level of TNF-α is also increased in case of obese human subjects and its exogenous administration has been linked to insulin resistance (Saghizadeh et al. 1996; Krogh-Madsen et al. 2006). CNS inflammation has been reported to cause muscle

atrophy. Muscle breakdown has been reported to be caused by inflammatory cytokines by their action within the brain (Braun et al. 2011). This effect of cytokines is dependent on the activation of hypothalamic–pituitary–adrenal (HPA) axis.

Foetal stage for the development of skeletal muscle is crucial as there is no net increase in muscle fibre number after birth. Stages in foetal muscle development include myogenesis, adipogenesis and fibrogenesis, which are derived from mesenchymal stem cell (MSC). A shift of MSC from myogenesis to adipogenesis and fibrogenesis results in increased intramuscular fat and connective tissue as well as reduced muscle fibre number, all of which have negative effect on offspring muscle function and properties. Therefore, maternal well being is necessary for proper development of skeletal muscles in the offspring. Maternal obesity leads to low-grade inflammation which changes the commitment of MSCs (Du et al. 2010). Poor foetal skeletal muscle development impairs glucose and fatty acid metabolism by skeletal muscle in response to insulin stimulation, and thus predisposes the offspring to diabetes and obesity later in life (Zambrano et al. 2005).

12.4.3 Obesity and Central Inflammation

Pathologically obesity-induced neuroinflammation plays the major role in regulating obesity and related metabolic disorders. The central nervous system, particularly hypothalamus, plays the regulatory role in maintaining metabolic homeostasis by regulating various physiological processes including feeding, body weight, energy expenditure and glucose metabolism via endocrine signalling, trophic actions, complex neuronal plasticity and projections into the autonomous control centres of the brain. Inflammation induced in the brain has negative impact on the neurohormonal signalling of the hypothalamic neurons and also contributes to the neurodegeneration and disruption of the neural stem cells. Circulating metabolic signals such as insulin, gut hormones, leptin and nutrients are sensed by mediobasal hypothalamus (MBH), which then commands the downstream neurohormonal networks to control different aspects of the metabolic physiology. In addition to modulation of the sympathetic and parasympathetic nervous system, hypothalamic neurons can project to the autonomic sites in the brain which further control metabolic activites.

12.4.3.1 Cellular Targets Involved in Obesity Induced Neuroinflammation

Obesity is linked to hypothalamic inflammation which involves both neuronal and non-neuronal populations and their crosstalk. Brain comprises of the more than 50 % of the non-neuronal cell population including glial, periventricular and vascular constituents. Astrocytes and microglia are among the most abundant cell types which play important role in maintaining the BBB, support neuronal metabolism and act as both guard and react to the local tissue injury. Both cell types show remarkable plasticity by altering genetic programmes and morphologies to combat

with different infections and insults. They both are involved in overnutrition-induced central inflammation.

High-fat diet leads to the infiltration and activation of microglia and astrocytes, thus resulting in the activation of inflammatory cascades and causing the production of local inflammatory cytokines. Diet-induced obesity results in the increased levels of microglial activation markers which have been observed throughout the hippocampus (Erion et al. 2014). Similarly astrocytes are also activated by different saturated fatty acids present in high fat diet, which further trigger the production of inflammatory cytokines and also cause reactive gliosis. Tomassoni et al. (2013) reported significant increase in the number of the glial fibillary acidic protein (GFAP) immunoreactive astrocytes throughout hippocampus, frontal and parietal cortices in obese Zucker rats.

Functionally, macrophages or microglia activation has two separate polarization states M1 and M2. M1 or the classically activated macrophages are induced by LPS or IFN-γ and are responsible for various inflammatory consequences through the production of various proinflammatory mediators like proinflammatory cytokines IL-1β, IL-6, TNF-α and various reactive oxygen and nitrogen species. M2 phenotype is anti-inflammatory and protective state of the microglia. It is also associated with the activity of PPARδ and PPARγ, well-known regulators of lipid metabolism and mitochondrial activity (Odegaard et al. 2011). Microglia or macrophages present in the adipose tissue assume the number of states along the M1/M2 spectrum depending upon fat depot location and the degree of fat deposition. Increase in adiposity results in the shift from the alternative M2 state to classically inflammatory M1 state (Aron-Wisnewsky et al. 2009). Lumeng et al. (2007) have reported that obesity induces switch of microglia or macrophages phenotype from M2 to M1 polarization. Obesity also affects lipid metabolism by affecting the activity of PPARδ and PPARγ.

The neuronal cell types regulating energy homeostasis are the orexigenic neuropeptide Y (NPY)/agouti-related peptide (AGRP) neurons and anorexigenic pro-opiomelanocortin (POMC) neurons. They both reciprocally regulate the energy homeostasis through negative and positive energy balance actions. Their functions are also regulated by leptin. These neurons get affected by obesity induced neuroinflammation which negatively impacts their regulatory cascades such as leptin and insulin signalling. This neuroinflammation also exerts the effect on neuroendocrine system by compromising the secretion of anorexigenic POMC derived α-melanocyte secreting hormone, cocaine and amphetamine-regulated transcript (CART) resulting in increase in appetite along with leptin and insulin resistance.

12.4.3.2 Molecular Targets and Mechanism Involved in Obesity-Induced Neuroinflammation

IKKβ/NF-κB pathway plays a key role in obesity linked hypothalamic inflammation by regulating the transcription of various inflammatory genes like iNOS, COX-2, inflammatory cytokines like TNF-α, IL-1β, IL-6, etc., and various other inflammatory mediators. This pathway is activated by both receptor dependent and independent processes. Pattern recognition receptors like TLR2, TLR4 and the

cytokines receptors have been shown to initiate the induction of obesity related neuroinflammation by activating NF-κB pathway. In addition to these receptors, receptor independent intracellular organelle stress and disturbances involving the endoplasmic reticulum stress (Zhang et al. 2008; Purkayastha et al. 2011), oxidative stress (Zhou et al. 2011) and defects in the autophagy (Meng et al. 2011) are also the contributors of hypothalamic inflammation which ultimately converge at the IKKβ/NF-κB inflammatory pathway. Endoplasmic reticulum stress activates NF-κB through the signalling crosstalk between the IKKβ/NF-κB pathway and unfolded protein response elements (UPR) via PKR-like ER kinase, inositol requiring enzyme-1 and activating transcription factor-6 (Deng et al. 2004; Hu et al. 2006; Yamazaki et al. 2009). The inflammasomes known as the immune cell sensors like Nod like receptor 3 (NLRP3), are also the mediators of the mitochondrial dysfunction and oxidative stress leading to obesity-mediated neuroinflammation. At the time of increased oxidative workload, higher level of ER activity is in demand such as protein synthesis which results in ER stress. Hypothalamic and extrahypothalamic brain regions show greater ER stress during obesity (Castro et al. 2013; Cakir et al. 2013), which has been implicated in perpetuating the development of obesity (Williams 2012). Hypothalamic ER stress ultimately activates IKKβ/NF-κB in the hypothalamus. In addition, cytosolic changes induced by overnutrition, such as dysfunctional ER and mitochondria can lead to autophagy defects. Excessive stress in ER can lead to apoptosis (Rao et al. 2004; Ron and Walter 2007), and eventually brain atrophy (Miller and Spencer 2014).

In an ongoing study in our lab, we have been using 30 % fat diet (by weight) fed Wistar strain rats as HFD induced obesity model. The animals were divided into four groups. First group (Control) was fed with regular chow feed. Second group (HFD) was fed with 30 % fat diet. Third group (C + E) was fed with regular chow feed supplemented with dry leaf powder of *Withania somnifera*. Fourth group (H + E) was fed with high-fat diet supplemented with dry leaf powder of *Withania somnifera*. The animals were kept on respective feeding regime for 12 weeks. After 12 weeks, the animals were sacrificed and their sera and brains were isolated. Western blotting was done for GFAP, OB-Rb (Leptin receptor) and proinflammatory cytokines such as TNFα, IL-1β, IL-6 from hypothalamus region of brain. The data is represented in Fig. 12.2 panel a. OB-Rb expression was reduced, whereas the level of all proinflammatory cytokines was increased with HFD and alleviated with Ashwagandha feeding. Panel (b) in Fig. 12.2 represents the data of serum leptin and insulin levels. The level of leptin has increased significantly in HFD group and Ashwagandha leaf powder supplementation was seen to suppress the deleterious effects of HFD on serum biomarkers as well as brain function impairments.

Besides direct entry of free fatty acids, cytokines and chemokines into the brain at BBB lacking areas, systemic inflammation and free fatty acids in excess also promote central inflammation by initiation of pro-inflammatory cytokine and prostaglandin cascade that stimulates centrally projecting neurons (Blatteis 2007). There are many molecular targets downstream of the IKKβ/NF-κB pathway which

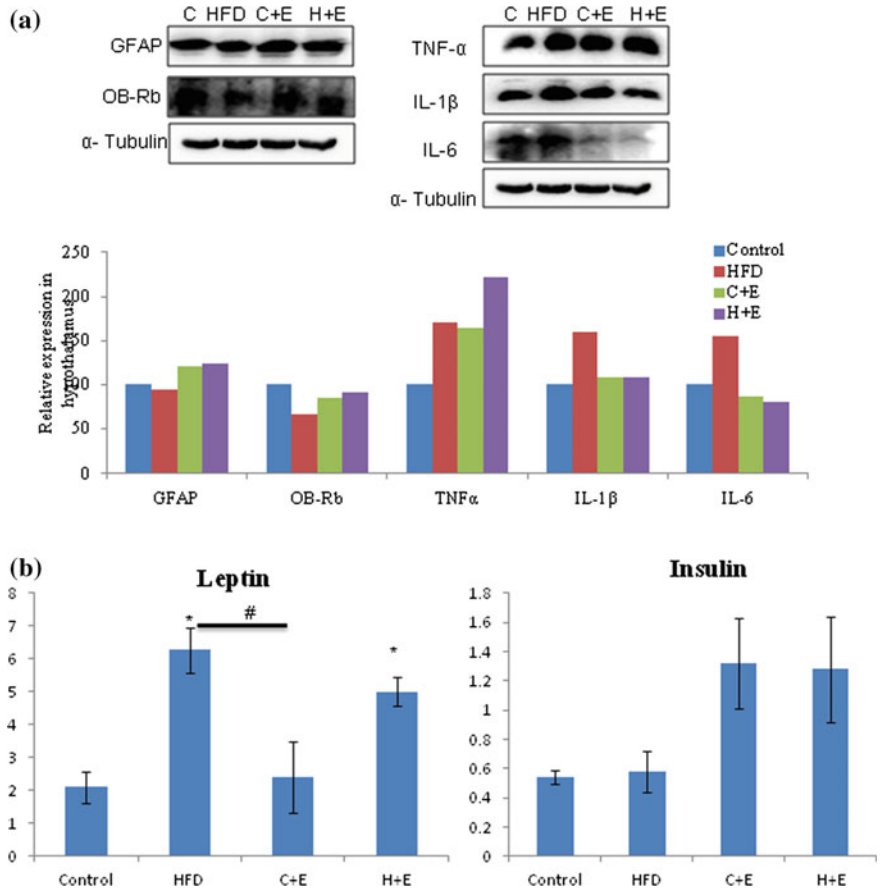

Fig. 12.2 High-Fat Diet-induced hypothalamic inflammation. **a** Western blotting data of GFAP, OB-Rb (Leptin receptor) and inflammatory cytokines (TNFα, IL-1β and IL-6) from animals fed with control chow diet, 30 % high-fat diet (HFD), control diet plus dry leaf powder of *Withania somnifera* (C + E) and 30 % fat diet plus dry leaf powder of *Withania somnifera* (H + E). The samples are pooled in all groups with $n = 2$ in each group. The levels of OB-Rb are inversely related to the levels of leptin in serum. *Withania somnifera* helps in regaining of the leptin levels. The levels of proinflammatory cytokines are elevated in high fat diet fed group which are alleviated with *Withania somnifera.* **b** Serum profile of leptin and insulin in high fat diet induced obesity model system. The expression of leptin and insulin was also affected by the high-fat diet, which ultimately leads to the development of insulin and leptin resistance

link obesity with various metabolic deficits such as suppressor of cytokine signalling-3 (SOCS3), an inhibitory signalling protein which inhibits both insulin and leptin signalling. Overnutrition-induced IKKβ/NF-κB activation can cause upregulation of SOCS3 gene expression in hypothalamus and induce hypothalamic insulin and leptin resistance. Another protein, Protein tyrosine phosphatase 1B (PTP1B), like SOCS3, inhibits leptin and insulin signalling and interestingly,

PTP1B has also been implicated in the IKKβ/NF-κB inflammatory pathway. Such neuroinflammation impairs intracellular hormonal signalling of regulatory neurons and disrupts neurogenesis through depletion of neural stem cells (NSCs). The progression of overnutrition-related diseases such as obesity and diabetes, characterized by hyperlipidemia and hyperglycemia, secondarily leads to pathophysiological overnutrition in the internal environment of the body, which exacerbates neuroinflammation.

12.5 Role of Blood–Brain Barrier in Obesity Induced Neuroinflammation

Blood–brain barrier (BBB) regulates the microenvironment for reliable neuronal signalling by allowing or restricting the entry of essential nutrients, blood borne toxins into the central nervous system. Elevated levels of sugars and fatty acids present in the high fat diet influence the brain by disrupting BBB integrity. Increasing BBB permeability allows peripheral cytokines and immune cells to enter the brain tissue. Anatomically MBH region in the hypothalamus is located at the most vulnerable position due to partially leaky BBB. Similarly, hypothalamic arcuate nucleus (ARC) and other circumventricular organs such as subfornical organs and area postrema lacking an effective BBB are more prone to various inflammatory factors. Therefore on exposure to prolonged feeding, oxidative stress and mitochondrial dysfunction in MBH neurons occur perhaps earlier than the induction in other cells. Rats on the Western diet for three months have showed decreased expression of tight junction proteins in choroid plexus and BBB (Kanoski et al. 2010). Also the rats fed with the high cholesterol and saturated fat diet for 6 months have shown reduced integrity of BBB and increased microgliosis in the hippocampus indicating that hippocampus may be more vulnerable to the diet-induced BBB disruption (Fig. 12.3).

The majority of research to study correlation of obesity with neurological and psychiatric diseases is predominately focused on people in Western nations and thus it is difficult to predict whether these results can be generalized to other world populations (Jakabek et al. 2011). Similarly, differences in eating behaviors between Western and non-Western patients with mental illness, dementia and cognitive decline has also received limited attention. Weight gain and metabolic disturbances are well known potential adverse events related to antipsychotic medication. A recently published meta-analysis shows that some second-generation antipsychotics (SGAs), such as olanzapine, cause more metabolic side effects than other SGAs (Rummel-Kluge et al. 2010). In recent years, the importance of physical health of patients suffering from mental illness and dementias has become a major concern of the medical community and, as a result, several guidelines and consensus recommendations (Marwaha et al. 2008) have been developed to manage the standards of physical health in this group of patients (Chacon et al. 2011; Cabral et al. 2011; Knochel et al. 2012). Association between obesity and depression has

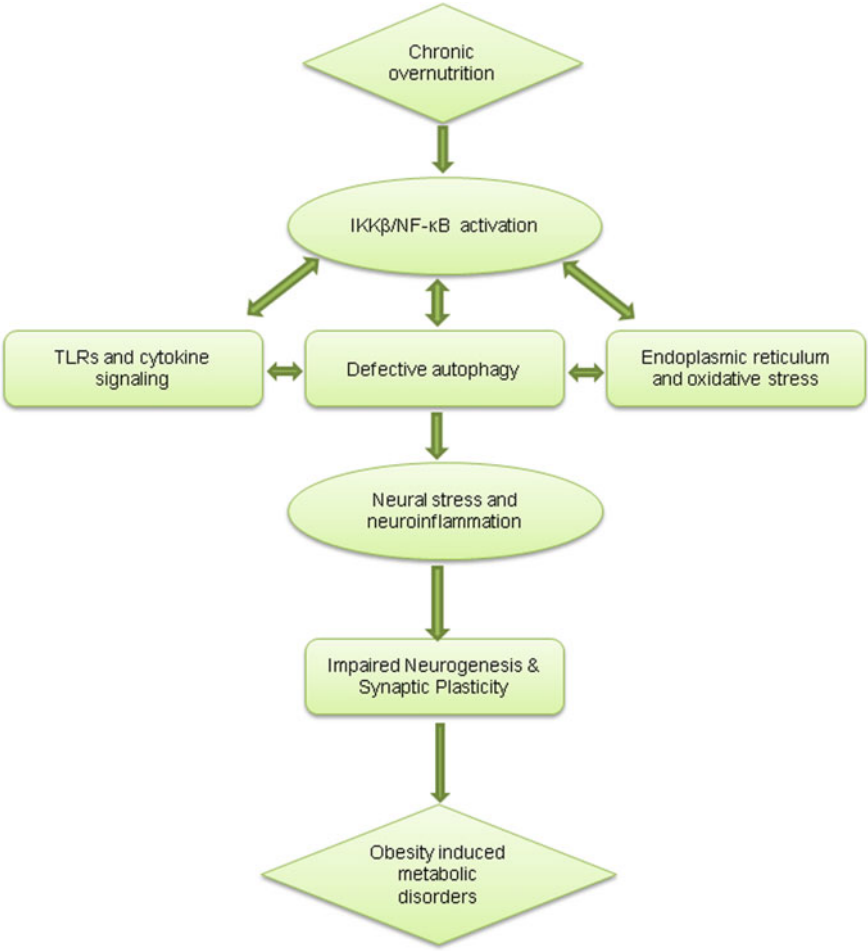

Fig. 12.3 Role of neuroinflammation in obesity-associated diseases. The mediobasal hypothalamus (MBH) is affected by chronic overnutrition, a prolonged nutritional change which primarily arises from environmental and sociobehavioral factors such as Western diet, sedentary lifestyle and disrupted diurnal rhythmicity. These lead to the IKKβ/NFκB-directed inflammatory response and intracellular organelle stress in the MBH. Many of these cellular and molecular components promote each other, resulting in overnutrition-related neuroinflammation

been reported in studies conducted in the United States (Atlantis and Baker 2008) and Western countries (Green et al. 2007). Moreover, search for biomarkers for diagnosis and prognosis of the mental health in patients suffering from metabolic syndrome is a growing concern among the clinicians (Martins-De-Souza et al. 2010a, b; Kluge et al. 2011).

Besides the lacunas in our current knowledge of molecular and cellular mechanisms that correlate obesity with neurodegenerative diseases, therapeutic prevention or reversal of acute or chronic neuronal injury caused by obesity also remains unknown. Therapeutically multiple therapies and lifestyle changes are required to reduce obesity that may or may not ameliorate neuronal insults. Recently, Nerurkar et al. (2011) reported that *Momordica charantia* (bitter melon) attenuates HFD-induced oxidative stress and neuroinflammation; and related studies in literature emphasize that herbal products may offer the distinctive therapeutic strategy to improve obesity-associated peripheral inflammation and neuroinflammation. Pawar et al. (2011) reported the anti-inflammatory and mucorestorative activity of *Withania somnifera* root extract in a rectal gel preparation used in TNBS-induced Inflammatory Bowel Disease. In Asian countries, *Gynostemma pentaphyllum* is widely used to treat dyslipidemia, type 2 diabetes and inflammation (Gauhar et al. 2012) and the ethanolic extract of *G. pentaphyllum* caused the reduction in body weight gain, liver weight, and blood cholesterol levels by activating AMP-activated protein kinase (AMPK) in the soleus muscle. Furthermore, bamboo leaves showed anti-inflammatory potency and has been used to treat metabolic disorders such as obesity and diabetes (Koide et al. 2011). Further exploration of the molecular mechanisms of potential interventions by natural products may offer a unique therapeutic strategy in amelioration of DIO-associated peripheral inflammation and neuroinflammation and associated neurological and neurodegenerative disorders.

Identification of obesity-associated neuroinflammation targets in brain and introducing innovative interventions/approaches based on the use of herbal products may help to plan therapeutic strategies to curtail the progression of neuropathogenesis in obese patients. This approach may also reduce the severity of associated psychopathological and physical illness such as hypertension, T2D, CVDs, etc. In particular, if parallel studies are done on brain and blood samples, and if defects are also observed in blood, then simple and non-invasive diagnostic assays could be established which eventually might help recognize the disease before its clinical onset.

References

Aron-Wisnewsky J, Tordjman J, Poitou C, Darakhshan F, Hugol D, Basdevant A, Aissat A, Guerre-Millo M, Clement K (2009) Human adipose tissue macrophages: m1 and m2 cell surface markers in subcutaneous and omental depots and after weight loss. J Clin Endocrinol Metab 94(11):4619–4623

Atlantis E, Baker M (2008) Obesity effects on depression: systematic review of epidemiological studies. Int J Obes 32(6):881–891

Barkin SL (2013) The relationship between executive function and obesity in children and adolescents: a systematic literature review. J Obes

Benigni A, Cassis P, Remuzzi G (2010) Angiotensin II revisited: new roles in inflammation, immunology and aging. EMBO Mol Med 2(7):247–257

Bjørbæk C, Hollenberg AN (2002) Leptin and melanocortin signaling in the hypothalamus. Vitam Horm 65:281–311

Blakemore AI, Froguel P (2010) Investigation of Mendelian forms of obesity holds out the prospect of personalized medicine. Ann N Y Acad Sci 1214(1):180–189

Blatteis CM (2007) The onset of fever: new insights into its mechanism. Prog Brain Res 162:3–14

Bogoyevitch MA, Ngoei KR, Zhao TT, Yeap YY, Ng DC (2010) c-Jun N-terminal kinase (JNK) signaling: recent advances and challenges. Biochimica et Biophysica Acta (BBA) Proteins Proteomics 1804(3):463–475

Braun TP, Zhu X, Szumowski M, Scott GD, Grossberg AJ, Levasseur PR, Graham K, Khan S, Damaraju S, Colmers WF, Baracos VE (2011) Central nervous system inflammation induces muscle atrophy via activation of the hypothalamic–pituitary–adrenal axis. J Exp Med 208 (12):2449–2463

Bray GA, Popkin BM (1998) Dietary fat intake does affect obesity! Am J Clin Nutr 68(6): 1157–1173

Buckman LB, Hasty AH, Flaherty DK, Buckman CT, Thompson MM, Matlock BK, Weller K, Ellacott KL (2014) Obesity induced by a high-fat diet is associated with increased immune cell entry into the central nervous system. Brain Behav Immun 35:33–42

Cabral P, Meyer HB, Ames D (2011) Effectiveness of yoga therapy as a complementary treatment for major psychiatric disorders: a meta-analysis. Prim Care Companion CNS Disord 13(4)

Cai D (2012) One step from prediabetes to diabetes: hypothalamic inflammation? Endocrinology 153(3):1010

Cai D (2013) Neuroinflammation and neurodegeneration in overnutrition-induced diseases. Trends Endocrinol Metab 24(1):40–47

Cai D, Liu T (2011) Hypothalamic inflammation: a double-edged sword to nutritional diseases. Ann N Y Acad Sci 1243(1):E1–E39

Cai D, Liu T (2012) Inflammatory cause of metabolic syndrome via brain stress and NF-κB. Aging (Albany NY) 4(2):98

Çakir I, Cyr NE, Perello M, Litvinov BP, Romero A, Stuart RC, Nillni EA (2013) Obesity induces hypothalamic endoplasmic reticulum stress and impairs proopiomelanocortin (POMC) post-translational processing. J Biol Chem 288(24):17675–17688

Calle EE, Kaaks R (2004) Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nat Rev Cancer 4(8):579–591

Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ (2003) Overweight, obesity, and mortality from cancer in a prospectively studied cohort of US adults. N Engl J Med 348(17):1625–1638

Castro G, Areias MFC, Weissmann L, Quaresma PG, Katashima CK, Saad MJ, Prada PO (2013) Diet-induced obesity induces endoplasmic reticulum stress and insulin resistance in the amygdala of rats. FEBS open bio 3:443–449

Cattin L, Bordin P, Fonda M, Adamo C, Barbone F, Bovenzi M, Manto A, Pedone C, Pahor M (1997) Factors associated with cognitive impairment among older Italian inpatients. J Am Geriatr Soc 45(11):1324–1330

Chacón F, Mora F, Gervás-Ríos A, Gilaberte I (2011) Efficacy of lifestyle interventions in physical health management of patients with severe mental illness. Ann Gen Psychiatry 10(22): 21929761

Challis BG, Pritchard LE, Creemers JW, Delplanque J, Keogh JM, Luan JA, Wareham NJ, Yeo GS, Bhattacharyya S, Froguel P, White A (2002) A missense mutation disrupting a dibasic prohormone processing site in pro-opiomelanocortin (POMC) increases susceptibility to early-onset obesity through a novel molecular mechanism. Hum Mol Genet 11(17):1997–2004

Clément K, Vaisse C, Lahlou N, Cabrol S, Pelloux V, Cassuto D, Gourmelen M, Dina C, Chambaz J, Lacorte JM, Basdevant A (1998) A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. Nature 392(6674):398–401

Davidson S, Judd F, Jolley D, Hocking B, Thompson S (2000) The general health status of people with mental illness. Australas Psychiatry 8(1):31–35

Deng J, Lu PD, Zhang Y, Scheuner D, Kaufman RJ, Sonenberg N, Harding HP, Ron D (2004) Translational repression mediates activation of nuclear factor kappa B by phosphorylated translation initiation factor 2. Mol Cell Biol 24(23):10161–10168

Du M, Yan X, Tong JF, Zhao J, Zhu MJ (2010) Maternal obesity, inflammation, and fetal skeletal muscle development. Biol Reprod 82(1):4–12

Elias MF, Elias PK, Sullivan LM, Wolf PA, D'agostino RB (2003) Lower cognitive function in the presence of obesity and hypertension: the Framingham heart study. International journal of obesity 27(2):260–268

Erion JR, Wosiski-Kuhn M, Dey A, Hao S, Davis CL, Pollock NK, Stranahan AM (2014) Obesity elicits interleukin 1-mediated deficits in hippocampal synaptic plasticity. J Neurosci 34(7): 2618–2631

Farooqi IS (2006) Monogenic human obesity syndromes. Prog Brain Res 153:119–125

Farooqi IS, Rahilly O (2004) Monogenic human obesity syndromes. Recent Prog Horm Res 59:409–424

Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, Perry JR, Elliott KS, Lango H, Rayner NW, Shields B (2007) A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science 316 (5826):889–894

Fung TT, Rimm EB, Spiegelman D, Rifai N, Tofler GH, Willett WC, Hu FB (2001) Association between dietary patterns and plasma biomarkers of obesity and cardiovascular disease risk. Am J Clin Nutr 73(1):61–67

Gariepy G, Nitka D, Schmitz N (2010) The association between obesity and anxiety disorders in the population: a systematic review and meta-analysis. Int J Obes 34(3):407–419

Gauhar R, Hwang SL, Jeong SS, Kim JE, Song H, Park DC, Song KS, Kim TY, Oh WK, Huh TL (2012) Heat-processed Gynostemma pentaphyllum extract improves obesity in ob/ob mice by activating AMP-activated protein kinase. Biotechnol Lett 34(9):1607–1616

Geller F, Reichwald K, Dempfle A, Illig T, Vollmert C, Herpertz S, Siffert W, Platzer M, Hess C, Gudermann T, Biebermann H (2004) Melanocortin-4 receptor gene variant I103 is negatively associated with obesity. Am J Hum Genet 74(3):572–581

Ghosh G, Wang VYF, Huang DB, Fusco A (2012) NF-κB regulation: lessons from structures. Immunol Rev 246(1):36–58

Green AI, Drake RE, Brunette MF, Noordsy DL (2007) Schizophrenia and co-occurring substance use disorder. Am J Psychiatry 164(3):402–408

Gregor MF, Hotamisligil GS (2011) Inflammatory mechanisms in obesity. Annu Rev Immunol 29: 415–445

Gunstad J, Paul RH, Cohen RA, Tate DF, Spitznagel MB, Gordon E (2007) Elevated body mass index is associated with executive dysfunction in otherwise healthy adults. Compr Psychiatry 48(1):57–61

Guyenet PG (2006) The sympathetic control of blood pressure. Nat Rev Neurosci 7(5):335–346

Haddad PM, Sharma SG (2007) Adverse effects of atypical antipsychotics. CNS drugs 21(11): 911–936

Halliwell B (2001) Role of free radicals in the neurodegenerative diseases. Drugs Aging 18 (9):685–716

Hashimoto T, Cook WS, Qi C, Yeldandi AV, Reddy JK, Rao MS (2000) Defect in peroxisome proliferator-activated receptor α-inducible fatty acid oxidation determines the severity of hepatic steatosis in response to fasting. J Biol Chem 275(37):28918–28928

He G, Yu GY, Temkin V, Ogata H, Kuntzen C, Sakurai T, Sieghart W, Peck-Radosavljevic M, Leffert HL, Karin M (2010) Hepatocyte IKKβ/NF-κB inhibits tumor promotion and progression by preventing oxidative stress-driven STAT3 activation. Cancer Cell 17(3): 286–297

Herbert A, Gerry NP, McQueen MB, Heid IM, Pfeufer A, Illig T, Wichmann HE, Meitinger T, Hunter D, Hu FB, Colditz G (2006) A common genetic variant is associated with adult and childhood obesity. Science 312(5771):279–283

Herrera BM, Lindgren CM (2010) The genetics of obesity. Curr DiabRep 10(6):498–505

Hinney A, Schmidt A, Nottebom K, Heibult O, Becker I, Ziegler A, Gerber G, Sina M, Gorg T, Mayer H, Siegfried W (1999) Several mutations in the melanocortin-4 receptor gene including

a nonsense and a frameshift mutation associated with dominantly inherited obesity in humans. J Clin Endocrinol Metab 84(4):1483–1486
Hinney A, Vogel CI, Hebebrand J (2010) From monogenic to polygenic obesity: recent advances. Eur Child Adolesc Psychiatry 19(3):297–310
Horton TJ, Drougas H, Brachey A, Reed GW, Peters JC, Hill JO (1995) Fat and carbohydrate overfeeding in humans: different effects on energy storage. Am J Clin Nutr 62(1):19–29
Hu P, Han Z, Couvillon AD, Kaufman RJ, Exton JH (2006) Autocrine tumor necrosis factor alpha links endoplasmic reticulum stress to the membrane death receptor pathway through IRE1α-mediated NF-κB activation and down-regulation of TRAF2 expression. Mol Cell Biol 26(8):3071–3084
Hursting SD, Dunlap SM (2012) Obesity, metabolic dysregulation, and cancer: a growing concern and an inflammatory (and microenvironmental) issue. Ann N Y Acad Sci 1271(1):82–87
Jakabek D, Quirk F, Driessen M, Aljeesh Y, Baune BT (2011) Obesity and nutrition behaviours in Western and Palestinian outpatients with severe mental illness. BMC Psychiatry 11(1):159
Kamimura D, Ishihara K, Hirano T (2003) IL-6 signal transduction and its physiological roles: the signal orchestration model. In: Reviews of physiology, biochemistry and pharmacology. Springer, Berlin, pp 1–38
Kang YM, Ma Y, Zheng JP, Elks C, Sriramula S, Yang ZM, Francis J (2009) Brain nuclear factor-kappa B activation contributes to neurohumoral excitation in angiotensin II-induced hypertension. Cardiovasc Res cvp073
Kanoski SE, Zhang Y, Zheng W, Davidson TL (2010) The effects of a high-energy diet on hippocampal function and blood-brain barrier integrity in the rat. J Alzheimers Dis 21(1):207
Karhunen LJ, Lappalainen RI, Vanninen EJ, Kuikka JT, Uusitupa MIJ (1997) Regional cerebral blood flow during food exposure in obese and normal weight women. Brain 120(9):1675–1684
Kluge W, Alsaif M, Guest PC, Schwarz E, Bahn S (2011) Translating potential biomarker candidates for schizophrenia and depression to animal models of psychiatric disorders
Knöchel C, Oertel-Knöchel V, O'Dwyer L, Prvulovic D, Alves G, Kollmann B, Hampel H (2012) Cognitive and behavioural effects of physical exercise in psychiatric patients. Prog Neurobiol 96(1):46–68
Koide CL, Collier AC, Berry MJ, Panee J (2011) The effect of bamboo extract on hepatic biotransforming enzymes–findings from an obese–diabetic mouse model. J Ethnopharmacol 133(1):37–45
Kousta E, Hadjiathanasiou CG, Tolis G, Papathanasiou A (2009) Pleiotropic genetic syndromes with developmental abnormalities associated with obesity. J Pediatr Endocrinol Metab 22 (7):581–592
Krogh-Madsen R, Plomgaard P, Møller K, Mittendorfer B, Pedersen BK (2006) Influence of TNF-α and IL-6 infusions on insulin sensitivity and expression of IL-18 in humans. Am J Physiol Endocrinol Metab 291(1):E108–E114
Lalouel JM, Rao DC, Morton NE, Elston RC (1983) A unified model for complex segregation analysis. Am J Hum Genet 35(5):816
Lee JY, Sohn KH, Rhee SH, Hwang D (2001) Saturated fatty acids, but not unsaturated fatty acids, induce the expression of cyclooxygenase-2 mediated through Toll-like receptor 4. J Biol Chem 276(20):16683–16689
Li W, Prakash R, Chawla D, Du W, Didion SP, Filosa JA, Zhang Q, Brann DW, Lima VV, Tostes RC, Ergul A (2013) Early effects of high-fat diet on neurovascular function and focal ischemic brain injury. Am J Physiol Regul Integr Compar Physiol 304(11):R1001–R1008
Lin WW, Karin M (2007) A cytokine-mediated link between innate immunity, inflammation, and cancer. J Clin Invest 117(5):1175
Linker R, Kleinewietfeld M, Lee DH, Muller D, Manzel A (2014) Dietary saturated fatty acids promote differentiation of Th1 and Th17 cells and aggravate neuroinflammation. Neurology, 82(10 Supplement):6–149
Lumeng CN, Bodzin JL, Saltiel AR (2007) Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest 117(1):175

Maayan L, Hoogendoorn C, Sweat V, Convit A (2011) Disinhibited eating in obese adolescents is associated with orbitofrontal volume reductions and executive dysfunction. Obesity 19 (7):1382–1387

Maes HH, Neale MC, Eaves LJ (1997) Genetic and environmental factors in relative body weight and human adiposity. Behav Genet 27(4):325–351

Martins-de-Souza D, Maccarrone G, Wobrock T, Zerr I, Gormanns P, Reckow S, Falkai P, Schmitt A, Turck CW (2010a) Proteome analysis of the thalamus and cerebrospinal fluid reveals glycolysis dysfunction and potential biomarkers candidates for schizophrenia. J Psychiatr Res 44(16):1176–1189

Martins-De-Souza D, Dias-Neto E, Schmitt A, Falkai P, Gormanns P, Maccarrone G, Turck CW, Gattaz WF (2010b) Proteome analysis of schizophrenia brain tissue. World J Biol Psychiatry 11(2):110–120

Marwaha S, Johnson S, Bebbington P, Angermeyer MC, Brugha T, Azorin JM, Kilian R, Kornfeld A, Toumi M, EuroSC Study Group (2008) Correlates of subjective quality of life in people with schizophrenia: findings from the EuroSC study. J Nerv Mental Dis 196(2):87–94

McNay DE, Briançon N, Kokoeva MV, Maratos-Flier E, Flier JS (2012) Remodeling of the arcuate nucleus energy-balance circuit is inhibited in obese mice. J Clin Investig 122(1):142

Meng Q, Cai D (2011) Defective hypothalamic autophagy directs the central pathogenesis of obesity via the IκB kinase β (IKKβ)/NF-κB pathway. J Biol Chem 286(37):32324–32332

Miller AA, Spencer SJ (2014) Obesity and neuroinflammation: a pathway to cognitive impairment. Brain Behav Immun 42:10–21

Molteni R, Barnard RJ, Ying Z, Roberts CK, Gomez-Pinilla F (2002) A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning. Neuroscience 112(4):803–814

Moraes JC, Coope A, Morari J, Cintra DE, Roman EA, Pauli JR, Romanatto T, Carvalheira JB, Oliveira AL, Saad MJ, Velloso LA (2009) High-fat diet induces apoptosis of hypothalamic neurons. PLoS One 4(4):e5045

Mueller K, Anwander A, Möller HE, Horstmann A, Lepsien J, Busse F, Mohammadi S, Schroeter ML, Stumvoll M, Villringer A, Pleger B (2011) Sex-dependent influences of obesity on cerebral white matter investigated by diffusion-tensor imaging. PLoS One 6(4):e18544

Naugler WE, Sakurai T, Kim S, Maeda S, Kim K, Elsharkawy AM, Karin M (2007) Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. Science 317(5834):121–124

Nerurkar PV, Johns LM, Buesa LM, Kipyakwai G, Volper E, Sato R, Shah P, Feher D, Williams PG, Nerurkar VR (2011) Momordica charantia (bitter melon) attenuates high-fat diet-associated oxidative stress and neuroinflammation. J Neuroinflammation 8(64):2094–2098

Nguyen JC, Killcross AS, Jenkins TA (2014) Obesity and cognitive decline: role of inflammation and vascular changes. Front Neurosci 8:375

Odegaard JI, Chawla A (2011) Alternative macrophage activation and metabolism. Ann Rev Pathol 6:275

Oeckinghaus A, Hayden MS, Ghosh S (2011) Crosstalk in NF-[kappa] B signaling pathways. Nat Immunol 12(8):695–708

Pan DA, Lillioja S, Kriketos AD, Milner MR, Baur LA, Bogardus C, Jenkins AB, Storlien LH (1997) Skeletal muscle triglyceride levels are inversely related to insulin action. Diabetes 46 (6):983–988

Park EJ, Lee JH, Yu GY, He G, Ali SR, Holzer RG, Österreicher CH, Takahashi H, Karin M (2010) Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. Cell 140(2):197–208

Paton JF, Waki H (2009) Is neurogenic hypertension related to vascular inflammation of the brainstem? Neurosci Biobehav Rev 33(2):89–94

Pawar P, Gilda S, Sharma S, Jagtap S, Paradkar A, Mahadik K, Ranjekar P, Harsulkar A (2011) Rectal gel application of Withania somnifera root extract expounds anti-inflammatory and

muco-restorative activity in TNBS-induced Inflammatory Bowel Disease. BMC Complement Altern Med 11(1):34

Pepping JK, Freeman LR, Gupta S, Keller JN, Bruce-Keller AJ (2013) NOX2 deficiency attenuates markers of adiposopathy and brain injury induced by high-fat diet. Am J Physiol Endocrinol Metab 304(4):E392–E404

Pilch PF, Lee J (2004) Insulin receptor family. Encyclopedia Biol Chem 2:436–440

Popkin BM, Gordon-Larsen P (2004) The nutrition transition: worldwide obesity dynamics and their determinants. Int J Obes 28:S2–S9

Purkayastha S, Zhang H, Zhang G, Ahmed Z, Wang Y, Cai D (2011) Neural dysregulation of peripheral insulin action and blood pressure by brain endoplasmic reticulum stress. Proc Natl Acad Sci 108(7):2939–2944

Rahmouni K, Correia ML, Haynes WG, Mark AL (2005) Obesity-associated hypertension new insights into mechanisms. Hypertension 45(1):9–14

Raji CA, Ho AJ, Parikshak NN, Becker JT, Lopez OL, Kuller LH, Hua X, Leow AD, Toga AW, Thompson PM (2010) Brain structure and obesity. Hum Brain Mapp 31(3):353–364

Rao RV, Ellerby HM, Bredesen DE (2004) Coupling endoplasmic reticulum stress to the cell death program. Cell Death Differ 11(4):372–380

Rao SM, Reddy JK (2004) PPARα in the pathogenesis of fatty liver disease. Hepatology 40 (4):783–786

Reilly JJ, Armstrong J, Dorosty AR, Emmett PM, Ness A, Rogers I, Steer C, Sherriff A (2005) Early life risk factors for obesity in childhood: cohort study. BMJ 330(7504):1357

Rivera P, Pérez-Martín M, Pavón FJ, Serrano A, Crespillo A, Cifuentes M, López-Ávalos MD, Grondona JM, Vida M, Fernández-Llebrez P, de Fonseca FR (2013) Pharmacological administration of the isoflavone daidzein enhances cell proliferation and reduces high fat diet-induced apoptosis and gliosis in the rat hippocampus. PLoS One 8(5):e64750

Ron D, Walter P (2007) Signal integration in the endoplasmic reticulum unfolded protein response. Nat Rev Mol Cell Biol 8(7):519–529

Rummel-Kluge C, Komossa K, Schwarz S, Hunger H, Schmid F, Lobos CA, Kissling W, Davis JM, Leucht S (2010) Head-to-head comparisons of metabolic side effects of second generation antipsychotics in the treatment of schizophrenia: a systematic review and meta-analysis. Schizophr Res 123(2):225–233

Russell JC, Graham SE, Richardson M (1998) Cardiovascular disease in the JCR: LA-cp rat. Mol Cell Biochem 188(1–2):113–126

Sabia S, Kivimaki M, Shipley MJ, Marmot MG, Singh-Manoux A (2009) Body mass index over the adult life course and cognition in late midlife: the Whitehall II Cohort Study. Am J Clin Nutr 89(2):601–607

Saghizadeh M, Ong JM, Garvey WT, Henry RR, Kern PA (1996) The expression of TNF alpha by human muscle. Relationship to insulin resistance. J Clin Inv 97(4):1111

Sakurai T, He G, Matsuzawa A, Yu GY, Maeda S, Hardiman G, Karin M (2008) Hepatocyte necrosis induced by oxidative stress and IL-1α release mediate carcinogen-induced compensatory proliferation and liver tumorigenesis. Cancer Cell 14(2):156–165

Schillaci G, Pirro M, Gemelli F, Pasqualini L, Vaudo G, Marchesi S, Siepi D, Bagaglia F, Mannarino E (2003) Increased C-reactive protein concentrations in never-treated hypertension: the role of systolic and pulse pressures. J Hypertens 21(10):1841–1846

Shi P, Diez-Freire C, Jun JY, Qi Y, Katovich MJ, Li Q, Sriramula S, Francis J, Sumners C, Raizada MK (2010) Brain microglial cytokines in neurogenic hypertension. Hypertension 56 (2):297–303

Shoelson SE, Herrero L, Naaz A (2007) Obesity, inflammation, and insulin resistance. Gastroenterology 132(6):2169–2180

Shu CJ, Benoist C, Mathis D (2012) The immune system's involvement in obesity-driven type 2 diabetes. In: Seminars in immunology, vol 24, no 6. Academic Press, pp 436–442

Simoneau JA, Colberg SR, Thaete FL, Kelley DE (1995) Skeletal muscle glycolytic and oxidative enzyme capacities are determinants of insulin sensitivity and muscle composition in obese women. FASEB J 9(2):273–278

Slentz CA, Houmard JA, Kraus WE (2009) Exercise, abdominal obesity, skeletal muscle, and metabolic risk: evidence for a dose response. Obesity 17(S3):S27–S33

Sokal J, Messias E, Dickerson FB, Kreyenbuhl J, Brown CH, Goldberg RW, Dixon LB (2004) Comorbidity of medical illnesses among adults with serious mental illness who are receiving community psychiatric services. J Nerv Ment Dis 192(6):421–427

Solinas G, Karin M (2010) JNK1 and IKKβ: molecular links between obesity and metabolic dysfunction. FASEB J 24(8):2596–2611

Sorensen TI, Echwald S, Holm JC (1996) Leptin in obesity. BMJ. Br Med J 313(7063):953

Speakman JR, Rance KA, Johnstone AM (2008) Polymorphisms of the FTO gene are associated with variation in energy intake, but not energy expenditure. Obesity 16(8):1961–1965

Strobel A, Issad T, Camoin L, Ozata M, Strosberg AD (1998) A leptin missense mutation associated with hypogonadism and morbid obesity. Nat genet 18(3):213–215

Stumpf C, John S, Jukic J, Yilmaz A, Raaz D, Schmieder RE, Daniel WG, Garlichs CD (2005) Enhanced levels of platelet P-selectin and circulating cytokines in young patients with mild arterial hypertension. J Hypertens 23(5):995–1000

Stutzmann F, Vatin V, Cauchi S, Morandi A, Jouret B, Landt O, Tounian P, Levy-Marchal C, Buzzetti R, Pinelli L, Balkau B (2007) Non-synonymous polymorphisms in melanocortin-4 receptor protect against obesity: the two facets of a Janus obesity gene. Hum Mol Genet 16 (15):1837–1844

Taki Y, Kinomura S, Sato K, Inoue K, Goto R, Okada K, Uchida S, Kawashima R, Fukuda H (2008) Relationship between body mass index and gray matter volume in 1428 healthy individuals. Obesity 16(1):119–124

Tanaka S, Takehashi M, Matoh N, Iida S, Suzuki T, Futaki S, Hamada H, Masliah E, Sugiura Y, Ueda K (2002) Generation of reactive oxygen species and activation of NF-κB by non-Aβ component of Alzheimer's disease amyloid. J Neurochem 82(2):305–315

Timmermans S, Bogie JF, Vanmierlo T, Lütjohann D, Stinissen P, Hellings N, Hendriks JJ (2014) High fat diet exacerbates neuroinflammation in an animal model of multiple sclerosis by activation of the renin angiotensin system. J Neuroimmune Pharmacol 9(2):209–217

Tomassoni D, Nwankwo IE, Gabrielli MG, Bhatt S, Muhammad AB, Lokhandwala MF, Tayebati SK, Amenta F (2013) Astrogliosis in the brain of obese Zucker rat: a model of metabolic syndrome. Neurosci Lett 543:136–141

Vaisse C, Clement K, Durand E, Hercberg S, Guy-Grand B, Froguel P (2000) Melanocortin-4 receptor mutations are a frequent and heterogeneous cause of morbid obesity. J Clin Inv 106 (2):253

van Dam RM, Willett WC, Rimm EB, Stampfer MJ, Hu FB (2002) Dietary fat and meat intake in relation to risk of type 2 diabetes in men. Diab Care 25(3):417–424

Wang X, Bao W, Liu J, OuYang YY, Wang D, Rong S, Xiao X, Shan ZL, Zhang Y, Yao P, Liu LG (2013) Inflammatory markers and risk of type 2 diabetes. Diabetes care 36(1):166–175

Wang Z, Liu D, Wang F, Liu S, Zhao S, Ling EA, Hao A (2012a) Saturated fatty acids activate microglia via toll-like receptor 4/NF-κB signalling. Br J Nutr 107(02):229–241

Wang Z, Liu D, Wang F, Liu S, Zhao S, Ling EA, Hao A (2012b) Saturated fatty acids activate microglia via toll-like receptor 4/NF-κB signalling. Br J Nutr 107(02):229–241

Ward MA, Carlsson CM, Trivedi MA, Sager MA, Johnson SC (2005) The effect of body mass index on global brain volume in middle-aged adults: a cross sectional study. BMC neurol 5(1):1

Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr (2003) Obesity is associated with macrophage accumulation in adipose tissue. J Clin Inv 112(12):1796

White MF (2003) Insulin signaling in health and disease. Science 302(5651):1710–1711

Williams LM (2012) Hypothalamic dysfunction in obesity. Proc Nutr Soc 71(04):521–533

Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H (2003) Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Inv 112(12):1821

Yamazaki H, Hiramatsu N, Hayakawa K, Tagawa Y, Okamura M, Ogata R, Huang T, Nakajima S, Yao J, Paton AW, Paton JC (2009) Activation of the Akt-NF-κB pathway by subtilase cytotoxin through the ATF6 branch of the unfolded protein response. J Immunol 183(2): 1480–1487

Yau PL, Castro MG, Tagani A, Tsui WH, Convit A (2012) Obesity and metabolic syndrome and functional and structural brain impairments in adolescence. Pediatrics 130(4):e856–e864

Yeo GS, Farooqi IS, Aminian S, Halsall DJ, Stanhope RG, O'Rahilly S (1998) A frameshift mutation in MC4R associated with dominantly inherited human obesity. Nat Genet 20(2): 111–112

Yi CX, Gericke M, Krüger M, Alkemade A, Kabra DG, Hanske S, Filosa J, Pfluger P, Bingham N, Woods SC, Herman J (2012) High calorie diet triggers hypothalamic angiopathy. Mol Metab 1(1):95–100

Yokum S, Ng J, Stice E (2012) Relation of regional gray and white matter volumes to current BMI and future increases in BMI: a prospective MRI study. Int J Obes 36(5):656–664

Zambrano E, Martínez-Samayoa PM, Bautista CJ, Deas M, Guillen L, Rodríguez-González GL, Guzman C, Larrea F, Nathanielsz PW (2005) Sex differences in transgenerational alterations of growth and metabolism in progeny (F2) of female offspring (F1) of rats fed a low protein diet during pregnancy and lactation. J Physiol 566(1):225–236

Zhang X, Dong F, Ren J, Driscoll MJ, Culver B (2005) High dietary fat induces NADPH oxidase-associated oxidative stress and inflammation in rat cerebral cortex. Exp Neurol 191(2): 318–325

Zhang X, Zhang G, Zhang H, Karin M, Bai H, Cai D (2008) Hypothalamic IKKβ/NF-κB and ER stress link overnutrition to energy imbalance and obesity. Cell 135(1):61–73

Zhou R, Yazdi AS, Menu P, Tschopp J (2011) A role for mitochondria in NLRP3 inflammasome activation. Nature 469(7329):221–225

Role of Microglia in Adult Neurogenesis

13

Payel Ghosh and Anirban Ghosh

Abstract

Microglia as resident macrophages are responsible to maintain the normal physiology and homeostasis in the central nervous system and undergo various structural changes to perform immunological functions. Microglial activation are widely implicated in both neuroprotection and neurodegeneration. Apart from its conventional neuromodulatory role, microglia are also associated with brain development, neuronal circuitry formation, neuroendocrine regulation as well as neurogenesis. In this review, we discuss critical role of microglia in regulating adult neurogenesis.

13.1 Introduction

Microglia are designated as a hybrid between glial cells and white blood cells in brain (Streit 2001). They are the monitor of wellbeing of brain environment with a plethora of supportive and protective activities to maintain normal physiology and homeostasis in CNS. From its discovery as the 'third element' of central nervous system by Cajal (1913) and characterization with its functions by del Rio Hortega (1932), these cells mostly established them as the chief immunomodulatory cells in brain (del Rio Hortega 1932; Aloisi 2001; Ghosh et al. 2013). However, from the past decade onwards the territory of microglial functions are widening up showing its diverse functional attributes, apart from or in lieu to its immune functions. They

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ensure active role in CNS development, neuronal circuitry formation, surveillance, neuro-endocrine regulation and psychological behavior, etc., including the process of neurogenesis (Nimmerjhan et al. 2005; Neumann et al. 2009; Paolicelli et al. 2011; Yirmiya et al. 2011). Basically being an immune competent cell, microglia possesses the inherent capacity to take part in inflammatory procedures in brain tissue. More specifically, this is the major determinant of exerting and controlling inflammatory conditions in brain (Aloisi 2001; Ekdahl et al. 2009). As microglia is capable of controlling the level of inflammation, they in turn control the immune effector mechanism there. So life and death of the newly formed neurons largely depends on the microglial activities. It is also found that neurogenic lineage cells are more sensitive to inflammation than gliogenic lineage cells and inflammatory microglia impairs neurogenesis in adult rats (Monje et al. 2003; Ekdahl et al. 2003).

Though neurogenesis is majorly an event which occurs in embryonic and perinatal stages during brain development, recently adult neurogenesis is accepted universally in some restricted pockets or niches of brain tissue (Abrous et al. 2005; Ernst and Frisén 2015). The presence of microglia in those places prompted us to investigate the role of such inflammatory mediators, particularly, in adult neurogenesis. In this article, participation of microglia in the process of adult neurogenesis has been discussed in the light of present knowledge achieved so far. Let us start with the occurrence of neurogenesis in adult, followed by microglial influence and participation in the process, their crosstalk with forming neurons and finally summarizing microglial role as inflammatory mediator in adult neurogenic events.

13.2 Historic Preview of Adult Neurogenesis

Early neuro-anatomists, including Santiago Ramón y Cajal, considered the nervous system fixed and incapable of regeneration. The first evidence of adult mammalian neurogenesis in the cerebral cortex was presented by Altman (1962), followed by a demonstration of adult neurogenesis in the dentate gyrus of the hippocampus in 1963 (Altman 1963). In 1969, Joseph Altman discovered and named the rostral migratory stream (RMS) as the source of adult generated granule cell neurons in the olfactory bulb (Altman 1969). In 1980s, the scientific community ignored these findings despite use of the most direct method of demonstrating cell proliferation in the early studies, i.e., ^{3}H-thymidine autoradiography. By that time, Shirley Bayer (and Michael Kaplan) again showed that adult neurogenesis exists in mammals (rats) (Bayer 1982; Bayer et al. 1982), and Nottebohm showed the same phenomenon in birds (Goldman et al. 1983) sparking renewed interest in the topic. The field did not recover from this until the late 1990s when researchers, including Elizabeth Gould, Fred Gage, and Peter Eriksson, published a series of papers that initiated an explosion of research on the existence, function, and implications of adult mammalian neurogenesis. Studies in the 1990s (Reynolds et al. 1992; Gage et al. 1995) finally put research on adult neurogenesis into a mainstream pursuit. Also in the early 1990s, hippocampal neurogenesis was demonstrated in nonhuman

primates and humans (Eriksson et al. 1998; Gould et al. 1999a, b). In 2008, neurogenesis in the cerebellum of adult rabbits has also been characterized (Ponti et al. 2008). Further, some authors (particularly Elizabeth Gould) have suggested that adult neurogenesis may also occur in regions within the brain not generally associated with neurogenesis including the neocortex (Gould et al. 1999a, b; Zhao et al. 2003; Shankle et al. 1999). However, others have questioned the scientific evidence of these findings, arguing that the new cells may be of glial origin (Rakic 2002). When bromodeoxyuridine (BrdU), the nucleotide analogue was used as lineage tracer to demonstrate neurogenesis, it showed that neurogenesis is an almost life long process in mammals including human (Kuhn et al. 1996; Eriksson et al. 1998). However, these neuronal developments are highly conserved in region specific manner among embryonic, early postnatal and particularly in adult brain.

13.3 Adult Neurogenesis: The Site and Process

Neurogenesis occurs throughout life in the adult mammalian brain including humans (Eriksson et al. 1998; Roy et al. 2000; Wang et al. 2011). In rodents new neurons are continually born throughout adulthood from neural stem/progenitor cells predominantly in two regions of the brain: (1) the subependyma of the lateral ventricles (SVZ) where neural stem cells and progenitor generate new neurons (Neuroblast) that migrate to the olfactory bulb via the rostral migratory stream (RMS) to form olfactory bulb interneurons and (2) subgranular zone (SGZ) of the hippocampal dentate gyrus to form mature granule cells, these two regions referred to as "neurogenic niches" (Gage 2000). Following injury such as stroke, neuroblasts generated in the subventricular zone migrate also into areas which are not normally neurogenic, e.g., striatum and cerebral cortex (Ekdahl et al. 2009). However, recent work has shown these cells migrate to the striatum in humans (Ernst et al. 2014) and not the olfactory bulb (Bergmann et al. 2012). Many of the newborn cells die shortly after they are born (Dayer et al. 2003) but a number of them become functionally integrated into the surrounding brain tissue (Toni et al. 2007, 2008).

During their development from neural stem cells/progenitors to mature functionally integrated neurons various choices are made, such as proliferation or quiescence, cell survival or death, migration or establishment, growth or retraction of processes, synaptic assembly or pruning, or tuning of synaptic transmission. The process is altered by physiological stimuli as well as several brain diseases. The process of adult neurogenesis is a tightly regulated and finely tuned dynamic event which is subjected to be modulated with different physiological and pathopharmacological cues. Mostly neurogenesis in adult brain with normal physiological condition is believed to be rare, but injury may trigger the process (Gould 2007). The process of generating new neurons, i.e., neurogenic process consists of four phases: proliferation, migration, differentiation, and survival after that functionally active new neurons integrated on the existing neural circuitry therefore contributes to various brain functions under both normal and disease state (Ming and Song 2011)

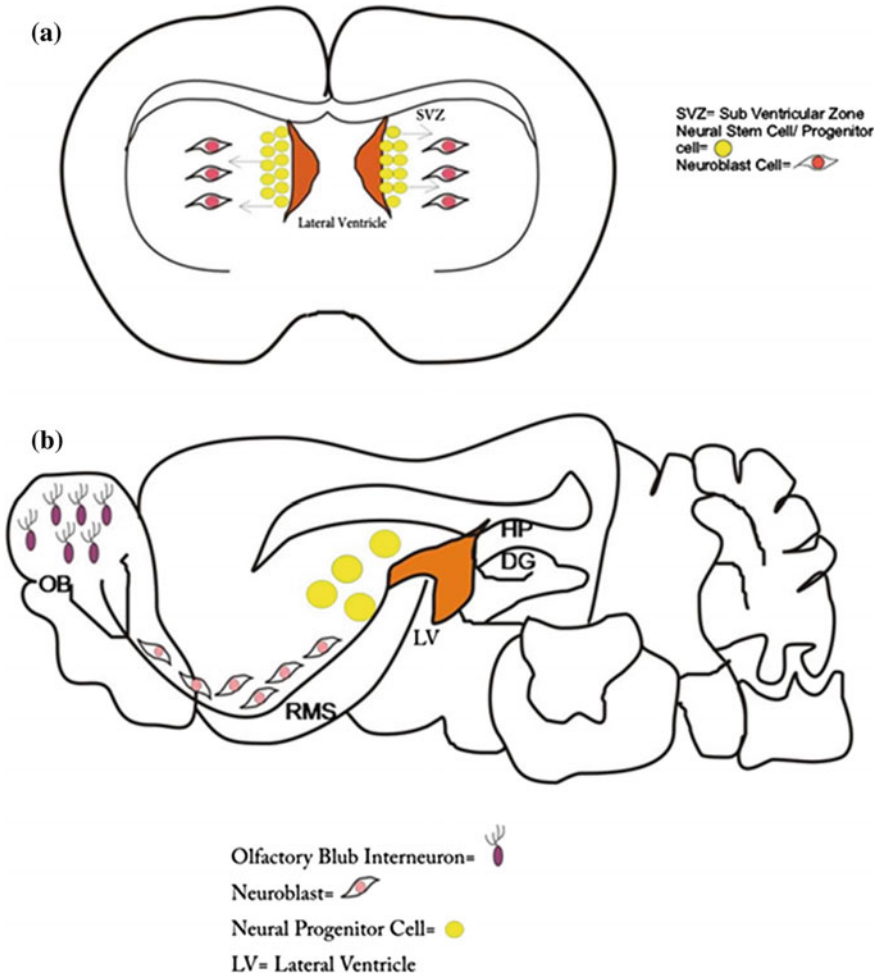

Fig. 13.1 Neurogenesis in "neurogenic" brain regions, i.e., in the adult Dentate Gyrus and Subventricular Zone. **a** A coronal section of rat brain tissue showing the formation of neuroblast cells from neural progenitor cells in SVZ region. **b** A sagittal section view of an adult rodent brain showing the two restricted regions that exhibit active adult neurogenesis: dentate gyrus (DG) in the hippocampal formation (HP); the lateral ventricle (LV) to the rostral migratory stream (RMS) to the olfactory bulb (OB)

(Fig. 13.1). Although a vast number of neurons are formed. Only a very small proportion of them becomes stable and integrated into the existing network and therefore survives for a long period of time throughout the life (Kempermann et al. 2003).

There are only two critical periods for neural progeny survival: (1) during transition from amplifying neuroprogenitors to neuroblast (Sierra et al. 2010; Platel et al. 2010); and (2) during the integration stage of the immature neurons (Tashiro et al. 2006; Mouret et al. 2008). In two months, the surviving neurons receive input

from other neurons (Van et al. 1999; Piatti et al. 2006), with some forming functional synapses, and possessing electrophysiological properties indistinguishable from those of the mature neuron (Ge et al. 2008).

A. ***Dentate gyrus of hippocampus as the site of adult Neurogenesis***:

In case of adult hippocampal neurogenesis, neuroprogenitor cells in the subgranular zone (SGZ) of dentate gyrus give rise to form newborn neuroblast which are incorporated in the circuitry as mature neurons at the end of a 4 week period and the majority of them are presumed to die by apoptosis at the immature neuron stage or in the first 1–4 days of their life, during the transition from amplifying neuroprogenitors to neuroblast. These apoptotic newborn cells are rapidly cleared out from the SGZ neurogenic niche through phagocytosis by unchallenged microglia, i.e., by the resident brain immune cells without producing inflammation (Sierra et al. 2010). After this time point, only very small changes in cell number occur (Fig. 13.2).

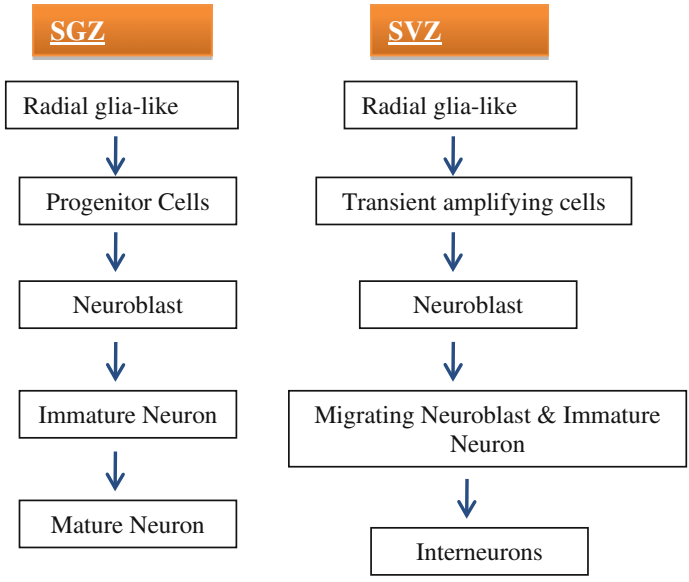

Fig. 13.2 Developmental stages of adult neurogenesis in the both SVZ and SGZ region of brain. Dentate gyrus of the hippocampus. Summary of five developmental stages during adult hippocampal neurogenesis: (1) activation of quiescent radial glia-like cell in both subgranular zone (SGZ) and subventricular zone (SGZ); (2) proliferation of intermediate progenitors in the SGZ region whereas transient amplifying cells generated from radial glia-like cells in SVZ region; (3) generation of neuroblasts; (4) migration and integration of immature neurons; (5) maturation of adult-born dentate granule cells in hippocampal region whereas in SVZ region after migration to specific region immature neurons differentiate into different subtypes of interneurons

From the SGZ, neuronal and glial progenitors with limited self-renewal capacity migrate into the granule cell layer and develop primarily into granule cells, a few becomes interneurons (Liu et al. 2003; Livneh and Mizrahi 2011; Seaberg and Vander Kooy 2002). The continuous integration of adult-born hippocampal neurons is important for synaptic transmission and bidirectional plasticity in the dentate gyrus.

In adult subgranular zone (SGZ), the intermediate progenitors are generated from proliferating radial and non-radial precursor cells and these intermediates give rise to neuroblast. The nascent or immature neurons move into the inner granular cell layer where they differentiate into hippocampus as dentate granular cells. There they extend dendrites to molecular layer and axons to the CA3 region. These new born neurons integrate with existing neuronal circuitry developing their synaptic integrity (Fig. 13.3) (Ming and Song 2011; Ge et al. 2008). Ambient GABA release followed by GABAergic and glutamatergic synaptic inputs initially activate those neurons. Output synapse may be formed on the appropriate target cells in CA3 and hilus. Axons of new born granular neurons in adults establish contacts with hilar interneurons, mossy cells as well as pyramidal cells in CA3 and glutamate is released as neurotransmitter (Toni et al. 2008). These new born neurons are featured by hyper-excitability and synaptic plasticity in comparison with mature granule cells which has been reduced with continued maturity as also reflected in their basic electro-physiological properties when measured (Schmidt-Hieber et al. 2004; Ming and Song 2011).

B. ***Subventricular zone of lateral ventricle and olfactory bulb as site of adult Neurogenesis***:

In adult SVZ, the neuroblast is generated from transient amplifying cells derived from proliferating glial cells. Through astrocytic tube neuroblast cell migrate towards olfactory bulb forming a chain of cells, which is named as rostral migratory stream (RMS) (Lois 1996). After reaching to olfactory bulb, nascent neurons detached from RMS, radially migrate and differentiate. When majority become GABAergic granular neurons lacking axons and forming dendro-dendritic synapses and appearing as tufted cells, others may be dopaminergic or glutamatergic (Lledo et al. 2006; Brill et al. 2009) (Fig. 13.2).

The adult neurogenic niche is majorly composed of mature neurons, different progeny of neuronal precursors along with endothelial cells, ependymal cells, astrocytes and microglia. Associations with vascular endothelial cells are appearing as the important regulator of proliferation of adult neuronal precursors. Adult SVZ astrocyte release glutamate, crucial for neuroblast survival, and also express Robo receptor related to immediate migration of neuroblast through RMS who express Slit1 (Kaneko et al. 2010; Platel et al. 2010). Ependymal cells act like a shield for neuronal progenitor spetecting the neurogenic niche and release Noggin, an antagonist of bone morphogenic protein (BMP) to form a migratory gradient (Lim et al. 2000). They also help to create a concentration gradient of molecules by beating their cilia to direct migration (Sawamoto et al. 2006).

13.4 Microglia: The Non-NSC Derivative in Adult Neurogenesis

Among different glial populations in brain, microglia holds its position unique as they are not derived from gliogenesis from neuro-glial stem or progenitor cells in brain. So these cells are the outsiders who entered, populate and integrated in the developing to matured brain tissue boundary. Presently, microglial myelo-monocytic lineage and blood relation is established (Prinz and Mildner 2011). There are controversies regarding time of entry of and origin/establishment of microglia in brain from precursor myeloid lineage cells. One strong view states that microglial progenitors which form resident microglial populations in brain are different from blood monocyte lineage and these myeloid lineage cells are derived from primitive yolk sac macrophages. They enter very early in embryonic brain development and populate between embryonic days 7–11 in developing brain (Dahlstrand et al. 1995; Chan et al. 2007; Ginhoux et al. 2013). This Myb-negative, Csf1 receptor positive and PU.1 transcription factor dependent cells, which are different from blood, are believed to populate forming brain in early embryo prior to neuronal expansion. Hence they become the prime candidate who contributes to the basic environment in rudimentary brain for future neurogenesis.

Though being a very important candidate in embryonic neurogenesis, its role in adult neurogenesis is also very important for its capability to modulate inflammatory condition and maintenance of the integrity and physiology of brain microenvironment. In general, aging brain shows substantive decrease of neurogenesis and inflammatory changes in microglia (Rao et al. 2006; Walter et al. 2011; Dilger and Johnson 2008). Microglia are found in the sites of neurogenesis, which have different functions in comparison with the non-neurogenic regions (Going et al. 2006). In the initial studies in rodents, brain inflammation and microglia activation were found to be detrimental for the survival of the new hippocampal neurons early after they had been born. The role of inflammation for adult neurogenesis has, however, turned out to be much more complex. Recent experimental evidence indicates that microglia under certain circumstances can be beneficial and support the different steps in neurogenesis, progenitor proliferation, survival, migration, and differentiation. Here, we summarize the current knowledge on the role of microglia in adult neurogenesis in the intact and injured mammalian brain.

13.4.1 Microglial Cells Influence Adult Neurogenesis

Microglia are located within the neurogenic niches and has become interesting candidates for modulating neurogenesis in both the healthy and injured brain. The microenvironment or the niche in which neural progenitor cells live critically influences the process of neurogenesis, which spans several steps including the proliferation of stem or progenitor cells; the survival of immature or mature neurons; the migration of new neuroblasts to their appropriate locations; and the

differentiation of neuroblasts to a neuronal phenotype and the construction of synaptic connectivity (Ekdahl et al. 2009). As an important component of the brain microenvironment and due to their invariant participation in most pathological processes in the CNS, microglia are increasingly implicated as a potential nonneural regulator of neurogenesis.

In the adult brain, resting or surveying microglia, which are characterized by many fine perpendicular processes extending from a few long prolongations, have been regarded as sensor cells for the detection of abnormalities or changes in the brain and help to maintain environmental homeostasis. Once stimulated by means of brain injury or immunological stimuli resting or ramified microglia transform into activated or amoeboid microglia and therefore migrate rapidly to the injury site along the chemokine gradient in vitro and also in response to chemoattractants including ATP&NO released directly or indirectly by the injured neurons. Thus, exert effect on the survival of neurons. Positive or negative effect of microglia on neuronal survival is context-dependent such as type of stimulus, timing of microglial activation and age of animals.

Microglia can release factors that influence adult neurogenesis and glial development (Butovsky et al. 2006). Microglial cells can thus exert dual effects. Inflammation-associated microglia can attenuate neurogenesis, whereas microglia activated by certain T helper cell cytokines promotes neurogenesis. The impact on oligodendrocyte development is of particular interest, as microglial cells migrate along white matter tracts during their postnatal invasion, at a time when oligodendrocytes are differentiating.

Whether microglia support or damage the survival and development of neural progenitor cells also remains controversial. Microglia instructed by T-cell master cytokines IL-4 and low concentrations of IFN-γ support adult oligodendrogenesis as well as neurogenesis and offer neuroprotection, involving complex regulation of insulin-like growth factor (IGF)-I and tumor necrosis factor (TNF)-α. By contrast, treatment with LPS or amyloid-β (Aβ) aggregates, which represent cytotoxic challenges, or with high levels of IFN-γ, do not support cell renewal; they may even impede it. IL-4 or IL-4-activated microglia can reverse this impediment (Hanisch and Kettenmann 2007).

However, just like the dual roles in neuroprotection, whether a specific cytokine-activated microglial cell will take a pro- or anti-neurogenic role is also context-dependent. For example, microglial cells activated by IFN-γ, a proinflammatory cytokine can be neurotoxic or supportive of neurogenesis, depending on the concentration of IFN-γ. TGF-β, which is considered to be beneficial to neurogenesis, can actually exert a negative influence on neurogenesis when it is chronically produced in the aged brain. Additionally, if other cytokines exist in the same niche simultaneously, the outcome will be determined by the balance among the various cytokines microglial dysfunction may also be involved in the down regulation of neurogenesis in the aged or diseased brain. The cellular source of IFN-γ and IL-4 in vivo is likely to be Tcells, therefore it is reasonable to assume that the T cell-mediated immune response is an integral part of the

regulation of microglial phenotype or function, and thus can influence neuronal survival or neurogenesis directly or indirectly (Luo and Chen 2012).

Besides neurogenesis there is some significant role of microglia remains as follows:

1. In the developing brain microglia helps in neuronal differentiation and in the regulation of neuronal apoptosis through the production of neurotrophins.
2. In response to neuronal injury they secrete neurotrophic factors such as IGF1, BDNF, TGFβ, NGF, etc., cytokines and plasminogen. Thus helps in tissue repair and neuronal regeneration.
3. They help in successful axonal regeneration by phagocyting the myelin debris that contains some inhibitory molecules that prevent axon growth. Thereby helps in neuronal survival.
4. During normal brain development they cause "Synaptic Pruning" and therefore help in new connection formation or regeneration.

13.4.2 Active Microglial Participation

During normal development of brain, neurogenesis and neuronal circuit formation is an obvious event which should be regulated with high precision. The resident macrophage of brain, i.e., microglia plays the key role to clear the incorrect connections or projections and apoptotic corps of erroneously placed neuronal bodies. For minimal basal level of such errors, the resident ramified or surveilling microglia in the adult SGZ niche are enough to rapidly phagocytose the apoptotic corps of such neurons (Sierra et al. 2010; Bachstetter and Gemma 2013). Under any abnormal condition or during any pathophysiological impact which can produce inflammatory conditions, microglia become activated and responds immediately transforming into the reactive form mostly transforming towards amoeboid morphology. This can be either beneficial or detrimental depending on the extent or level of activation, duration of activity as well as site of their action in the neurogenic niches. The whole event is highly dependent on the balance between secreted pro- and anti-inflammatory molecular mediators in the microenvironment (Ekdahl et al. 2009). Some studies also hinted that microglial activation and T-cell recruitment are also required for enriched environment induced SGZ neurogenesis (Ziv et al. 2006).

Microglia modulates hippocampal neurogenesis by pruning of newborn cells during the first critical period of survival. Ramified microglia more efficiently clears apoptotic newborn neurons by a special modification of their process forming phagocytic pouches independently from the cell body either in terminal or en passant branches in an immunologically silent process. Efficiency of phagocytosis is measured by the clearance time, the Ph-index and by the phagocytic capacity (Sierra et al. 2010). In response to disease or injury or trauma, i.e., any kind of tissue damage, damage associated molecular patterns (DAMPs) are released which

in turn activated microglia into amoeboid form therefore engulf the apoptotic cell less efficiently than ramified microglia (Bachstetter and Gemma 2013).

Phagocytosis of necrotic and apoptotic cells during brain development, neurodegenerative diseases, and senescence, is essential for the maintenance of tissue homeostasis throughout the lifespan. In the adult brain phagocytosis of apoptotic debris is performed by microglia (Neumann et al. 2009) as it is beneficial because it reduces the secretion of pro-inflammatory cytokines, chemoattractants and the migration of T lymphocytes.

In the basal condition, phagocytosis of apoptotic newborn neurons is performed by ramified unchallenged microglia through terminal or en passant branches forming "ball and chain" structures more efficiently as it was observed that high proportion of apoptotic cells (phagocytic index >90 %, phagocytic capacity >35 %) completely engulfed within 1.2–1.5 h (Sierra et al. 2010) than the phagocytosis performed by amoeboid microglia observed during neurodegeneration (Hanisch and Kettenmann 2007). The rest of the apoptotic cells which are not engulfed by microglia, i.e., <10 % due to delay between the onset of the apoptotic program and the exposure of signals primed the local unchallenged microglia to execute phagocytosis. High phagocytic potential of unchallenged microglia can be even further enhanced during neurodegeneration. Removal of structures (cells, dendrites, or synapses from damaged neurons) that have lost their function by microglia is beneficial for the system as it makes space for new connections and thereby helping the system to regenerate.

13.4.3 Ramified Microglia and Their Effect on Adult Hippocampal Neurogenesis

In intact brain, microglia regulates several steps of adult hippocampal neurogenesis. In the SGZ, progenitor cells migrate to the granule cell layer and differentiate into a neuronal phenotype, with most NPCs dying in the first few days of life. Within two months, the surviving neurons receive input, form functional synapses with their target cells, and exhibit electrophysiological properties indistinguishable from those of mature neurons. In intact brain, ramified microglia eliminates apoptotic newborn cells during the first few days of their life by phagocytosis. This phagocytosis occurs by a special modification of the microglial processes, which form phagocytic pouches that engulf the apoptotic cells. Microglia can also affect proliferation, differentiation, and survival, through the secretion of neurotrophic factors. Finally microglia communicates with nearby neurons through the CX3CR1/CX3CL1 signaling. Interactions between CX3CL1 and CX3CR1 contribute to the ability of microglia to maintain a surveillant/ramified phenotype. Disruption of this signaling results in a change in microglia phenotype and function. This leads to the decreased of hippocampal neurogenesis (Bachstetter and Gemma 2013) (Fig. 13.3).

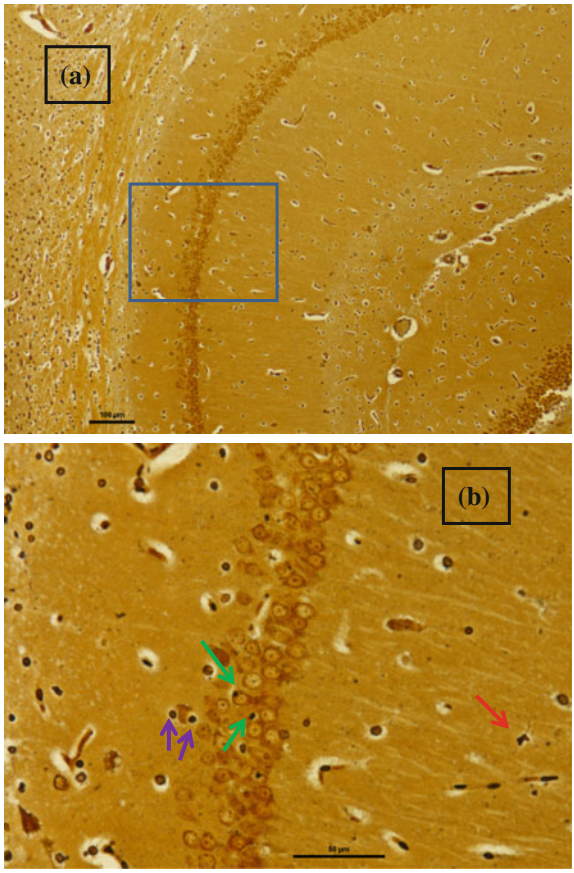

Fig. 13.3 Silver-Gold staining of young adult rat brain tissue of SGZ region of brain. **a** Neuronal cell layer of hippocampus just above the dentate gyrus region showing different cellular distribution within this region, **b** enlarged view of marked region shows the presence of densely stained cell probable microglia found in CA1 region of hippocampus. Neuron-microglia interaction in this region is visible prominently (indicated by *green arrows*). Both amoeboid (indicated by *violet arrows*) and ramified, irregular shaped microglia (indicated by *red arrow*) present in hippocampal region to exhibit their involvement in neurogenesis. Photographed taken by Nikon Eclipse TS 100 microscope with CCD camera (5 megepixel) Nikon DS-Fi2 (Nikon Corporation, Japan)

13.4.4 Microglia Derived Factors in Neurogenesis

Besides phagocytosis microglia also supports neurogenesis by several different mechanism: (1) microglia could have a direct instructive role in dictating the commitment to a neuronal phenotype, (2) microglia could promote proliferation through secretion of neurotrophic factors, and (3) microglia could produce factors that regulate survival of neuronal cells.

In vitro studies demonstrate that microglia have the capacity to guide the differentiation of precursor cells isolated from embryonic brain as well as adult mouse neural precursor cells toward a neuronal phenotype (Aarum et al. 2003). Microglia can affect proliferation and survival, in addition to neuron differentiation. Data showing that addition of microglia-conditioned media in SVZ-derived culture increased neuroblast production (Walton et al. 2006).

Furthermore, loss of inducible Neurogenesis was paralleled by microglia depletion in proliferating culture. While a number of growth factors secreted by microglia could be responsible for such effect, evidence suggests that microglia are capable of producing growth factors, such as Insulin-like growth factor1(IGF-1) and Brain-derived neurotrophic factor (BDNF), which promote neurogenesis (Ziv and Schwartz 2008). Following an enriched environment or physical activity, beneficial microglia increase, and this increase correlates with an increase in hippocampal neurogenesis (Ziv et al. 2006; Choi et al. 2008). However, other studies have shown no correlation or an inverse correlation in the role of microglia in neurogenesis stimulated by environmental enrichment (Gebara et al. 2013).

Microglia is an essential component of the neurogenic niche and therefore controls various steps of neurogenesis such as neuronal proliferation, differentiation, and survival of newborn neurons into the existing neuronal circuitry. However, the specific molecular and cellular mechanisms remains to be explore. A deeper understanding of the physiological function of microglia in the different steps of neurogenesis is needed.

13.5 Crosstalk Between Neuron and Microglia

A number of neuronal signals were found that can regulate microglia activation (Biber et al. 2007), suggesting a neuron-microglia dialogue. Neurons may also deliver signals that keep microglia in their surveillance mode indicating normal function. Under physiological conditions several neuron-mediated signals have an anti-inflammatory action at the level of the microglia. Cluster of differentiation CD200 (also called OX2), CD47, CD55, CX3CL1 (fractalkine), are all neuro-immunoregulatory proteins constitutively expressed in healthy neurons with a cognate receptor on microglia (Kierdorf and Prinz 2013). Microglia can regulate neurogenesis at a number of steps in the neurogenic process. Therefore, a bidirectional regulation of neurons/neurogenesis and microglia might provide a means to fine tune the neurogenic process. Neuronal signal that regulates microglia function is the chemokine fractalkine. Fractalkine is constitutively expressed at high levels on healthy neurons. The receptor for fractalkine, CX3CR1, is more highly expressed on microglia, than macrophages. Data suggest that fractalkine signaling may be involved in neuron-microglia dialogue in the neurogenic niche that regulates neurogenesis. It is shown by genetic deletion or pharmacological antagonism of CX3CR1 in young adult rats that CX3CR1 is important for the maintenance of hippocampal neurogenesis, as animals with decreased CX3CR1 have less

neurogenesis (Bachstetter et al. 2011). Furthermore, levels of fractalkine, which are abundantly expressed in young healthy rodent brains, were decreased in aged rodents (Bachstetter et al. 2011). It was suggested that the decrease in fractalkine signaling may contribute to the increased neuroinflammation and decreased hippocampal neurogenesis seen in the aged rodent brain.

Loss of fractalkine/CX3CR1 signaling in a non-disease model, not only affects neurogenesis, it can cause impairments in motor learning, cognitive function and synaptic plasticity through increased microglia activation and inflammation in the CNS (Rogers et al. 2011). Microglia through the CX3CR1 receptor plays a physiological role in adult hippocampal neurogenesis and cognitive function. During explaining the dual roles of microglia, i.e., either neuroprotective or neurotoxic nature of microglia we mainly stressed to the influence of microglia on neurons. However, many studies indicate that neurons are not merely passive targets of microglia but rather exert control over microglial activities (Biber et al. 2007). There are considerable interactions between neurons and microglia. Microglia is not merely surveyors of brain tissue but also receives and actively responds to signals coming from neurons. Depending on whether they are healthy or injured, neurons send "on" or "off" signals to influence microglial activation. We can observe the close association of microglia with neurons in different places of CNS including hippocampus in adult (Fig. 13.4).

It was hypothesized that activation of microglia as a consequence of neuronal injury is primarily aimed at neuroprotection, with the loss of specific communications between neurons and microglia leading to the neurotoxic behavior of microglia (Polazzi and Contestabile 2002).

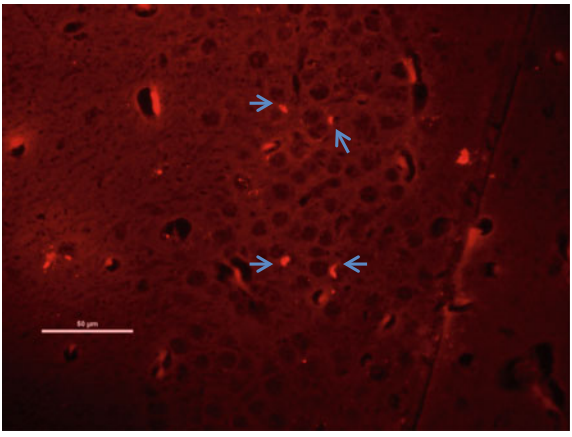

Fig. 13.4 Iba-1 expression showing the presence of microglial cells in SGZ region of adult rat brain tissue. Existences of microglia in SGZ region, both amoeboid and elongated microglia are found in the hippocampus. Photographed taken by Nikon Eclipse TS 100 microscope with CCD camera (5 megepixel) Nikon DS-Fi2 (Nikon Corporation, Japan)

Microglial activation is tightly restricted by signaling coming from neurons:

1. CD200-CD200R has been identified as one of the critical pathways in attenuating microglial activation. CD200 is a member of the immunoglobulin superfamily and is expressed on the neuronal membrane surface, while the CD200 receptor (CD200R) is primarily present in the macrophage lineage, which includes microglia. This interaction helps microglia to maintain their quiescent or surveying state. The disruption of CD200-CD200R interactions results in an accelerated microglial response, whereas intensified CD200-CD200R interactions contribute to attenuation in neurodegeneration (Chitnis et al. 2007).
2. Apart from direct interactions through receptor-ligand combinations, electrical activity and soluble factors such as neurotrophins and anti-inflammatory agents released from intact neurons also maintain microglial quiescence. In a neuron-glia co-culture, the blockade of neuronal electrical activity by tetrodotoxin or a glutamate receptor antagonist facilitated microglial activation induced by IFN-γ (Neumann 2001).
3. Injured neurons induced either neuroprotective or neurotoxic behaviors in microglia depending on the manner of injury.

13.6 Microglia, Neuroinflammation, and Neurogenesis—Exterior to Interior

Overall, microglial activities in the sites of neurogenesis in adult and inflammatory influences vary with spatial, behavioral and obviously in pathological conditions. Several studies demonstrated that proinflammatory condition adversely affect the process of neurogenesis in adult (Ekdahl et al. 2003), Butovsky et al. (2006) demonstrated that selective cytokines in dose dependent manner is capable to continue neurogenesis in adult. In presence of any kind of stress, injury or insult, or bacterial or viral infection induce neuroinflammatory reaction in brain as a result CNS combat these challenges bi-directionally. On one hand, an inflammatory cascade becomes activated to eliminate the pathogen whereas on the other hand a repair process characterized by enhanced Neurogenesis. Depending on the nature of different challenges experienced by the brain, adult neurogenesis is either enhanced or blocked. Uncontrolled immune response impairs neural progenitor cells (NPCs) survival, proliferation and blocks repair processes whereas well-controlled immune response can support NPCs survival and promote recovery (Butovsky et al. 2006). The regenerative process involves the directed migration of NPCs to the site of injury/inflammation, subsequent differentiation and final integration into the neuronal circuits (Abrous et al. 2005). Inflammatory process in the neurogenic regions of the brain greatly alters the microenvironment and thereby influences the fate of these NPCs.

Activation of microglia due to damage or insult helps in production of pro and anti-inflammatory cytokines and chemokines such as IL-1β, TNFα, IL-6, IL-18, MCP-1, SDF-1, various reactive oxygen and nitrogen species (Rock et al. 2004). In presence of LPS or endotoxin insult (uncontrolled local immune response) in adult rat brain causing acute neuroinflammation specified by microglial activation in dentate gyrus region of SGZ showing a sharp decline in hippocampal neurogenesis (Monje et al. 2003) as microglia upon activation release an array of proinflammatory mediators which are anti-neurogenic in nature such as IL-1β, IL-6, IL-18 and TNFα. Thus, microglial activation by endotoxin blocks both neurogenesis and oligodendrogenesis. However, whether the effect of activated microglia on injured or inflamed CNS either positive or negative is determined by the type of activation. Microglia that encounters well-controlled adaptive immunity in the form of CD4+ T cells showing correlation with a protective phenotype. Microglia activated by Th1 and Th2 cytokine mediated adaptive immune response such as IL-4 and IFN-γ neuronal survival. IL-4 induced microglia precisely induce oligodendrogenesis via the insulin-like growth factor (IGF-1) signaling whereas low level of IFN-γ stimulated microglia show bias towards neurogenesis (Butovsky et al. 2005). Proinflammatory mediators released from microglia culminate in neurodegeneration by altering neural stem cell niche. Beside this, MCP-1 and SDF-1 released by activated microglia functions as a positive chemoattractant which promotes the survival and proliferation of NPCs and also directs the migration towards the site of infections or injury via its cognate receptor CCR2 and CXCR4, respectively, expressed on NPCs, thus promoting regenerative homeostasis (Das and Basu 2008).

There are several experimental proofs of microglial response and inflammatory changes with the exteriorly altered or interiorly modified conditions, which ultimately modify the neurogenesis. In enriched environment (EE) condition SGZ neurogenesis is found associated with increased MHCII expression of local microglia and inflammation (Ziv et al. 2006). Different Iba1 expression was found in dentate gyrus (DG), but not in hippocampal CA1 and CA3 regions and suppress inflammation in the region in EE condition (Williamson et al. 2012). Wheel running experiments also show considerable effect to suppress age-related microglia induced inflammation resulting in increase in adult neurogenesis (van Praag et al. 2005). This exercise increases IGF-1, anti-inflammatory cytokine IL-1ra and chemokine CX3CL1 expressing microglia, but reduces MHCII, proinflammatory TNFα expression and T-cells in the sites of DG showing their resting state (Olah et al. 2009; Kohman et al. 2012; Pervaiz and Hoffman-Goetz 2011). Status epilepticus (SE) model shows excitatory signals affect microglial state and neurogenesis by increasing IGF-1 expression to activate microglia (Choi et al. 2008).

The role of microglia in postnatal and adult neurogenesis differs from the general mechanism of action in embryonic neurogenesis. Cunningham and team reported that microglia regulate the pool of neuronal precursor cells (NPC) by phagocytosing Tbr^{2+} and Pax^{6+} cells in late embryos (Cunningham et al. 2013). Mostly embryonic microglia varies from morphological and functional features of postnatal and adult microglia. Pre and early postnatal microglia are mostly amoeboid, active expressing Runt-related transcription factor 1 (Runx1) (Zusso et al. 2012; Ghosh et al. 2015).

They are also showing active Notch1 signaling with Jagged-1 and Delta-1 ligand (Cao et al. 2008). In that state they are active and involved with proinflammatory and phagocytic states. This state of microglia actively supports neurogenesis and oligodendrogliogenesis in perinatal phases (Butovsky et al. 2014). But with gaining adulthood microglia transforms to resting ramified state. TGFβ signaling plays important role in this transition (Erblich et al. 2011). In normal matured brain microglia produce proinflammatory mediators generally in pathogenic conditions. As SVG is a site of lifelong neurogenesis presence of microglia is observed in that region in adult brain. These microglia in postnatal phase initially increases neurogenesis and oligodendrogliogenesis via proinflammatory cytokine mediated fashion where cytokines like IL-1β, IL-6, TNFα, IFNγ, etc., are involved (Shigemoto-Mogami et al. 2014). A progressive reduction of neurogenesis and gaining of resting phenotype of microglia in this region is also observed, which may indicate tight regulation of neurogenesis in adult. The SVZ region is vascularized with plexus of blood vessels. Several soluble factors release from blood vessels which help to accumulate microglia when needed (Ihrie and Alvarez-Buylla 2011). Some signaling like CXCL12/CXCR4 may be involved in such accumulation in the NSC/NPC niche of adult SVZ (Arno et al. 2014).

13.7 Conclusion

Adult neurogenesis seems to recapitulate several processes that occur during neurogenesis and neuronal circuit formation in embryonic development. However, the microenvironmental detailing of niche organization, clonal properties, cellular and molecular regulatory mechanisms and their differences in both the process are still to be deduced clearly. However, presence and active participation of microglia in both embryonic and adult neurogenesis are clearly depicted in the studies so far, and their important regulatory roles in embryonic and adult neurogenesis are beyond any doubt now. Particularly, microglial active participation in inflammatory state in adult brain neurogenesis is becoming the crucial for recovery from brain injury and various neuronal damages. Therefore, from the above discussions it can be stated that various inflammatory mediators from normal to pathological conditions help to shape the process of neurogenesis. Where in neonatal stages microglial proinflammatory morpho-functional conditions and neurogenesis are not in direct conflict, but the situation is not the same in adult. Inflammation and activated microglia, in general, are found detrimental to adult neurogenesis and repair. But plenteous experimental evidences are showing that several inflammatory mediators secreted from microglia or present in microenvironment have positive effect on neurogenesis or neuronal healing in adult. The nature and balance of inflammatory mediators are important to direct and optimize the process (Das and Basu 2008; Ekdahl et al. 2009). Microglia also indicates that it may receive signals from adjacent inner to distant outer environment in and around the site of neurogenesis or sense internal–external cues to figure out the tempo and state of the process of

neurogenesis (Sato 2015). Therefore, microglia in normal and pathogenic brain can act as a very important component contributing inflammatory development and controlling neurogenesis in adult.

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References

Aarum J, Sandberg K, Haeberlein SL, Persson MA (2003) Migration and differentiation of neural precursor cells can be directed by microglia. Proc Natl Acad Sci USA 100(26):15983–15988

Abrous DN, Koehl M, Le Moal M (2005) Adult neurogenesis: from precursors to network and physiology. Physiol Rev 85:523–569

Aloisi F (2001) Immune function of microglia. Glia 36(2):165–179

Altman J (1962) Are new neurons formed in the brains of adult mammals? Science 135 (3509):1127–1128

Altman J (1963) Autoradiographic investigation of cell proliferation in the brains of rats and cats. Anatomical Rec 145(4):573–591

Altman J (1969) Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. J Comp Neurol 137(4):433–457

Arno B, Grassivaro F, Rossi C, Bergamaschi A, Castiglioni V et al (2014) Neural progenitor cells orchestrate microglia migration and positioning into the developing cortex. Nat Commun 5:5611

Bachstetter AD, Gemma C (2013) The role of microglia in adult hippocampal neurogenesis. Front Cell Neurosci 7(229):1–5

Bachstetter AD, Morganti JM, Jernberg J, Schlunk A, Mitchell SH, Brewster KW et al (2011) Fractalkine and CX(3)CR1 regulate hippocampal neurogenesis in adult and aged rats. Neurobiol Aging 32(11):2030–2044

Bayer SA (1982) Changes in the total number of dentate granule cells in juvenile and adult rats: a correlated volumetric and ^{3}H-thymidine autoradiographic study. Exp Brain Res 46(3):315–323

Bayer SA, Yackel JW, Puri PS (1982) Neurons in the rat dentate gyrus granular layer substantially increase during juvenile and adult life. Science 216(4548):890–892

Bergmann O, Liebel J, Bernard S, Alkass K, Yeung MSY, Steier P, Kutschera W, Johnson L, Landen M, Druid H, Spalding KL, Frisen J (2012) The age of olfactory bulb neurons in humans. Neuron 74:634–639

Biber K, Neumann H, Inoue K, Boddeke HW (2007) Neuronal 'On' and 'Off' signals control microglia. Trends Neurosci 30:596–602

Brill MS, Ninkovic J, Winpenny E, Hodge RD, Ozen I, Yang R, Lepier A, Gascon S, Erdelyi F, Szabo G et al (2009) Adult generation of glutamatergic olfactory bulb interneurons. Nat Neurosci 12:1524–1533

Butovsky O, Talpalar AE, Ben-Yaakov K, Schwartz M (2005) Activation of microglia by aggregated beta-amyloid or lipopolysaccharide impairs MHC-II expression and renders them cytotoxic whereas IFN-gamma and IL-4 render them protective. Mol Cell Neurosci 29(3): 381–393

Butovsky O et al (2006) Microglia activated by IL-4 or IFN-γ differentially induce neurogenesis and oligodendrogenesis from adult stem/progenitor cells. Mol Cell Neurosci 31:149–160

Butovsky O, Jedrychowski MP, Moore CS, Cialic R, Lanser AJ, Gabriely G, Koeglsperger T, Dake B, Wu PM, Doykan CE et al (2014) Identification of a unique TGF-beta-dependent molecular and functional signature in microglia. Nat Neurosci 17:131–143

Cao Q, Lu J, Kaur C, Sivakumar V, Li F, Cheah PS, Dheen ST, Ling EA (2008) Expression of Notch-1 receptor and its ligands Jagged-1 and Delta-1 in amoeboid microglia in postnatal rat brain and murine BV-2 cells. Glia 56:1224–1237

Chan WY, Kohsaka S, Rezaie P (2007) The origin and cell lineage of microglia: new concepts. Brain Res Rev 53(2):344–354

Chitnis T, Imitola J, Wang Y, Elyaman W, Chawla P, Sharuk M, Raddassi K, Bronson RT, Khoury SJ (2007) Elevated neuronal expression of CD200 protects Wlds mice from inflammation-mediated neurodegeneration. Am J Pathol 170(5):1695–1712

Choi SH, Veeraraghavalu K, Lazarov O, Marler S, Ransohoff RM, Ramirez JM et al (2008) Non-cell-autonomous effects of presenilin 1 variants on enrichment-mediated hippocampal progenitor cell proliferation and differentiation. Neuron 59(4):568–580

Cunningham CL, Martinez-Cerdeno V, Noctor SC (2013) Microglia regulate the number of neural precursor cells in the developing cerebral cortex. J Neurosci 33(10):4216–4233

Dahlstrand J, Lardelli M, Lendahl U (1995) Nestin mRNA expression correlates with the central nervous system progenitor cell state in many, but not all, regions of developing central nervous system. Brain Res Dev Brain Res 84(1):109–129

Das S, Basu A (2008) Inflammation: a new candidate in modulating adult neurogenesis. J Neurosci Res 86(6):1199–1208

del Rio-Hortega P (1932) Microglia. In: Penfield W (ed) Cytology and cellular pathology of the nervous system, Hoeber, New York, pp 481–584

Dayer AG, Ford AA, Cleaver KM, Yassaee M, Cameron HA (2003) Short-term and long-term survival of new neurons in the rat dentate gyrus. J Comp Neurol 460(4):563–572

Dilger RN, Johnson RW (2008) Aging, microglial cell priming, and the discordant central inflammatory response to signals from the peripheral immune system. J Leukoc Biol 84 (4):932–939

Ekdahl CT, Claasen JH, Bonde S, Kokaia Z, Lindvall O (2003) Inflammation is detrimental for neurogenesis in adult brain. Proc Natl Acad Sci 100(23):13632–13637

Ekdahl CT, Kokaia Z, Lindvall O (2009) Brain inflammation and adult neurogenesis: the dual role of microglia. Neuroscience 158(3):1021–1029

Erblich B, Zhu L, Etgen AM, Dobrenis K, Pollard JW (2011) Absence of colony stimulation factor-1 receptor results in loss of microglia, disrupted brain development and olfactory deficits. PLoS ONE 6(10):e26317

Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage FH (1998) Neurogenesis in the adult human hippocampus. Nat Med 4:1313–1317

Ernst A, Frisén J (2015) Adult neurogenesis in humans-common and unique traits in mammals. PLoS Biol 13(1):e1002045. doi:10.1371/journal.pbio.1002045

Ernst A, Alkass K, Bernard S, Salehpour M, Perl S, Tisdale J, Possnert G, Druid H, Fisen J (2014) Neurogenesis in the striatum of the adult human brain. Cell 156(5):1072–1083

Gage FH (2000) Mammalian neural stem cells. Science 287(5457):1433–1438

Gage FH, Ray J, Fisher LJ (1995) Isolation, characterization, and use of stem cells from the CNS. Annu Rev Neurosci 18:159–192

Ge S, Sailor KA, Ming GL, Song H (2008) Synaptic integration and plasticity of new neurons in the adult hippocampus. J Physiol 586:3759–3765

Gebara E, Sultan S, Kocher-Braissant J, Toni N (2013) Adult hippocampal neurogenesis inversely correlates with microglia in conditions of voluntary running and aging. Front Neurosci 7:145

Ghosh A, Streit WJ, Minghetti L, Basu A (2013) Microglia in development and disease. Clin Dev Immunol, Article ID-736459, 2 p

Ghosh P, Mukherjee N, Ghosh K, Mallick S, Pal C, Laskar A, Ghosh A (2015) Prospective microglia and brain macrophage distribution pattern in normal rat brain shows age sensitive dispersal and stabilization with development. Indian J Exp Biol 53(9):561–567

Ginhoux F, Hoeffel G, Low D, Huber T (2013) Origin and differentiation of microglia. Front Cell Neurosci 7(45):1–14
Goings GE, Kozlowski DA, Szele FG (2006) Differential activation of microglia in neurogenic versus non-neurogenic regions of the forebrain. Glia 54(4):329–342
Goldman SA, Nottebohm F (1983) Neuronal production, migration, and differentiation in a vocal control nucleus of the adult female canary brain. Proc Natl Acad Sci USA 80(8):2390–2394
Gould E (2007) How widespread is adult neurogenesis in mammals? Nat Rev Neurosci 8:481–488
Gould E, Reeves AJ, Graziano MS, Gross CG (1999a) Neurogenesis in the neocortex of adult primates. Science 286(5439):548–552
Gould E, Reeves AJ, Fallah M, Tanapat P, Gross CG, Fuchs E (1999b) Hippocampal neurogenesis in adult Old World primates. Proc Natl Acad Sci USA 96(9):5263–5267
Hanisch UK, Kettenmann H (2007) Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat Neurosci 10(11):1387–1394
Ihrie RA, Alvarez-Buylla A (2011) Lake-front property: a unique germinal niche by the lateral ventricles of the adult brain. Neuron 70(4):674–686
Kaneko N, Marin O, Koike M, Hirota Y, Uchiyama Y et al (2010) New neurons clear the path of astrocytic processes for their rapid migration in the adult brain. Neuron 67(2):213–223
Kempermann G, Gast D, Kronenberg G, Yamaguchi M, Gage FH (2003) Early determination and long-term persistence of adult-generated new neurons in the hippocampus of mice. Development 130(2):391–399
Kierdorf K, Prinz M (2013) Factors regulating microglia activation. Front Cell Neurosci 7:44
Kohman RA, DeYoung EK, Bhattacharya TK, Lindsey N, Peterson LN, Rhodes JS (2012) Wheel running attenuates microglia proliferation and increases expression of a proneurogenic phenotype in the hippocampus of aged mice. Brain Behav Immun 26(5):803–810
Kuhn HG, Dickinson-Anson H, Gage FH (1996) Neurogenesis in the dentate gyrus of the adult rat: age related decrease of neuronal progenitor proliferation. J Neurosci 16(6):2027–2033
Lim DA, Tramontin AD, Trevejo JM, Herrera DG, Garcia-Verdugo JM, Alvarez-Buylla A (2000) Noggin antagonizes BMP signaling to create a niche for adult neurogenesis. Neuron 28 (3):713–726
Liu S, Wang J, Zhu D, Fu Y, Lukowiak K, Lu YM (2003) Generation of functional inhibitory neurons in the adult rat hippocampus. J Neurosci 23(3):732–736
Livneh Y, Mizrahi A (2011) Long-term changes in the morphology and synaptic distributions of adult-born neurons. J Comp Neurol 519(11):2212–2224
Lledo PM, Alonso M, Grubb MS (2006) Adult neurogenesis and functional plasticity in neuronal circuits. Nat Rev Neurosci 7(3):179–193
Lois C, Garcia-Verdugo JM, Alvarez-Buylla A (1996) Chain migration of neuronal precursors. Science 271(5251):978–981
Luo XG, Chen SD (2012) The changing phenotype of microglia from homeostasis to disease. Transl Neurodegener 1:9
Ming GL, Song H (2011) Adult Neurogenesis in the mammalian brain: significant answers and significant questions. Neuron 70(4):687–702
Monje ML, Toda H, Palmer TD (2003) Inflammatory blockade restores adult hippocampal neurogenesis. Science 302(5651):1760–1765
Mouret A, Gheusi G, Gabellec MM, de Chaumont F, Olivo-Marin JC, Lledo PM (2008) Learning and survival of newly generated neurons: when time matters. J Neurosci 28(45):11511–11516
Neumann H (2001) Control of glial immune function by neurons. Glia 36(2):191–199
Neumann H, Kotter MR, Franklin RJ (2009) Debris clearance by microglia: an essential link between degeneration and regeneration. Brain 132(Pt 2):288–295
Nimmerjahn A, Kirchhoff F, Helmchen F (2005) Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308(5726):1314–1318
Olah M, Ping G, De Haas AH, Brouwer N, Meerlo P, Van Der Zee EA, Biber K, Boddeke HW (2009) Enhanced hippocampal neurogenesis in the absence of microglia T cell interaction and microglia activation in the murine running wheel model. Glia 57(10):1046–1061

Paolicelli RC, Bolasco G, Pagani F, Maggi L, Scianni M, Panzanelli P, Giustetto M, Ferreira TA, Guiducci E, Dumas L, Ragozzino D, Gross CT (2011) Synaptic pruning by microglia is necessary for normal brain development. Science 333(6048):1456–1458

Pervaiz N, Hoffman-Goetz L (2011) Freewheel training alters mouse hippocampal cytokines. Int J Sports Med 32(11):889–895

Piatti VC, Esposito MS, Schinder AF (2006) The timing of neuronal development in adult hippocampal neurogenesis. Neuroscientist 12(6):463–468

Platel JC, Dave KA, Gordon V, Lacar B, Rubio ME, Bordey A (2010) NMDA receptors activated by subventricular zone astrocytic glutamate are critical for neuroblast survival prior to entering a synaptic network. Neuron 65(6):859–872

Polazzi E, Contestabile A (2002) Reciprocal interactions between microglia and neurons: from survival to neuropathology. Rev Neurosci 13(3):221–242

Ponti G, Peretto B, Bonfanti L, Reh Thomas A (2008) Genesis of neuronal and glial progenitors in the cerebellar cortex of peripuberal and adult rabbits. PLoS ONE 3(6):e2366

Prinz M, Mildner A (2011) Microglia in the CNS: immigrants from another world. Glia 59 (2):177–187

Rakic P (2002) Adult neurogenesis in mammals: an identity crisis. J Neurosci 22(3):614–618

Rao MS, Hattiangady B, Shetty AK (2006) The window and mechanisms of major age-related decline in the production of new neurons within the dentate gyrus of the hippocampus. Aging Cell 5(6):545–558

Reynolds BA, Weiss S (1992) Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science 255(5052):1707–1710

Rock RB, Gekker G, Hu S, Sheng WS, Cheeran M, Lokensgard JR, Peterson PK (2004) Role of microglia in central nervous system infections. Clin Microbiol Rev 17(4):942–964

Rogers JT, Morganti JM, Bachstetter AD, Hudson CE, Peters MM, Grimmig BA et al (2011) CX3CR1 deficiency leads to impairment of hippocampal cognitive function and synaptic plasticity. J Neurosci 31(45):16241–16250

Roy NS, Wang S, Jiang L, Kang J, Benraiss A, Harrison-Restelli C et al (2000) In vitro neurogenesis by progenitor cells isolated from the adult human hippocampus. Nat Med 6 (3):271–277

Sato K (2015) Effects of microglia on neurogenesis. Glia 63(8):1394–1405

Sawamoto K, Wichterle H, Gonzalez-Perez O, Cholfin JA, Yamada M, Spassky N, Murcia NS, Garcia-Verdugo JM, Marin O, Rubenstein JL et al (2006) New neurons follow the flow of cerebrospinal fluid in the adult brain. Science 311(5761):629–632

Schmidt-Hieber C, Jonas P, Bischofberger J (2004) Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus. Nature 429:184–187

Seaberg RM, Vander Kooy D (2002) Adult rodent neurogenic regions: the ventricular subependyma contains neural stem cells, but the dentate gyrus contains restricted progenitors. J Neurosci 22(5):1784–1793

Shankle WR, Rafii MS, Landing BH, Fallon JH (1999) Approximate doubling of numbers of neurons in postnatal human cerebral cortex and in 35 specific cytoarchitectural areas from birth to 72 months. Pediatr Dev Pathol 2(3):244–259

Shigemoto-Mogami Y, Hoshikawa K, Goldman JE, Sekino Y, Sato K (2014) Microglia enhance neurogenesis and oligodendrogenesis in the early postnatal subventricular zone. J Neurosci 34 (6):2231–2243

Sierra A, Encinas JM, Deudero JJ, Chancey JH, Enikolopov G, Overstreet Wadiche LS et al (2010) Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. Cell Stem Cell 7(4):483–495

Streit WJ (2001) Microglia and macrophages in the developing CNS. Neurotoxicology 22(5):619–624

Tashiro A, Sandler VM, Toni N, Zhao C, Gage FH (2006) NMDA receptor mediated, cell-specific integration of new neurons in adult dentate gyrus. Nature 442:929–933

Toni N, Teng EM, Bushong EA, Aimone JB, Zhao C, Consiglio A, van Praag H, Martone ME, Ellisman MH, Gage FH (2007) Synapse formation on neurons born in the adult hippocampus. Nat Neurosci 10(6):727–734

Toni N, Laplagne DA, Zhao C, Lombardi G, Ribak CE, Gage FH, Schinder AF (2008) Neurons born in the adult dentate gyrus form functional synapses with target cells. Nat Neurosci 11 (8):901–907

Van Praag PH, Christie BR, Sejnowski TJ, Gage FH (1999) Running enhances neurogenesis, learning, and long-term potentiation in mice. Proc Natl Acad Sci USA 96(23):13427–13431

Van Praag H, Shubert T, Zhao C, Gage FH (2005) Exercise enhances learning and hippocampal neurogenesis in aged mice. J Neurosci 25(38):8680–8685

Walter J, Keiner S, Witte OW, Redecker C (2011) Age-related effects on hippocampal precursor cell subpopulations and neurogenesis. Neurobiol Aging 32(10):1906–1914

Walton NM, Sutter BM, Laywell ED, Levkoff LH, Kearns SM, Marshall GP et al (2006) Microglia instruct subventricular zone neurogenesis. Glia 54(8):815–825

Wang C, Liu F, Liu YY, Zhao CH, You Y, Wang L et al (2011) Identification and characterization of neuroblasts in the subventricular zone and rostral migratory stream of the adult human brain. Cell Res 21(11):1534–1550

Williamson LL, Chao A, Bilbo SD (2012) Environmental enrichment alters glial antigen expression and neuroimmune function in the adult rat hippocampus. Brain Behav Immun 26 (3):500–510

y Cajal SR (1913) Contribución al conocimiento de la neuroglía del cerebro humano. Trab Lab Invest Biol 11:255–315

Yirmiya R, Goshen I (2011) Immune modulation of learning, memory, neural plasticity and neurogenesis. Brain Behav Immun 25(2):181–213

Zhao M, Momma S, Delfani K, Carlen M, Cassidy RM, Johansson CB, Brismar H, Shupliakov O, Frisen J, Janson AM (2003) Evidence for neurogenesis in the adult mammalian substantia nigra. Proc Natl Acad Sci USA 100(13):7925–7930

Ziv Y, Schwartz M (2008) Immune-based regulation of adult neurogenesis: implications for learning and memory. Brain Behav Immun 22(2):167–176

Ziv Y, Ron N, Butovsky O, Landa G, Sudai E, Greenberg N, Cohen H, Kipnis J, Schwartz M (2006) Immune cells contribute to the maintenance of neurogenesis and spatial learning abilities in adulthood. Nat Neurosci 9:268–275

Zusso M, Methot L, Lo R, Greenhalgh AD, David S, Stifani S (2012) Regulation of postnatal forebrain amoeboid microglial cell proliferation and development by the transcription factor runx1. J Neurosci 32(33):11285–11298