Role of Microglia in Autism: Recent Advances

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Key Words

Autism · Microglia · Autism spectrum disorder

Abstract

The neurobiological basis for autism remains poorly understood. However, the neuroinflammation processes play an important role in the induction of autistic behavioral changes. Microglial cells can exhibit widely differing functions during brain development, including synaptogenesis and stem cell proliferation, in addition to playing a role in the innate immunity. Mounting evidence indicates that microglial activation or dysfunction can profoundly affect neural development, resulting in neurodevelopmental disorders, including autism. These mechanisms in autism have been investigated using neuropathological studies of human autopsy brains, a large number of murine experimental models and in vivo neuroimaging studies of the human brain. The purpose of this review is to discuss microglial activation or dysfunction and to highlight the detrimental role that microglia play in the development of autism. The recent advances presented in this review support that further elucidation of the mechanisms and kinetics of microglial responses will help to establish a window for therapeutic intervention in individuals with autism.

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Introduction

Autism is a set of heterogeneous neurodevelopmental conditions characterized by early-onset difficulties in social communication and unusually restricted, repetitive behavior and interests. The worldwide population prevalence is approximately 1%, and 2–3 times more males are affected than females. Genetics play a key role in the etiology of autism, in conjunction with developmentally early environmental factors [1]. The neurobiological basis for autism remains poorly understood. However, numerous investigations have suggested that immune abnormalities are one of the most important contributing factors in the development of autism [2, 3]. Serum antibodies against central nervous system (CNS) antigens and maternal antibodies to fetal brain proteins have been associated with autism [4–6]. Maternal IgG reactive to fetal brain proteins has been experimentally shown to contribute toward autism development via the induction of behavioral alterations in offspring mice or monkeys. These offspring had been prenatally exposed to serum IgG obtained from the mothers of autistic children [7, 8]. Therefore, neuroinflammation processes in the brain may play an important role in the induction of autistic behavioral changes [9, 10].

Deficits in synaptic maturation, which are characterized by weak functional connectivity across brain regions, may play a role in the pathophysiology of neurodevelopmental disorders, including autism [11]. Microglia are the representative mononuclear phagocytes in the CNS and ultimately have a myeloid origin [12]. The concept of repopulating brain microglia from bone marrow-derived cells in adult mice under normal physiological conditions is controversial [13]. However, there is a major wave of migration of the primitive myeloid progenitors into the CNS to become resident microglia [14], and these microglial cells are suggested to contribute to brain development, including synaptic maturation. This review critically summarizes the recent advances that support the important role that microglia play in regulating the development of autism.

Functions of Microglia

Microglia can exhibit widely differing functions at different stages in life, both physiologically and during various pathological situations [15]. The functions of the microglia during CNS development include the following: (1) phagocytic activity during neuronal/synaptic development (probably reflected in the pruning of redundant neurons and connections), (2) neuronal development influenced by the secretion of cytokines, neurotrophins and growth factors, (3) the removal of cell debris facilitating plasticity and synaptogenesis and (4) the regulation of stem cell proliferation [16]. Microglia colonize the neuronal proliferative zones in the developing neocortex and phagocytose neuronal precursor cells during the late stages of cortical neurogenesis [17]. Augmenting the in utero activation of fetal microglia through maternal immune activation decreases the number of neural precursor cells, while the in utero deactivation or elimination of fetal microglia increases them. Conversely, the enhancement of neurogenesis and oligodendrogenesis by activated microglia is demonstrated in the early postnatal subventricular zone [18]. These results suggest that any factors that alter the number or activation state of microglia either in utero or during the early postnatal period can profoundly affect neural development, thus resulting in neurodevelopmental disorders, including autism.

Similar to macrophages, microglia adopt different activation phenotypes in response to CNS insults. The activation of microglia results in a range of responses that include morphological alterations, migration to the site of injury and proliferation (microgliosis), as well as an increased expression of several factors, including im-

mune mediators. Moreover, microglial cells may transform into highly phagocytic cells, thereby removing dead cells, accumulated debris, protein aggregates, and bacterial and viral pathogens [19]. The definitions of such types of microglial activation are initially based on the peripheral monocytes/macrophages characterized in in vitro experiments and are not entirely identical to those for macrophages. However, two activation phenotypes have been investigated: the classical proinflammatory and neurotoxic phenotype M1 and the alternate anti-inflammatory phenotype M2 [20] (fig. 1).

Exposure of microglial cell cultures to stimuli such as bacterial lipopolysaccharides (LPS) [21, 22], TNF- α [23], IFN- γ [24], necrotic neurons [25], oligomers of A β [26], and α -synuclein [27, 28] induce the M1 phenotype. The classical M1 phenotype is characterized by the activation of mitogen-activated protein kinase (ERK1/2 and p38) [22], the expression of MHC-II (major histocompatibility complex type II) cell surface glycoprotein, the secretion of proinflammatory cytokines (TNF- α , IL-1 β , IL-6 and IL-12), and the production of reactive oxygen species (ROS). In addition, the upregulation of inducible nitric oxide (NO) synthase (iNOS or NOS2), glutaminase and inducible COX-2 (cyclooxygenase-2) leads to an increase of NO, glutamate and prostaglandins, respectively. Most of these factors released by microglia are neurotoxic for neuronal cell cultures [29]. The alternative M2 phenotype is neuroprotective and can be induced in primary microglial cells by the cytokines IL-4 and IL-13, which are secreted in vivo by Th2 lymphocytes [30]. The M2 phenotype is characterized by the expression of heparin-binding lectin Ym1, cysteine-rich protein FIZZ1 and arginase 1 by activated microglia [30]. In vitro, IL-4 is found to decrease iNOS activity, superoxide and TNF- α production in LPS- and TNF- α -activated microglia, along with the rescue of neurons from neurotoxicity [31]. IL-4 also increases the phagocytic activity of microglia, namely the uptake of oligomeric A β species through the scavenger receptor CD36 [32]. Additionally, in cell cultures, IL-13 and IL-10, which are anti-inflammatory cytokines produced by macrophages, increase the microglial secretion of activin A, a neuroprotective TGF- β superfamily member that also promotes oligodendrocyte differentiation [33] (fig. 1).

Microglial Activation in Autism

Microglial Activation in the Human Brain

Autism involves early brain overgrowth and dysfunction, which is most strongly evident in the prefrontal cor-

Fig. 1. Microglial activation phenotypes. M1 is the classical proinflammatory/neurotoxic phenotype, and M2 is the alternate anti-inflammatory/neuroprotective phenotype. MAPK = Mitogen-activated protein kinase; ↑ = increase or upregulation; ↓ = decrease or downregulation.

tex. An excess amount of neurons in the prefrontal cortex signals a disturbance in prenatal development and may be associated with an abnormal cell type and laminar development [34]. In postmortem studies of autistic brains, lower numbers of neurons have been reported in the amygdala, the fusiform gyrus of the temporal lobe and the cerebellum [35]. One current line of research emphasizes alterations in the basic columnar organization of the neocortex [36]. Several investigations have indicated microglial activation in human autistic brains. Neuropathological studies of autopsy brains with autism demonstrated the presence of active neuroinflammatory processes in the cerebral cortex, white matter and, most notably, the cerebellum. Immunocytochemical studies have shown a marked activation of microglia and astroglia, and cytokine profiling has indicated that macrophage chemoattractant protein-1 and tumor growth factor-β1, which are derived from neuroglia, were the most prevalent cytokines in the brain tissues [37]. An immunohistochemical study in autopsy brains with autism and matched controls showed significant increased densities of microglia in two functionally and anatomically disparate cortical areas, namely the frontoinsular and visual cortices, suggesting the dense distribution of microglia throughout the cerebral cortex in brains with autism [38]. Microglial and neuronal organization was examined in the dorsolat-

eral prefrontal cortex, which is a region of pronounced early brain overgrowth during the development of autism, of 13 male postmortem autism subjects and 9 controls. The autism brains exhibited increased short-distance microglia-neuron interaction, including the encirclement of neurons by microglial processes. In the autism brains, neuron-neuron clustering increased with advancing age. However, microglia-microglia organization was normal at all ages, suggesting that the aberrantly close microglia-neuron association in autism is not a result of altered microglial distribution but a neuron-specific reaction [39]. In some children with autism, the amygdala has an aberrant growth trajectory marked by an early enlargement followed by a normal or even reduced volume by adulthood [40]. In the adult postmortem examination of the amygdala from individuals with autism, there was evident heterogeneity within the autism cohort; however, 2 of the 8 autism brains displayed strong microglial activation [41]. There were fewer oligodendrocytes in the amygdala of older adult individuals with autism, suggesting increasing cognitive difficulties in the later stages of life in autism patients. In addition, a human transcriptome analysis in the control and autistic cortical brains revealed a strong, negative correlation between two differentially co-expressed modules, the activated M2-state microglia genes and the synaptic transmission genes [42]. The M2-

Table 1. Experimental models of autism due to maternal inflammatory activation involving offspring microglial alteration

Methods	Mechanism of inflammatory activation	Pathological findings	Behaviors relevant to autism	Refs.
Poly I:C	Activation of NF- κ B; production of TNF- α , IL-6 and IL-12	Decreased expression of reelin; increased expression of GFAP; increased number of activated microglia	Impairments in nonspatial memory and learning tasks; motor activity similar to autistic behaviors	52
Influenza A virus (H3N2)	Genetic alterations in maternal Th1 responses	Changes in catecholaminergic neurons; microglial density in males	Alterations in social and aggressive behaviors; increased locomotor behaviors, particularly in males	53
LPS	Enhancement of the NOX-PI3K pathway	Hyperproliferation of neural stem and progenitor cells; forebrain microglial proliferation	Abnormal autism-associated behaviors	55

GFAP = Glial fibrillary acidic protein; Refs. = references.

activation state microglia genes were altered in the autistic brains, potentially driven by type I IFN responses. This process may drive changes in neuronal progenitor cell proliferation and connectivity, with subsequently altered activity-dependent neural expression profiles during postnatal development [42]. These results highlight the interplay between the innate immunity and neuronal activity in the etiology of autism.

In vivo investigations have also been reported. The peripheral benzodiazepine receptor, known as the 18-kDa translocator protein (TSPO), is a cholesterol transporter protein expressed in the membrane of mitochondria of cells throughout the body [43]. A positron emission tomography analysis of TSPO using the radiocarbon [^{11}C](R)-PK11195 enables the visualization of the activated microglia in vivo in the whole brain [44]. This procedure demonstrated the [^{11}C](R)-PK11195 binding potential values to be significantly higher in multiple brain regions in young adults with autism compared to those of controls, suggesting excessive microglial activation in multiple brain regions in autism patients [45]. Historically, the increase in TSPO expression was attributed to the activation of the microglia within the CNS [46]. However, there is now growing evidence that reactive astrocytes also show an increase in TSPO binding after brain insult [47]. A new TSPO ligand, ^{18}F -GE-180, is able to reveal sites of activated microglia in both gray and white matter; however, the signal increases with the presence of activated astrocytes [48]. These investigations suggest that glial reaction may also be involved in the development of autism in concert with the microglial activation in the brain.

Maternal Inflammatory Activation

In addition to the strong evidence for the genetic transmission of autism, the maternal inflammatory response is one of the most contributing environmental risk factors in the etiology of autism [49]. Potential pathological effects have been investigated by using a wide variety of experimental models (table 1).

Polyriboinosinic-polyribocytidilic acid (poly I:C), a synthetic double-stranded RNA shown to bind to Toll-like receptor 3, leads to the activation of NF- κ B (nuclear factor κ -light-chain-enhancer of activated B cells) and the production of proinflammatory cytokines such as TNF- α , IL-6 and IL-12 [50]. Poly I:C is often referred to as a viral mimetic as it activates the immune system and produces dose-dependent cytokine responses comparable to those occurring during naturally occurring or opportunistic viral infections [51]. In spiny mouse experiments, a single subcutaneous injection of a low dose of poly I:C at mid-gestation induces subclinical infections such as the common cold during pregnancies. However, the offspring showed significant impairments in nonspatial memory and learning tasks and demonstrated motor activity similar to autistic behaviors. A brain histological examination revealed a significantly decreased expression of reelin, an increased expression of glial fibrillary acidic protein and an increased number of activated microglia, specifically in the hippocampus [52]. These investigations imply that the prenatal subclinical infection and resultant activation of the maternal immune system could be risk factors for neurodevelopmental disorders such as autism.

Pregnant mice with a BALB/c background exposed to influenza A virus (H3N2) on gestational day 9 bore autistic offspring (both male and female) with dose-dependent alterations in social and aggressive behaviors and increased locomotor behaviors, particularly in the males. This experiment also demonstrated changes in the catecholaminergic and microglial cell density in the brainstem tissues of male flu-exposed offspring only, suggesting an association between sex-specific alterations and dopamine metabolism and brainstem inflammation [53]. The BALB/c mice used in this experiment are known to be more sensitive to the H3N2 virus than those with the C57Bl/6 background. The BALB/c background has a greater Th1-type response than the C57Bl/6 background, which has a greater Th2-type response [54]. The authors suggested that the genetic alterations in the maternal Th1 responses contribute to the developmental abnormalities in the offspring after gestational viral exposure [53].

Maternal inflammation during critical periods of embryonic development can cause brain overgrowth and autism-associated behaviors as a result of altered neural stem cell function [55]. ROS at nontoxic levels can increase stem cell self-renewal and neurogenesis through the reversible inactivation of the tumor suppressor gene *PTEN* protein and the subsequent enhancement of the PI3K pathway [56]. The maternal inflammatory response stimulates the generation of ROS through the actions of various cytokines and the activation of the NOX (NADPH oxidase) enzyme, which enhances signal transduction for many growth and trophic factors that are important for normal brain development [57]. Pregnant mice treated with low-dose LPS at embryonic day 9 had offspring with brain overgrowth, and a more pronounced effect was observed in the *PTEN* heterozygotes. The exposure to maternal inflammation also enhanced the NOX-PI3K pathway signaling, stimulated the hyperproliferation of neural stem and progenitor cells, increased the number of forebrain microglia, and produced abnormal autism-associated behaviors in affected pups. It has not yet been clarified whether the increase in microglia plays a role in stimulating progenitor proliferation [58] or in the phagocytic pruning of cells produced in excess following maternal inflammatory response exposure [17]. However, these murine models of maternal inflammation support the concept that prenatal neuroinflammatory dysregulation in neural stem cell redox signaling can act in concert with underlying genetic susceptibilities to affect the cellular responses to environmentally altered cellular levels of ROS [55].

Mast Cell-Microglia Interaction

Microglia respond to proinflammatory signals released from nonneuronal cells, principally those of immune origin. Mast cells are of particular relevance in this context. They are derived from a distinct precursor in the bone marrow and mature under the influence of stem cell factor and various cytokines [59]. Mast cells also reside in the brain. It has been reported that rat brain mast cells, which were exclusively concentrated within the pia mater surrounding the diencephalon during embryonic stages, migrate along the penetrating vessels and enter the thalamus during development [60]. Nearly 97% of all brain mast cells lie on the abluminal side of the blood vessels. Mast cells are capable of migrating across the blood-brain barrier in situations where the barrier is compromised as a result of CNS pathology [61]. The high incidence of autism patients suffering from food or skin allergies [62] suggests the possibility that such mast cell-microglia interactions may contribute to the pathophysiology of neurodevelopmental disorders.

Among the inbred mouse strains that have been tested for abnormal behaviors, BTBR mice are among the most autistic-like strains. BTBR mice show low reciprocal social transference for food, high levels of repetitive self-grooming, low levels of social approach and juvenile play, and an unusual pattern of ultrasonic vocalization; these traits are consistent with the core symptoms of autistic humans such as impaired communication, repetitive behavior and lowered reciprocal social interactions [63–66]. In evaluations of the immune system of BTBR mice, this strain showed the following: (1) significantly elevated levels of serum IgG and IgE, IgG anti-brain antibodies and IgG and IgE deposited in the brain, (2) an elevated expression of cytokines and (3) an increased proportion of MHC-II-expressing microglia compared to B6 mice. This study suggested that neuroinflammation may be due to the activated microglia or the increased presence of mast cells, which are prominent in circumventricular organs, the hippocampal fissure and the perivascular spaces in the posterior lateral thalamus. As a result, these areas may play a role in the occurrence of BTBR behavioral abnormalities [9].

Microglial Dysfunction in Autism

The possibility of deficits in the elimination of synapses during synaptic maturation (so-called ‘pruning’) has been investigated and may explain some of the behavioral and circuit-level deficits found in autism [67]. Mi-

croglia play a critical role in pruning synapses during development [68]. Mice that have a transient reduction of microglia in the brain due to the failure to respond to the neuronally expressed chemokine fractalkine (fractalkine receptor knockout mice, *Cx3cr1*^{KO}) show an excess amount of weak excitatory synapses due to the consequence of their failure to eliminate immature synaptic connections during the second and third postnatal weeks [69]. The *Cx3cr1*^{KO} mice also demonstrated that reduced synaptic pruning during development is associated with persistent deficits in synaptic multiplicity, reduced functional connectivity between the brain regions, impaired social interaction, and increased repetitive behavior phenotypes in autism. These results suggest the possibility that a primary deficit in the microglia may contribute to circuit-level deficits across neurodevelopmental disorders, including autism [67].

Mecp2-null microglia are reported to be toxic to neurons in vitro through the production of high levels of glutamate [70]. In a murine model of Rett syndrome, the transplantation of wild-type bone marrow into irradiation-conditioned *Mecp2*-null hosts resulted in the engraftment of brain parenchyma by bone marrow-derived myeloid cells of microglial phenotype and the arrest of disease development. These benefits mediated by wild-type microglia were diminished when the phagocytic activity was inhibited. These findings demonstrate that microglia play an important role in the pathophysiology of Rett syndrome [71], thereby suggesting the

possibility of bone marrow transplantation as a potentially effective therapeutic approach for patients with autism.

Conclusions and Future Perspectives

Autism spectrum disorders are extremely heterogeneous. The rapidly growing list of genes and alleles that contribute to autism susceptibility suggests that many more genes will be discovered in the future. As we herein reviewed, microglia have a significant impact on several important etiological factors of autism such as the brain immune function, synaptic plasticity, brain circuitry, stem cell development, and the genetic interface for environmental stimuli. Further elucidation of the mechanisms and kinetics of microglial responses will therefore help to establish a window for therapeutic intervention in individuals with autism.

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The author has no conflicts of interest to disclose.

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