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The influence of neuroinflammation in Autism Spectrum Disorder

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ABSTRACT

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterised by deficits in social communication and restricted or repetitive behaviours. The clinical presentation of ASD is highly variable and diagnosis is based on the presence of impaired social communication and repetitive and/or restricted behaviours. Although the precise pathophysiologies underlying ASD are unclear, growing evidence supports a role for dysregulated neuroinflammation. The potential involvement of microglia and astrocytes reactive to inflammatory stimuli in ASD has generated much interest due to their varied roles including in mounting an immune response and regulating synaptic function. Increased numbers of reactive microglial and astrocytes in both ASD postmortem tissue and animal models have been reported. Whether dysregulation of glial subtypes exacerbates alterations in neural connectivity in the brain of autistic patients is not well explored. A role for the gut-brain axis involving microbial-immune-neuronal cross talk is also a growing area of neuroinflammation research. Greater understanding of these interactions under patho/physiological conditions and the identification of consistent immune profile abnormalities can potentially lead to more reliable diagnostic measures and treatments in ASD.

1. Introduction

Chronic inflammation of the central nervous system (CNS) is an emerging concept that has been gaining increasing credence in its role in health and disease over the past five years (Heneka et al., 2015; Najjar et al., 2013; Philips and Robberecht, 2011), including in Autism Spectrum Disorder (ASD) (Onore et al., 2012). Neuroinflammation is characterized by sustained activity and proliferation of glial cells (astrocytes and microglia) and arises following injury, infection or disease and is proposed to alter brain function. It is well established that microglia and astrocytes undergo morphological changes and release multiple pro-inflammatory mediators in response to these pathologies (Hanisch and Kettenmann, 2007; Kierdorf and Prinz, 2013). These mediators consist primarily of cytokines and chemokines that coordinate immune responses under physiological conditions as well as regulating neuronal function and connectivity. Therefore, increased microglia or astrocyte activity can lead to abnormal immune profiles and subsequently affect neural signalling and cognitive function.

A vast proportion of the literature to date refers to phenotypic changes in glia as 'activated' (Dubbelaar et al., 2018; Placone et al., 2015). Astrocytes and microglia are, however, highly dynamic and in addition to regulating neuroinflammation they are involved in homeostatic processes such as synapse formation, pruning and plasticity. Because use of the term 'activated' implies that these cells are inactivated or in an 'off' state under physiological conditions, we have opted to refer to microglia and astrocytes that have changed their phenotypes in response to changes in the microenvironment as 'reactive'.

2. Microglia

During development, microglia originate in the yolk sac as early as embryonic day 8 to 10 in mice and immediately colonize the brain (Reemst et al., 2016). During synaptogenesis, they induce apoptosis of excess neurons and may even regulate developmental synapse formation through the release of thrombospondins (Bessis et al., 2007). Microglia are thought to be the first glial cell type to respond to pathological changes in the brain and release cytokines and prostaglandins to recruit astrocytes and further exacerbate inflammation (Kreutzberg, 1995). During neuroinflammation in the CNS, microglia remove cellular debris by phagocytosis, a function comparable to that of macrophages in the periphery (Vilhardt, 2005). While neuroprotective effects

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of microglial activity are well documented (Nakajima and Kohsaka, 2004), unnecessary or prolonged activity in the absence of immune challenge is implicated in neurotoxicity that occurs in multiple CNS diseases (Dheen et al., 2007). Microglia are not quiescent under physiological conditions; but are highly motile and in a constant state of surveillance of the surrounding microenvironment via distinctly ramified processes (Napoli and Neumann, 2009). Importantly, microglia modify their morphology to rapidly adapt to the environment. Following neuronal injury, microglia undergo morphological changes in response in response to stimulatory signals, such as glutamate or ATP release (Biber et al., 2007) or reduced inhibitory signals such as Transforming Growth Factor-beta (TGF-β) or CD200 (Cluster of Differentiation 200) released from a number of cells, particularly by neurons, astrocytes and oligodendrocytes. Consequently, reactive microglia mount an immune response, remove damaged cells and repair tissue via phagocytosis (Colonna and Butovsky, 2017; Kreutzberg, 1996; Lenz and Nelson, 2018). During this process, their ramified, long-branching processes retract and thicken, and the soma enlarges to adopt an amoeboid shape to enable even greater motility (Rock et al., 2004). Within the human brain, microglia are categorized into four main phenotypes: (1) ramified microglia, with small round somata and highly ramified processes, (2) primed microglia, with larger, somewhat elongated somata and highly ramified processes, (3) reactive microglia, with an amoeboid soma and very few ramified processes and (4) amoeboid microglia, with one or two unramified processes projecting from an elongated somata (Torres-Platas et al., 2014).

3. Astrocytes

Astrocytes are derived from the neuroectoderm and populate the brain parenchyma from approximately embryonic day 18 until at least postnatal day 7 in mice (Reemst et al., 2016). Astrocytes are critical in maintaining physiological homeostasis within the CNS, with important roles in supporting neuronal function, glial transmission and signalling via Ca^{2+} release and uptake (Araque and Navarrete, 2010). For example, astrocytic glutamate release can affect both pre- and post-synaptic neuronal activity. The idea that astrocytes are integral to pre- and post-synaptic function has led to the concept of a 'tripartite' synapse and places astrocytes amongst the central pathways of neuronal function (Perea et al., 2009). Cytokines released during the initiation of the immune response stimulate astrocytes to undergo reactive gliosis characterized by upregulation of astrocyte intermediate filament proteins, most notably glial fibrillary acidic protein (GFAP) (Pekny et al., 2014). Similar to microglia, reactive astrocytes undergo morphological changes and proliferate to mount an immune response. In contrast with microglia, however, they extend rather than retract their processes and undergo hypertrophy to enable this process (Sofroniew and Vinters, 2010).

Astrocytes and microglia express different biochemical markers. Specifically, astrocytes express excitatory amino acid transporters (EAATs) 1–5, whereas microglia lack EAAT4 (Liang et al., 2008). Moreover, astrocytes express a larger number of EAATs than microglia and as a result, glutamate uptake by astrocytes plays a significant role in maintaining extracellular glutamate levels (Anderson and Swanson, 2000). A prominent role in glutamate uptake enables astrocytes to facilitate precise spatial and temporal transmission of glutamatergic neurotransmission, thus regulating the activity of surrounding synapses (Marcaggi and Attwell, 2004). Astrocytes are also less morphologically reactive than microglia, potentially indicating a secondary role in responding to infiltrating stimuli. Indeed, it has been a point of controversy as to whether microglia or astrocytes are the initial responders to external threats to the immune system, with recent evidence suggesting that reactive microglia drive the induction of neuroprotective effects (Shinozaki et al., 2017) or neurotoxic activity (Liddelow et al., 2017) in a subset of reactive astrocytes. Nonetheless, it is clear that both microglial and astrocytic populations are also modulated via bidirectional communication (Gao et al., 2013).

4. Reactive microglia and astrocytes in ASD

Immune dysfunction has been identified in ASD (Careaga et al., 2010; Onore et al., 2012; Pardo and Eberhart, 2007; Pardo et al., 2005) but it is unclear whether it contributes to ASD onset or is a consequence of the disorder. In a key study by Vargas and colleagues, extensive microglial activity, indicated by an increased fractional area of immunocytochemical staining for the microglial marker, human leukocyte antigen-DR (HLA-DR), was reported in cortical regions, white matter and, most prominently, the cerebellum of postmortem ASD patient tissue compared to neurotypical controls (Vargas et al., 2005). Similar increases were seen for astrocyte immunoreactivity, particularly in the granular cell layer and white matter of the cerebellum, whereas GFAP protein expression, measured through western analysis, was increased significantly in the cerebellum, middle frontal gyrus and anterior cingulate gyrus of autistic patients (Vargas et al., 2005). In the same study, these observations correlated with marked histological changes including decreased numbers of Purkinje and granular cells together with reduced numbers of axons within the Purkinje cell layer of the cerebellum. An increased density of reactive microglia in the dorsolateral prefrontal cortex of individuals with ASD has also been observed (Morgan et al., 2010). Furthermore, altered microglial activity characterized by morphological changes including increased microglial soma size, retraction and thickening of processes and extension of filopodia from microglial processes as well as increased microglial density based on quantitative analyses of immunocytochemical labeling was observed in both the fronto-insular and visual cortex of ASD patient postmortem tissue samples (Tetreault et al., 2012), raising the suggestion that this pattern may be consistent throughout the cerebral cortex in ASD. Moreover, these findings were supported by increased gene expression of microglial markers TREM2 (Triggering receptor expressed on myeloid cells 2), DAP12/KARAP (killer cell activating receptor-associated protein) and CX3CR1 (CX3C chemokine receptor 1)) within the prefrontal cortex (PFC). The levels of these same microglial markers (with the addition of ionised calcium-binding adapter molecule 1 (IBA-1/Allograft inflammatory marker 1 (AIF1))), however, were decreased in the cerebellum (Edmonson et al., 2014) suggesting regional-specific modulation of microglia. In addition, the same study highlighted increased expression of astrocyte markers (GFAP; glial fibrillary acidic protein) in ASD PFC and cerebellar tissue. Relevant to these findings, GFAP levels in CSF samples from ASD patients are on average three times higher relative to controls (Ahlsen et al., 1993; Rosengren et al., 1992). Increased GFAP levels in ASD postmortem superior frontal, parietal and cerebellar cortex samples have also been demonstrated in comparison with healthy controls (Laurence and Fatemi, 2005).

Altered spatial organisation of microglia in ASD brains has also been reported. Morgan and colleagues reported that density analyses of IBA-1 stained brain sections showed that microglia and neurons in the dorsolateral prefrontal cortex of ASD patients are more frequently located in closer proximity compared to neurotypical controls, an interaction which was characterised by the encircling of neurons by microglia, suggesting increased interactivity (Morgan et al., 2012). This reduction in proximity of microglia and neurons could indicate altered functionality in ASD. For example, microglia located adjacent to neurons may actively increase degradation of dysfunctional synapses as a neuroprotective function or engage in excessive degradation of healthy neurons, which may exacerbate synaptic dysfunction present in ASD brain tissue.

Immune dysfunction and synaptic deficits have also been reported in mouse models of ASD; specifically models of maternal immune activation (MIA), which has been identified as a risk factor for ASD (Atladottir et al., 2010; Malkova et al., 2012). Stimulation of the immune system in rodents via administration of lipopolysaccharide (LPS; a component of gram negative bacterial cell walls) or

Polyinosinic:polycytidylic (Poly IC; a viral mimetic) during gestation has provided insights into neuroimmune mechanisms with male offspring mice exhibiting altered microglial receptor function and deficits in synaptic pruning in the hippocampus (Fernandez de Cossio et al., 2017), as well as immune dysregulation (Hsiao et al., 2012) and ASD-relevant behaviours (Hsiao et al., 2013). However, the number of activated and total microglia, investigated using microglial marker $CD11b^+$, remains unchanged in MIA offspring (Hsiao et al., 2012). Reducing the immune response, specifically by inhibiting the activity of interleukin-6 (IL-6) via administration of anti-inflammatory reagents, luteolin and diosmin, results in alleviation of the autism-like behaviours in MIA offspring (Parker-Athill et al., 2009). Moreover, MIA offspring show altered small intestine permeability as measured by gene expression encoding tight junction proteins when compared to control mice, as well as altered cytokine and chemokine expression, including decreased IL-12p40/p70 and MIP-1α (Hsiao et al., 2013).

Neuroimmune disturbances are observed across multiple animal models of autism and neurodevelopmental disorders. Fragile × Syndrome is the most common monogenic cause of autism (Budimirovic and Kaufmann, 2011) and similar to reports in MIA offspring, microglial expression remains unchanged in mice in which the FMRP (Fragile × Mental Retardation Protein) is deleted (Pacey et al., 2015). In the same study, however, astrocytes remained in a reactive state during postnatal development and during adulthood, as measured by increased GFAP expression and increased expression of Tumor Necrosis Factor Receptor 2 (TNFR2). Glial activation is also enhanced in the VPA600 model of autism (Lucchina and Depino, 2014), mimicking the increased risk of autism in babies born to pregnant mothers prescribed valproate for seizures (Christensen et al., 2013). Lucchina and Depino reported chronic hippocampal and cerebellar glial activation in mice treated with 600 mg/kg valproic acid (VPA600), alongside an exacerbated inflammatory response following LPS exposure. Specifically, VPA600 mice exhibited increased hippocampal microglial density compared to controls, and increased expression of the proinflammatory cytokines TNF-α and interleukin (IL)-6 in the cerebellum. Furthermore, Black and Tan Brachyury (BTBR) inbred mice display autism-like behaviours and express mutations associated with neurodevelopmental disorders including autism and schizophrenia such as the DISC1 (Disrupted in Schizophrenia 1) gene which is involved in learning and memory functions (Kilpinen et al., 2008). These mice demonstrate increased brain cytokine levels of IL-33, IL-18 and IL-1β, increased number of $CD4^+$ T cells in the spleen, mesenteric lymph nodes and peripheral blood, and an increased proportion of microglial cells compared to controls (Heo et al., 2011). Other models of neurodevelopmental disorders, including Reeler mice that exhibit inverted cortical layering display altered levels of IL-18 in brain and serum compared to WT mice (Businaro et al., 2016). Specifically, brain IL-18 is increased, whereas IL-18 levels in serum are lower in Reeler mutants compared to WT mice. Collectively, these findings support an association between abnormal neurodevelopment and aberrant inflammatory responses in rodent models relevant to autism.

5. Microglial-astrocytic interactions in neuroinflammation

Much of the bidirectional communication between microglia and astrocytes stems from the increased secretion of signaling molecules and cytokines. Reactive microglia release a range of cytokines and chemokines which are able to trigger astrocyte reactivity (Zhang et al., 2010). Indeed, a number of these, including IL-1, IL-2, IL-6, Tumour Necrosis Factor alpha (TNF-α) and Interferon gamma (IFN-γ), have been found to induce reactive astrogliosis, as measured through GFAP immunoreactivity, when injected into stab wound sites in neonatal mice (Balasingam et al., 1994). Reactive astrocytes, on the other hand, release ATP to trigger microglial activation. Together, reactive microglia and astrocytes form a glial scar at the site of injury to serve as a structural barrier to prevent further damage and promote tissue repair (Adams and Gallo, 2018). Following CNS injury, a major component of the glial scar, chondroitin sulfate proteoglycans (CSPGs), are upregulated in astrocytes (Asher et al., 2000). CSPGs bind to, and increase the signaling from, molecules involved in the recruitment of reactive microglia and other immune cells to the injury site (Rolls et al., 2008), suggesting that increased astrocyte activity can signal to further recruit microglia following injury. The prolonged activity of reactive microglia and astrocytes, heightened by their bidirectional communication, results in sustained release of these pro-inflammatory molecules, as well as reactive oxygen species (ROS), which can cause vasculo-endothelial dysfunction and structural damage to tissue in the surrounding CNS environment (Mittal et al., 2014). These substances act in concert to increase permeability at the blood-brain barrier (BBB) through a number of different mechanisms, including altering expression and inducing reorganization of the cytoskeletal and tight junction proteins forming the BBB (Capaldo and Nusrat, 2009; Pun et al., 2009). The infiltration by peripheral immune cells is facilitated by the expression of chemokines, such as CXCL12, on endothelial cells which bind to their receptor on leukocytes to facilitate binding and transmigration into the brain (Takeshita and Ransohoff, 2012). Due to increased levels of pro-inflammatory stimuli, microglia recruit immune cells from the bloodstream to exacerbate the CNS inflammatory response (Hanisch and Kettenmann, 2007). Astrocytes then adopt a protective role by secreting a number of regulatory factors such as glial-derived neurotrophic factor and transforming growth factor-β, which interact with endothelial cells to limit the entry of peripheral leukocytes and macrophages through the compromised BBB and facilitate its repair (Bush et al., 1999; Takeshita and Ransohoff, 2012). Hence, prolonged activity of reactive microglial and astrocytes, typically beyond a period of weeks or months, are considered a pathological hallmark of neuroinflammation. Reactive microglia secrete a number of cytokines and chemokines that promote the neuroinflammation response including via stimulating astrocyte activity.

Astrocytic and microglial proliferation rates are also altered during increased microglia activity in addition to morphological changes in these cell types. Changes in microglial and astrocyte populations can be quantified by western blot analysis of microglial marker proteins such as IBA-1, CD45 or CX3CR1, or markers of astrocytes such as GFAP. Furthermore, these techniques can be complemented by cellular immunofluorescence studies which enable the analysis of morphological changes relating to reactive state and cell numbers in specific regions of the brain. Such changes can be analysed in a high throughput and unbiased manner using software packages such as Neurolucida neuron tracing software (MBF Bioscience) to quantify glial soma size, the ramification of processes and proliferation in models of disease or infection (Abdolhoseini et al., 2016; Karperien et al., 2013; Kongsui et al., 2014).

6. The role of glia in synaptic plasticity

In addition to modulating inflammation, microglia and astrocytes contribute to maintaining homoeostasis in the brain by regulating synaptic function and plasticity (Ben Achour and Pascual, 2010) (Fig. 1). Indeed, mice expressing a mutation in the gene encoding the microglial protein DAP12 display altered synaptic function. Specifically, DAP12 loss-of-function results in enhanced hippocampal LTP (Roumier et al., 2004) and has been used to demonstrate that the induction of prenatal inflammation in these mice leads to further dysfunction to glutamatergic synapses in the adult offspring (Roumier et al., 2008). Interestingly, post-mortem data showed that DAP12 expression levels are increased in prefrontal cortex, and decreased in the cerebellum in ASD patients compared to healthy controls (Edmonson et al., 2014) suggesting regional-specific and clinically relevant changes in ASD. In addition to an active role in phagocytosis of dead or infected cells, microglia influence the rate of apoptosis of neurons and associated synapses (Paolicelli et al., 2011). They are also able to perform more

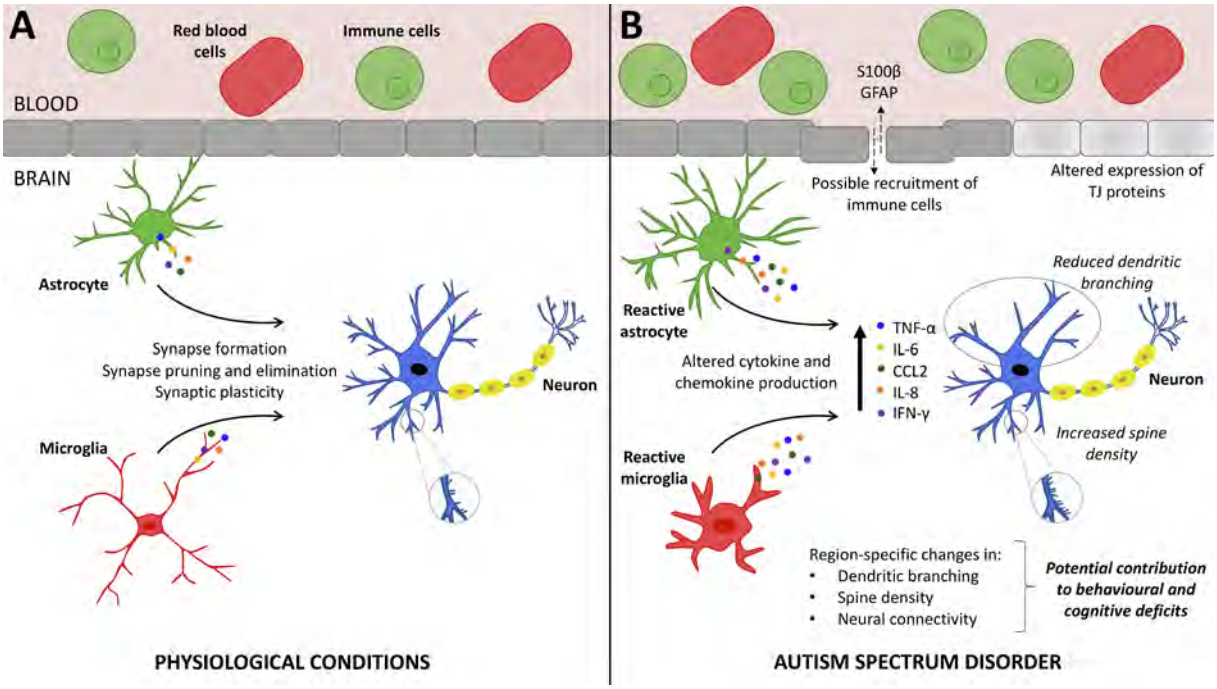

Fig. 1. Potential effects of reactive microglia and astrocytes in the ASD brain. A: Under physiological conditions, astrocytes and microglia regulate synapse formation, pruning and elimination as well as plasticity via appropriate release of cytokines and chemokines. **B:** Disruptions to the BBB, recruitment of immune cells and aberrant neuroinflammation triggers glial phenotypic changes and altered secretion of cytokines and chemokines, including increased TNF-α, IL-6, CCL2, IL-8 and IFN-γ in the brain. These changes may disrupt synapse maintenance and modify neuronal dendritic branching and spine density to contribute to under- and hyper-connectivity of specific brain regions. Such changes in neuronal networks may contribute to core ASD diagnostic traits. Reactive microglia represented as a single phenotype for simplicity. BBB: Blood-brain barrier.

precise and refined modulation of synaptic function by pruning dendritic spines, and are even capable of removing entire dendritic trees (Kettenmann et al., 2013). The presence of ramified microglia also appears to be important in maintaining normal cognitive function. Indeed, an absence of the microglial receptor, CX3C-chemokine ligand 1 (CX3CL1/fractalkine) results in impaired LTP and significant cognitive deficits, specifically in hippocampal-dependent learning and memory, as well as motor learning, which may be indicative of cerebellar or striatal-dependent learning impairments in these mice (Rogers et al., 2011). These deficits are attributed to increased signaling by IL-1β (Rogers et al., 2011) and highlight the role of microglia in the physiological regulation of synaptic plasticity and cognitive function, specifically memory and learning.

As microglia only make brief, periodic contact with synapses, they are required to be quite dynamic and motile in extending their processes to prune and modify dendritic spines and trees. Astrocytes, conversely, are in close structural and functional contact with pre-synaptic and postsynaptic neurons, as part of the tripartite synapse. They also extend and retract their fine processes to contact different neuronal synapses. This communication between synapses and astrocytes are facilitated by a number of neurotransmitter receptors and transporters expressed by astrocytes. Astrocytes also secrete thrombospondins, extracellular matrix proteins involved in the formation of synapses, which can additionally inhibit glutamate release at the presynaptic neuron and alter expression of receptors on the postsynaptic neuron to modulate synaptic transmission (Chung et al., 2015). They also contribute to synapse elimination by triggering phagocytic pathways through the expression of MEGF10 (Multiple epidermal growth factor-like domains protein 10) and MERTK (Tyrosine-protein kinase MER) (Chung et al., 2013). Alternatively, evidence also supports a role for astrocytes in indirectly triggering synapse elimination through the secretion of TGF-β, which induces C1q expression by retinal neurons to initiate microglial-mediated phagocytosis (Bialas and Stevens, 2013). As a further indication of the effects of astrocytes on neuronal function, GFAP-knockout mice display reduced long-term depression at cerebellar Purkinje cell synapses (Shibuki et al., 1996). These findings suggest that astrocyte function influences highly dynamic and plastic neuronal circuits in the adult brain and potentially contributes to the pathophysiology of ASD (Clarke and Barres, 2013).

Both microglia and astrocytes influence the formation, maintenance and elimination of neuronal synapses in early brain development and throughout life (Chung et al., 2015; Tay et al., 2017). Thus, the regulatory roles of microglia and astrocytes in shaping synapse function suggest that they, and the cytokines they release (Donzis and Tronson, 2014), are essential for synaptic plasticity and therefore the formation of memories and learning (Yirmiya and Goshen, 2011). Glial cell involvement in these processes is enabled by their close anatomical proximity with neurons. The morphology of astrocytes and microglia, specifically their ramified processes, enable them to envelop neuronal synapses within the CNS. These cell types also release a number of neuroactive substances termed ‘gliotransmitters’ such as glutamate, ATP and D-serine, and therefore actively regulate synaptic function (Araque and Navarrete, 2010; Fields and Stevens-Graham, 2002). Based on this intricate relationship between glia and the CNS, it is proposed that morphological and functional changes in astrocytes and microglia in the brain modulate synapse function.

7. Altered neural connectivity in ASD

Alterations in synaptic activity are well documented in ASD patients and likely contribute to the core phenotypes of impaired social communication and repetitive behaviours. Both reduced and hyper-connectivity in the brain are reported in a regional specific manner in ASD, suggesting changes in both anatomical and functional connectivity. Comprehensive functional MRI studies report reduced activation and functional connectivity in language areas (Just et al., 2004) and reduced synchronization of neural activity between frontal and parietal regions (Just et al., 2007) in ASD patients. Koshino and colleagues

showed that the fusiform face area of the brain, responsible for facial recognition, has less synchronized neural connectivity with frontal areas of the brain in ASD patients (Koshino et al., 2008). Similarly, Kleinhans and colleagues demonstrated reduced connectivity between the left amygdala and posterior cingulate (Kleinhans et al., 2008). Furthermore, underconnectivity between specific brain regions is correlated with ASD symptom severity. Greater social impairment is associated with reduced neural connectivity of the fusiform face area to the amygdala, and increased connectivity to the right inferior frontal gyrus (Kleinhans et al., 2008). Functional hyper-connectivity, however, has also been reported in the primary sensory, paralimbic and association areas in autistic children involving both short and long-range connections within and between multiple brain regions (Supekar et al., 2013). This widespread hyper-connectivity was also found to be associated with greater social impairment.

Regional under- and hyper-connectivity reported in children with ASD is supported by abnormalities in neuronal morphology. Specifically, the morphology and density of neuronal dendrites and spines differ in ASD patients compared to neurotypical controls (Martinez-Cerdeno, 2017). CA1 and CA4 hippocampal neurons have significantly fewer dendritic branches (Raymond et al., 1996), a reduced density of dendritic spines on some Golgi-impregnated pyramidal neurons (Williams et al., 1980) and fewer dendrites in the dorsolateral prefrontal cortex (Mukaetova-Ladinska et al., 2004) in ASD postmortem tissues compared to neurotypical controls. Conversely, Hutsler and Zhang (2010) reported greater spine densities in Golgi-impregnated pyramidal neurons from layer II of the frontal, temporal and parietal lobe regions and in layer V of the temporal lobe (Hutsler and Zhang, 2010). However, further work is needed to clarify regional changes in neuron morphology in ASD as studies to date are limited by the reduced availability of postmortem brain tissue.

It is clear however, that altered dendritic numbers, synaptic dysfunction and abnormal neuronal connectivity are frequently observed in both ASD post-mortem tissue and animal models. Because neural activity is modulated by microglia and astrocyte function, abnormal inflammatory processes in the brain involving these glial populations may contribute to cognitive and behavior abnormalities characterizing ASD. The involvement of microglia and astrocytes in regulating synaptic plasticity therefore provides potential targets for designing new therapies to improve outcomes for patients.

8. Altered cytokine network in ASD

Reactive microglia and astrocytes release a cascade of cytokines and chemokines which signal between cells and coordinate responses to changes in the nervous system environment. These pleiotrophic proteins are involved in cell signalling and are released by a number of immune cells, including macrophages and microglia, astrocytes, B lymphocytes, T lymphocytes and mast cells. Thus, they direct the inflammatory response in the CNS and peripheral immune system following alterations in the microenvironment. Multiple studies indicate differences in cytokine and chemokine expression in autistic and typically developing patients in both the CNS (Table 1) and periphery (Table 2).

8.1. Peripheral modulation of immunity

Immune cell recruitment via a compromised BBB in cases of chronic neuroinflammation can exacerbate inflammation in the CNS. Moreover, both central and peripheral inflammation can influence microglia reactivity and alter neuronal synaptic function in the brain (Di Filippo et al., 2013). While the precise influence of peripheral cytokines on the CNS immune environment in ASD has yet to be elucidated, abnormalities in gene expression associated with BBB integrity and function have been reported (Fiorentino et al., 2016). Furthermore, autoantibodies to brain tissue in the serum of children with ASD have also been detected (Wills et al., 2007), suggesting disrupted BBB permeability and function (Kanner et al., 2003; Marchi et al., 2003). For instance, elevated serum levels of S100β, a calcium-binding protein expressed mainly by astrocytes, were commonly identified in ASD including frequently in samples from children with severe ASD (Al-Ayadhi and Mostafa, 2012b) suggesting correlations of S100β protein levels with ASD behaviour. Moreover, in the periphery, recent analyses from 75 Chinese children diagnosed with ASD suggest that serum GFAP levels greater than 1.28 ng/ml are associated with a 9.88-fold increased risk of ASD (Wang et al., 2017). Thus, in considering cytokine alterations in the CNS, it is also important to assess for changes in the periphery. Cytokine alterations in the blood could potentially be used to provide information on inflammation and changes in synapse connectivity in the brain. Although transport systems at the BBB enable migration of some cytokines, including IL-1β, IL-6 and TNF-α, from the periphery into the brain (Banks et al., 1995), BBB disruption could also allow other cytokines to enter the CNS. Therefore, blood-borne cytokines and chemokines could also influence cognitive function and behaviour in autism. Thus, a summary of studies investigating cytokine levels in peripheral samples from autistic patients is presented in Table 2.

Importantly a number of cytokines dysregulated in ASD have also been correlated with symptom severity and performance in ASD diagnostic tests (Masi et al., 2017). Characterising specific cytokine profiles in autism may therefore be valuable as clinical biomarkers to aid in early detection and diagnosis (Rose and Ashwood, 2014). This would require identification of a number of cytokines that are consistently elevated in ASD. However, reports of cytokine dysregulation are highly variable between studies. This variability could be due in part to differences in study design, sample size, the type of sample collected (e.g. plasma, serum, PBMC, amniotic fluid), whether the cells have been stimulated, the specific stimulus (e.g. PHA, LPS, tetanus toxoid, other cytokines) and whether ASD subsets have been accounted for (i.e. symptom severity, verbal and non-verbal, regressive and non-regressive, presence and absence of GI issues).

9. CNS and peripheral immune profiles in ASD

Complex cytokine networks regulate the development, maintenance and homeostasis of the CNS. Disruption to these networks occurs following inflammation of brain tissue. While this response can be neuroprotective (i.e. by contributing to repair of damaged tissue), prolonged dysregulation of CNS cytokines results in the disruption of neuronal function via their roles in cognition and synapse regulation (McAfoose and Baune, 2009; Prieto and Cotman, 2017). In ASD, alterations to this cytokine network have been reported in the cerebrospinal fluid (CSF) and post-mortem brain tissue of autistic patients. These findings support an increased role for pro-inflammatory cytokines, including IL-6 and TNF-α, in autism pathophysiology.

IL-6 is typically produced during astrogliosis to promote neuronal survival, however prolonged elevation of IL-6 levels has been implicated in a number of CNS pathologies. IL-6 is produced by microglia, astrocytes and neurons in the brain (Gruol, 2015), and is also involved in neuronal and synaptic modification. Wei and colleagues further investigated the consequences of IL-6 overexpression, reporting impairments to cell adhesion and migration, as well as facilitating the formation of excitatory, but not inhibitory, synapses between mouse cerebellar granule cells (Wei et al., 2011). Data from animal studies support IL-6 as an important mediator of autistic-like behaviours (Wei et al., 2012, 2013). A recent study investigating the IL-6 signalling pathway in LPS-stimulated MIA offspring found that pioglitazone, which confers anti-inflammatory effects, reduced autistic-like behaviours possibly by reducing elevated IL-6 levels, highlighting the potential role of this cytokine in autism (Kirsten et al, 2018). Tumor necrosis factor-α (TNF-α) is another immunomodulatory molecule produced by neurons and glial cells in the CNS (Pan et al., 1997; Sawada et al., 1989) with a critical role in regulating synaptic strength

Table 1
CNS cytokine and chemokine profile in ASD.

Cytokine	Findings		Sample	Sample size	References	Comments
TNF-α	Increased		CSF: Serum	ASD: 10	(Chez et al., 2007)	Increased ratio of TNF-α in CSF: serum Lacks TD control; ratio was compared to CSF: serum ratio in other pathological states
			Brain	ASD: 8 TD: 8	(Li et al., 2009)	Specifically increased in the FCC
TGF-β1	Increased		Brain tissue	ASD: 7 TD: 7	(Vargas et al., 2005)	Specifically increased in the ACG, MFG and CBL
IL-6	Increased		Brain tissue	ASD: 6 TD: 6	(Wei et al., 2011)	Specifically increased in the CBL
				ASD: 7 TD: 7	(Vargas et al., 2005)	Specifically increased in the ACG, no change in MFG and CBL
				ASD: 8TD: 8	(Li et al., 2009)	Specifically increased in the FCC
IL-1β	No change		Brain tissue	ASD: 8 TD: 8	(Li et al., 2009)	Specifically increased in the FCC
GM-CSF	Increased		Brain tissue	ASD: 8 TD: 8	(Li et al., 2009)	Specifically increased in the FCC
Th1	IFN-γ	Increased	Brain tissue	ASD: 8 TD: 8	(Li et al., 2009)	Specifically increased in the FCC
			CSF	ASD: 6 TD: 6	(Vargas et al., 2005)	
Th2	IL-4	No change	Brain tissue	ASD: 8 TD: 8	(Li et al., 2009)	Investigated in the FCC
	IL-5	No change	Brain tissue	ASD: 8 TD: 8	(Li et al., 2009)	Investigated in the FCC
Th2/Treg	IL-10	Increased	Brain tissue	ASD: 7 TD: 7	(Vargas et al., 2005)	Specifically increased in the ACG, no change in MFG
		No change	Brain tissue	ASD: 8 TD: 8	(Li et al., 2009)	Investigated in the FCC
CCL2	Increased		Brain tissue	ASD: 7 TD: 7	(Vargas et al., 2005)	Specifically increased in the ACG and CBL, no change in MFG
			CSF	ASD: 6 TD: 6		
IL-8	Increased		Brain tissue	ASD: 8 TD: 8	(Li et al., 2009)	Specifically increased in the FCC
				ASD: 7 TD: 7	(Vargas et al., 2005)	
			CSF	ASD: 6 TD: 6	(Vargas et al., 2005)	
Eotaxin	Increased		Brain tissue	ASD: 7 TD: 7	(Vargas et al., 2005)	Specifically increased in the ACG, no change in MFG and CBL
MIP-1β	Increased		CSF	ASD: 6 TD: 6	(Vargas et al., 2005)	

CSF: cerebrospinal fluid; TD: typical development; DD: developmental delay; ACG: Anterior Cingulate Gyrus; MFG: Middle Frontal Gyrus; CBL: Cerebellum; FCC: Frontal Cerebral Cortex.

(Steinmetz and Turrigiano, 2010) and synaptic plasticity. This is thought to occur via inhibition of long-term potentiation of synapses (Cunningham et al., 1996; Tancredi et al., 1992), therefore a chronic increase of TNF-α may potentially hinder learning and memory in disorders such as autism.

Interestingly, IL-1β also contributes to the inhibition of LTP induction and is involved in reducing synaptic strength (Bellinger et al., 1993), as well as modulating memory and learning under physiological conditions (Goshen et al., 2007). In contrast to IL-6 and TNF-a for example, IL-1β levels appear to be unchanged in the CNS of autistic patients (Li et al., 2009). These findings do not necessarily negate a role for IL-1β in ASD as it has been reported to cross the BBB in mice (Banks et al., 1991, 1995) and a number of studies suggest that IL-1β levels are increased in the periphery of autistic patients (Ashwood et al., 2011c; Enstrom et al., 2010; Jyonouchi et al., 2001; Krakowiak et al., 2017; Ricci et al., 2013; El Din et al., 2006; Suzuki et al., 2011; Xie et al., 2017). Therefore, additional studies with larger data sets are required to determine if increased blood IL-1β influences CNS levels. IL-1β function is particularly important in the brain due to its involvement in the activation of serotonin transporters (Zhu et al., 2006) and modulation of inhibitory synapses (Hellstrom et al., 2005).

TGF-β1 also has multifaceted roles in attenuating neuroinflammation (Cekanaviciute et al., 2014) and synapse loss in mouse models of disease, as well as being implicated in astrocyte-mediated synapse formation (Diniz et al., 2014). Indeed, TGF-β1 acts as a multifunctional cytokine and is secreted by all cells of leukocyte lineage with dual roles in promoting and supressing inflammatory responses, depending on the target cell type, state of differentiation and cytokine array present (Letterio and Roberts, 1998). TGF-β1 knockout mice exhibit significant inflammation and autoimmune disorders (Shull et al., 1992), an absence of microglia in the CNS (Butovsky et al., 2014), increased neuronal death (Brionne et al., 2003) and synaptic dysfunction (Koeglsperger et al., 2013). Only one study has investigated TGF-β1 in the brain of autistic patients (Vargas et al., 2005) and reported an increase in TGF-β1 levels, despite overwhelming evidence supporting a decrease in TGF-β1 in the peripheral blood (Ashwood et al., 2008; El Gohary et al., 2015; Hashim et al., 2013; Okada et al., 2007). This may suggest dual roles for TGF-β1 in the brain and periphery of autistic patients; however, as with IL-1β, it may be premature to draw conclusions based on this research given the limited evidence available regarding alterations in brain TGF-β1 levels.

T-lymphocytes play a fundamental role in mediating adaptive immunity by secreting cytokines to regulate local immune responses (Filiano et al., 2017). A deficiency of T-cells in the blood of mice has been shown to induce cognitive and behavioural deficits, highlighting the influence of T-cells on CNS function (Kipnis et al., 2004). T-lymphocytes consist of two T cell subsets: $CD8^+$ cytotoxic T cells and $CD4^+$ helper T cells. $CD8^+$ cytotoxic T cells detect antigens presented by MHC

Table 2
Peripheral cytokine and chemokine profile in ASD.

Cytokine	Findings	Sample	Sample size	References	Comments
TNF-α	No change	Plasma	ASD: 18 TD: 18	(Singh, 1996)	
			ASD: 28 TD: 28	(Suzuki et al., 2011)	Non-significant trend for increase in ASD patients
			ASD: 15 TD: 20	(Tonhajzerova et al., 2015)	
	Increased	PBMC	ASD: 71 HS: 23 TD: 17	(Jyonouchi et al., 2001)	• Increased in ASD compared to TD and HS without stimulation • Increased in ASD compared TD and HS following stimulation with LPS, PHA and IL-12p70 • Increased in ASD compared to TD (but not HS) following stimulation with Tetanus toxoid and IL-18.
			ASD: 37 TD: 35	(Ashwood et al., 2011b)	• Increased in ASD compared to TD following stimulation with PHA • Associated with more stereotyped behaviours
		Serum	ASD: 30 TD: 30	(Ghaffari et al., 2016)	
			ASD: 29 TD: 29	(Ricci et al., 2013)	
			ASD: 38 TD: 13–15	(Tsilioni et al., 2015)	TNF decreased significantly following treatment with luteolin-containing dietary formulation
			ASD: 32 TD: 28	(Xie et al., 2017)	Positive correlation with ASD symptom severity
		Plasma	ASD: 77 TD: 77	(Al-Ayadhi, 2005)	
			ASD: 87 TD: 41	(Hu et al., 2018)	
		Amniotic fluid	ASD: 331 TD: 698	(Abdallah et al., 2013)	
TGF-β1	Increased	Plasma	ASD: 87 TD: 41	(Hu et al., 2018)	
	Decreased	Serum	ASD: 19 TD: 21	(Okada et al., 2007)	No correlation with behaviour
		Plasma	ASD: 30 TD: 30	(El Gohary et al., 2015)	Negative correlation with ASD symptom severity
			ASD: 50 DD: 50 TD: 50	(Hashim et al., 2013)	Negative correlation with ASD symptom severity
			ASD: 75 TD: 68	(Ashwood et al., 2008)	Negative correlation with ASD symptom severity
IL-1β	No change	Amniotic fluid	ASD: 331 TD: 698	(Abdallah et al., 2013)	
		Serum	ASD: 50 TD: 30	(Basheer et al., 2018)	
			ASD: 22 TD: 28	(Emanuele et al., 2010)	
		Plasma	ASD: 15 TD: 20	(Tonhajzerova et al., 2015)	
			ASD: 25 HS: 25	(Napolioni et al., 2013)	• No change compared to TD • Increased in ASD patients with GI issues compared to those without GI issues • Increased in regressive ASD patients compared to non-regressive ASD patients
	Increased	Serum	ASD: 32 TD: 28	(Xie et al., 2017)	
			ASD: 29 TD: 29	(Ricci et al., 2013)	
		PBMC	ASD: 17 TD: 16	(Enstrom et al., 2010)	Increase measured following TLR 2 and TLR 4 stimulation
			ASD: 71 HS: 23 TD: 17	(Jyonouchi et al., 2001)	• Increased in ASD compared to TD (but not HS) without stimulation • Increased in ASD compared TD and HS following stimulation with LPS
		Plasma	ASD: 97 TD: 87 DD: 39	(Ashwood et al., 2011c)	Increased ASD compared to TD (but not DD) and in regressive ASD patients compared to non-regressive ASD patients
			ASD: 21 TD: 21	(El Din et al., 2006)	
			ASD: 28 TD: 28	(Suzuki et al., 2011)	
		Neonatal blood spots	ASD: 214 DD: 27 TD: 62	(Krakowiak et al., 2017)	
	Decreased	PBMC	ASD: 17 TD: 16	(Enstrom et al., 2010)	Decrease measured following TLR 9 stimulation

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Table 2 (*continued*)

Cytokine	Findings	Sample	Sample size	References	Comments
IL-6	No change	Whole blood Serum	ASD: 13 TD: 13	(Croonenberghs et al., 2002)	Non-significant trend for an increase in whole blood samples from ASD patients
		Serum	ASD: 38 TD: 13–15	(Tsilioni et al., 2015)	• Non-significant Increase • IL-6 decreased significantly following treatment with luteolin-containing dietary formulation
			ASD: 22 TD: 28	(Emanuele et al., 2010)	
			ASD-FGID: 14 TD-FGID: 15 TD: 6	(Luna et al., 2017)	No change in ASD patients with functional gastrointestinal disorders (FGID) compared to TD patients with or without FGID
		PBMC	ASD: 37 TD: 35	(Ashwood et al., 2011b)	• No change compared to TD upon stimulation with PHA
		Plasma	ASD: 28 TD: 28	(Suzuki et al., 2011)	
			ASD: 25 HS: 25	(Napolioni et al., 2013)	• No change compared to TD • Increased in ASD patients with GI issues compared to those without GI issues
			ASD: 16 TD: 16	(Singh, 1996)	
		$CD4^+$ T cells $CD8^+$ T cells	ASD: 20 TD: 20	(Gupta et al., 1998)	
	Increased	PBMC	ASD: 17 TD: 16	(Enstrom et al., 2010)	Increase measured following TLR 2 stimulation
			ASD: 71 HS: 23 TD: 17	(Jyonouchi et al., 2001)	
		Plasma	ASD: 97 TD: 87 DD: 39	(Ashwood et al., 2011a)	• Increased in ASD compared to TD and DD • Increased only in patients with regressive ASD (but not non-regressive ASD) to TD (but not DD)
			ASD: 77 TD: 77	(Al-Ayadhi, 2005)	
			ASD: 21 TD: 21	(El Din et al., 2006)	
		Serum	ASD: 50 TD: 30	(Basheer et al., 2018)	
			ASD: 29 TD: 29	(Ricci et al., 2013)	
		PBMC	ASD: 17 TD: 16	(Enstrom et al., 2010)	Decreased following TLR 9 stimulation
	Decreased	Amniotic fluid	ASD: 359 TD: 741	(Abdallah et al., 2012b)	
		Plasma	ASD: 99 TD: 40	(Manzardo et al., 2012)	
IL-23	Increased	Serum	ASD: 29 TD: 29	(Ricci et al., 2013)	
	Decreased	Serum	ASD: 50 DD: 50 TD: 50	(Hashim et al., 2013)	Negative correlation with ASD symptom severity
		Plasma	ASD: 40 TD: 20	(Enstrom et al., 2008)	Decreased more significantly in patients with early onset ASD compared to regressive ASD
		PBMC	ASD: 34 TD: 26	(Onore et al., 2009)	Negative correlation with social interaction skills
GM-CSF	No change	Plasma	ASD: 97 DD: 39 TD: 87	(Ashwood et al., 2011a)	
		Neonatal blood specimen	ASD: 84 DD: 49 TD: 159	(Zerbo et al., 2014)	
		Amniotic fluid	ASD: 331 TD: 698	(Abdallah et al., 2013)	
	Increased	Plasma	ASD: 25 HS: 25	(Napolioni et al., 2013)	• No change compared to TD Increased in non-verbal ASD patients compared to verbal ASD patients
		PBMC	ASD: 37 TD: 35	(Ashwood et al., 2011b)	• Increased in ASD compared to TD following stimulation with PHA Associated with better socialisation and reduced lethargy
	Decreased	PBMC	ASD: 17 TD: 16	(Enstrom et al., 2010)	Decreased following TLR 9 stimulation

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Table 2 (*continued*)

Cytokine	Findings		Sample	Sample size	References	Comments
Th1	IL-2	No change	Serum	ASD: 32 TD: 28	(Xie et al., 2017)	
			Plasma	ASD: 97 DD: 39 TD: 87	(Ashwood et al., 2011a)	
				ASD: 25 HS: 25	(Napolioni et al., 2013)	• No change in ASD compared to TD • Increased in ASD patients with GI issues compared to those without GI issues
			Amniotic fluid	ASD: 359 TD: 741	(Abdallah et al., 2012b)	
		Increased	PBMC	ASD: 20 TD: 20	(Molloy et al., 2006)	
		Decreased	$CD4^+$ T cells $CD8^+$ T cells	ASD: 20 TD: 20	(Gupta et al., 1998)	
	IFN-γ	No change	Plasma	ASD: 97 DD: 39 TD: 87	(Ashwood et al., 2011a)	
				ASD: 87 TD: 41	(Hu et al., 2018)	
				ASD: 28 TD: 28	(Suzuki et al., 2011)	Non-significant increase
			PBMC	ASD: 71 HS: 23 TD: 17	(Jyonouchi et al., 2001)	No change upon stimulation with PHA, Tetanus toxoid, IL-12p70, IL-18 or dust mite
				ASD: 37 TD: 35	(Ashwood et al., 2011b)	• No change upon stimulation with PHA • Associated with more impaired communication
			Amniotic fluid	ASD: 359 TD: 741	(Abdallah et al., 2012b)	
		Increased	PBMC	ASD: 20 TD: 20	(Molloy et al., 2006)	
		Decreased	$CD4^+$ T cells $CD8^+$ T cells	ASD: 20 TD: 20	(Gupta et al., 1998)	
Th2	IL-4	No change	Serum	ASD: 32 TD: 28	(Xie et al., 2017)	
				ASD: 50 TD: 30	(Basheer et al., 2018)	
			Plasma	ASD: 97 DD: 39 TD: 87	(Ashwood et al., 2011a)	
				ASD: 28 TD: 28	(Suzuki et al., 2011)	Non-significant increase
			PBMC	ASD: 45 TD: 69	(Akintunde et al., 2015)	
		Increased	$CD4^+$ T cells $CD8^+$ T cells	ASD: 20 TD: 20	(Gupta et al., 1998)	
			PBMC	ASD: 20 TD: 20	(Molloy et al., 2006)	
			Amniotic fluid	ASD: 331 TD: 698	(Abdallah et al., 2013)	
			Neonatal blood spots	ASD: 214 DD: 27 TD: 62	(Krakowiak et al., 2017)	
		Decreased	Amniotic fluid	ASD: 359 TD: 741	(Abdallah et al., 2012b)	
	IL-5	No change	Plasma	ASD: 97 DD: 39 TD: 87	(Ashwood et al., 2011a)	
				ASD: 25 HS: 25	(Napolioni et al., 2013)	No change compared to TD; increased in regressive ASD patients compared to non-regressive ASD patients
			PBMC	ASD: 37 TD: 35	(Ashwood et al., 2011b)	• Non-significant increase in ASD compared to TD upon stimulation with PHA • Associated with better fine motor skills, better visual reception and more expressive language
		Increased	Plasma	ASD: 28 TD: 28	(Suzuki et al., 2011)	
				ASD: 25 TD: 25	(Napolioni et al., 2013)	Increased specifically in patients with regressive ASD
			PBMC	ASD: 20 TD: 20	(Molloy et al., 2006)	
				ASD: 71 HS: 23 TD: 17	(Jyonouchi et al., 2001)	Increased in ASD compared to TD (but not HS) following stimulation with IL-12p70 and IL-18

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Table 2 (*continued*)

Cytokine	Findings		Sample	Sample size	References	Comments
Th17	IL-17/IL-17A	Increased	Plasma	ASD: 50 TD: 30	(Basheer et al., 2018)	Increase in IL-17A more common and higher in children with severe ASD compared to mild to moderate ASD
				ASD: 25 HS: 25	(Napolioni et al., 2013)	Increased in children
				ASD: 28 TD: 28	(Suzuki et al., 2011)	
			Serum	ASD: 50 DD: 50 TD: 50	(Hashim et al., 2013)	
				ASD: 32 TD: 28	(Xie et al., 2017)	
				ASD: 45 TD: 40	(Al-Ayadhi & Mostafa, 2012a)	Increased IL-17A more common and higher in severe ASD than mild to moderate ASD
		No change	Plasma	ASD: 40 TD: 20	(Enstrom et al., 2008)	
				ASD: 25 HS: 25	(Napolioni et al., 2013)	• No change compared to TD • Increased in regressive ASD patients compared to non-regressive ASD patients
			PBMC	ASD: 34 TD: 26	(Onore et al., 2009)	
	IL-17F	No change	Serum	ASD: 32 TD: 28	(Xie et al., 2017)	
Th2/Treg	IL-10	No change	Serum	ASD: 32 TD: 28	(Xie et al., 2017)	
			Plasma	ASD: 22 TD: 28	(Emanuele et al., 2010)	
				ASD: 97 DD: 39 TD: 87	(Ashwood et al., 2011a)	
				ASD: 25 HS: 25	(Napolioni et al., 2013)	• No change compared to TD • Increased in non-verbal ASD patients compared to verbal ASD patients
				ASD: 28 TD: 28	(Suzuki et al., 2011)	
			PBMC	ASD: 20 TD: 20	(Molloy et al., 2006)	
				ASD: 37 TD: 35	(Ashwood et al., 2011b)	• No change in response to PHA stimulation • Associated with better expressive language
			$CD4^+$ T cells $CD8^+$ T cells	ASD: 20 TD: 20	(Gupta et al., 1998)	
			Amniotic fluid	ASD: 331 TD: 698	(Abdallah et al., 2013)	
		Decreased	PBMC	ASD: 71 HS: 23 TD: 17	(Jyonouchi et al., 2001)	• Decreased in ASD and HS compared to TD following IL-18 stimulation. • No change when stimulated with PHA, Tetanus toxoid, IL-12p70 or dust mite
CCL2	No change		Plasma	ASD: 87 TD: 41	(Hu et al., 2018)	
				ASD: 25 HS: 25	(Napolioni et al., 2013)	
	Increased		Plasma	ASD: 80 DD: 37 TD: 58	(Ashwood et al., 2011c)	• Increased in ASD compared to both TD and DD • Increased plasma CCL2 correlated with symptom severity, defined by lower cognitive scores
			Serum	ASD-FGID: 14 TD-FGID: 15 TD: 6	(Luna et al., 2017)	• Increased in ASD patients with FGID compared to TD patients without FGID • No change in ASD-FGID compared to TD-FGID • MCP-1 associated significantly with abdominal pain
			Neonatal blood specimen	ASD: 84 DD: 49 TD: 159	(Zerbo et al., 2014)	
			Amniotic fluid	ASD: 331 TD: 698	(Abdallah et al., 2012a)	
IL-8	Increased		Plasma	ASD: 28 TD: 28	(Suzuki et al., 2011)	
				ASD: 97 TD: 87 DD: 39	(Ashwood et al., 2011a)	Increased compared only to TD and in both regressive and non-regressive ASD patients
				ASD: 15 TD: 20	(Tonhajzerova et al., 2015)	
			PBMC	ASD: 37 TD: 35	(Ashwood et al., 2011b)	• Increased in ASD compared to TD when unstimulated • No change when stimulated with PHA • Associated with more impaired communication
			Neonatal blood specimen	ASD: 84 DD: 49 TD: 159	(Zerbo et al., 2014)	

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Table 2 (*continued*)

Cytokine	Findings	Sample	Sample size	References	Comments
RANTES	No change	Plasma	ASD: 28 TD: 28	(Suzuki et al., 2011)	
	Increased	Plasma	ASD: 80 DD: 37 TD: 58	(Ashwood et al., 2011c)	Increased in ASD compared to both TD and DD
			ASD: 42 TD: 35	(Shen et al., 2016)	
	Decreased	Neonatal blood specimen	ASD: 84 TD: 159 DD: 49	(Zerbo et al., 2014)	
Eotaxin	No change	Plasma	ASD: 28 TD: 28	(Suzuki et al., 2011)	Non-significant increase
		Neonatal blood specimen	ASD: 84 DD: 49 TD: 159	(Zerbo et al., 2014)	
	Increased	Plasma	ASD: 80 DD: 37 TD: 58	(Ashwood et al., 2011c)	Increased in ASD compared to both TD and DD
			ASD: 87 TD: 41	(Hu et al., 2018)	
		Serum	ASD-FGID: 14 TD-FGID: 15 TD: 6	(Luna et al., 2017)	• Increased in ASD patients with FGID compared to TD patients without FGID • No change in ASD-FGID compared to TD-FGID • Eotaxin associated significantly with abdominal pain
MIP-1α	No change	Plasma	ASD: 28 TD: 28	(Suzuki et al., 2011)	
			ASD: 87 TD: 41	(Hu et al., 2018)	
		Neonatal blood specimen	ASD: 84 DD: 49 TD: 159	(Zerbo et al., 2014)	
	Increased	Plasma	ASD: 45 TD: 35	(Shen et al., 2016)	
	Decreased	Plasma	ASD: 99 TD: 40	(Manzardo et al., 2012)	
MIP-1β	No change	Plasma	ASD: 80 DD: 37 TD: 58	(Ashwood et al., 2011c)	Increased in ASD only compared to DD, not compared to TD
			ASD: 28 TD: 28	(Suzuki et al., 2011)	
			ASD: 87 TD: 41	(Hu et al., 2018)	Non-significant increase
	Increased	Plasma	ASD: 45 TD: 35	(Shen et al., 2016)	
	Decreased	Plasma	ASD: 99 TD: 40	(Manzardo et al., 2012)	

PBMC: peripheral blood mononuclear cells; TD: typical development; DD: developmental delay (other than ASD); HD: healthy sibling; GI: gastrointestinal; PHA: phytohemagglutinin; LPS: Lipopolysaccharide; FGID: functional gastrointestinal disorder.

I molecules on virtually all cells in the body (except erythrocytes), and $CD4^+$ helper T cells detect antigens produced by antigen-presenting cells and present on MHC-II molecules. These antigen presenting cells include dendritic cells, macrophages and B cells (Filiano et al., 2017). Activated $CD4^+$ T cells undergo proliferation and differentiate into T-helper (Th) cell subsets. These subtypes include Th1, Th2, Th17 and regulatory T (Treg) cells and differentiate selectively, dependent on the nature of the inflammatory stimuli. Th1 and Th2 cells aid in advancing the immune response. Specifically, Th1 cells act to stimulate phagocytosis via the secretion of IFN-γ and IL-2, whereas Th2 cells produce IL-4, IL-5 and IL-13 to stimulate B-cell antibody secretion and allergy responses (Zhou et al., 2009).

Th17 cells secrete IL-17/IL-17A, IL-17F, IL-21 and IL-22, and also play an important role in eliminating invading pathogens, particularly extracellular bacteria (Korn et al., 2009). Activation of Th17 cells by TGF-β and IL-6 are involved in preserving tissue integrity and reducing inflammation, whereas activation by IL-23 has been implicated in chronic inflammation during infection and autoimmunity (Gaffen et al., 2014). While IL-17 is increased in patients with ASD (Al-Ayadhi and Mostafa, 2012a; Basheer et al., 2018; Hashim et al., 2013; Napolioni et al., 2013; Suzuki et al., 2011; Xie et al., 2017) the reported decrease in IL-23 (Enstrom et al., 2008; Hashim et al., 2013; Onore et al., 2009) suggests that IL-17 and IL-6 may play a protective role in autism by preventing excessive tissue damage.

Treg cells are involved in preventing autoimmunity and maintaining self-tolerance through the release of anti-inflammatory cytokines IL-10 and TGF-β (Corthay, 2009; Kondelkova et al., 2010). IL-10 can be secreted by both Th2 and Treg cells, as well as a number of other immune cells, to limit the excessive production of pro-inflammatory cytokines and chemokines during infection and minimize tissue damage (Couper et al., 2008). However, IL-10 levels remain unchanged in the peripheral blood of ASD patients (Ashwood et al., 2011a; Ashwood et al., 2011b; Emanuele et al., 2010; Gupta et al., 1998; Molloy et al., 2006; Napolioni et al., 2013; Suzuki et al., 2011; Xie et al., 2017). Whether this IL-10-mediated defense mechanism is triggered by neuroinflammation in the brain is unclear, with conflicting findings in brain tissue obtain from ASD patients (Li et al., 2009; Vargas et al., 2005). Numbers of T regulatory cells can be increased by the pro-inflammatory and regulatory cytokine, granulocyte macrophage colony stimulating factor (GM-CSF). However, findings are highly variable as to whether this cytokine in increased (Ashwood et al., 2011b; Napolioni et al., 2013), decreased (Enstrom et al., 2010) or unchanged (Abdallah et al., 2013; Ashwood

et al., 2011a; Zerbo et al., 2014) in peripheral samples, including blood and amniotic fluid, from patients with ASD compared to controls.

Chemokines are a subset of cytokines that guide the migration of cells. There are two major families of chemokines: the CC chemokines (including CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β, CCL5/RANTES, CCL11/Eotaxin) and CXC chemokines (Laing and Secombes, 2004). A number of chemokines are involved in recruiting other immune cells to the site of tissue damage or infection. They are also involved in communication between astrocytes, microglia and neurons under physiological and pathological conditions (de Haas et al., 2007). Monocyte chemoattractant protein-1 (also known as C–C Motif Chemokine Ligand 2) (MCP-1/CCL2) was the first characterised and most extensively studied chemokine and has is consistently elevated in both the brain and blood of autistic patients (Abdallah et al., 2012a; Ashwood et al., 2011c; Vargas et al., 2005; Zerbo et al., 2014). CCL2 is produced by resident astrocytes and microglia in the CNS and modulates the migration, proliferation and reactivity of microglia (He et al., 2016; Hinojosa et al., 2011) and excitatory synaptic transmission in the brain (Zhou et al., 2011). Increased secretion of CCL2 is also thought to increase BBB permeability during inflammation by altering expression of tight junction proteins (Song and Pachter, 2004; Stamatovic et al., 2005). In this scenario, increased recruitment of blood-borne monocytes and lymphocytes from the periphery, including T-lymphocytes could combat neuroinflammation in the brain. Interleukin-8 (IL-8/CXCL8) is also increased in the brain of autistic patients (Li et al., 2009; Vargas et al., 2005). This chemokine actively promotes neutrophil infiltration into the brain following injury. Evidence also supports a role for IL-8 in altering ion channel opening frequency to enhance excitatory transmission (Lax et al., 2002) and impacting neuronal excitability through calcium channel modulation (Puma et al., 2001).

10. The Gut-brain axis in ASD

Communication between the gastrointestinal tract, its resident microbes and the brain modulate mood and behaviour via bidirectional gut-brain axis pathways. Multiple lines of evidence suggest that gastrointestinal dysfunction impacts inflammatory responses and brain function via neural, hormonal and immunological signals, although the precise biological mechanisms involved remain unclear (Cryan and Dinan, 2012; Dinan and Cryan, 2017; Dinan et al., 2015). In addition to increased risk for dysregulated inflammatory responses, patients with ASD are more likely to suffer from GI symptoms, including constipation, diarrhea or abdominal pain, compared to neurotypical controls (McElhanon et al., 2014) as well as other disorders such as inflammatory bowel disease (IBD) (Lee et al., 2018). Abnormal intestinal permeability is also more common in people diagnosed with ASD compared to neurotypical controls (de Magistris et al., 2010; White, 2003) and can contribute to increased levels of circulating cytokines which may cross the BBB.

10.1. Gut dysbiosis in ASD

Autistic patients exhibit altered gut microbial diversity compared to controls and an increased abundance of toxin-secreting fungi and bacteria, genera *Clostridium* (Luna et al., 2017) and *Candida* (Li et al., 2017). Luna et al., reported that mucosal microbial and blood signatures in ASD patients differ from controls with GI pain (Luna et al., 2017). Specifically, this work showed altered cytokine levels (including increased CCL2, eotaxin (CCL proteins) and IFN-α2, and decreased growth-related oncogene-α in the blood and changes in tryptophan homeostasis.

Several established animal models of ASD including offspring of MIA- (Hsiao et al., 2013) and VPA-treated (de Theije et al., 2014; Liu et al., 2018; Sgritta et al., 2019) rodents as well as the naturally occurring BTBR strain (Sgritta et al., 2019) show dysbiosis. Mice lacking the synaptic adhesion protein Shank3 (Sgritta et al., 2019; Tabouy et al., 2018) also demonstrate altered microbial profiles. This emerging pattern includes fecal microbial dysbiosis, including decreased levels of Lactobaccilus reuteri in the Shank 3 knockout mice (Sgritta et al., 2019; Tabouy et al., 2018) and a similar profile to autism patients in valproate-treated rats (Liu et al., 2018). Importantly, social impairments in several of these models were rescued via manipulation of microbial populations (Hsiao et al., 2013; Sgritta et al., 2019). These findings suggest that neuroinflammation may contribute to the behavioural phenotypes of these mice.

10.2. Influence of gut microbes on microglia

Seminal findings by Erny and colleagues identified that gut microbial populations are required for the structural and functional maturation of microglia. This work illustrated marked differences in microglial mRNA expression profiles, increased microglial density in multiple brain regions and extended processes and branching complexity of microglia in germ-free compared to mice bred in a specific pathogen free (SPF) environment (Erny et al., 2015). In germ-free mice, microglial volumes overlapped more often in the absence of altered cytokine and growth factor levels in the local environment compared to SPF mice. Similarly, in mice colonized with a less complex gut microbial population (i.e. only three microbial strains), microglial density was increased. Following viral infection, microglia in germ-free mice showed reduced innate immune responses suggesting that host microbial populations support microglial resistance to bacterial and viral stimuli.

The important role of the gastrointestinal tract in CNS function and behaviour is evidenced by studies in germ-free mice (Desbonnet et al., 2014; Turnbaugh et al., 2009). Germ-free mice display decreased sociability and increased repetitive self-grooming behaviour compared to SPF control mice bred in a specific pathogen free environment (Desbonnet et al., 2014). As outlined above, germ-free mice display immature and malformed microglia with increased ramification and branching, and reduced microglial immune response to LPS treatment or viral infection (Erny et al., 2015). Critically, the social avoidance phenotype and microglial phenotype is rescued following postnatal recolonization of the gut with microbiota derived from a typically developing mouse, indicating a role for microbes in regulating behavior. Because microglial and inflammatory changes in the CNS could in part originate from microbial dysbiosis it is crucial to analyze changes in mucosal barrier integrity and gut microbial populations in models of disease.

10.3. Neural pathways

The vagal nerve consists predominantly of afferent fibers suggesting that this pathway is mainly utilized to relay messages to the brain from the gut. In support of a role in gut-brain axis signaling, vagotomy reduces the impact of bacterial infections including Citrobacter rodentium (Lyte et al., 2006), Camplobacter jejuni (Gaykema et al., 2004) and Salmonella typhimurium (Wang et al., 2002) in animal models. Within the gastrointestinal tract, the enteric nervous system regulates gastrointestinal motility and secretion as well as increasing evidence indicating its role as a modulator of immune function. The nervous system plays multiple roles in preserving mucosal barrier integrity. The mucosal epithelium is a major component of the mucosal barrier and replenishes approximately every 3–5 days in mammals. In addition, a mucus biofilm and secreted antimicrobial proteins protect the tissue from invasion by microbes, metabolites and food debris in the lumen of the gastrointestinal tract.

10.4. Mucosal barrier integrity

The mucosal barrier of the gastrointestinal tract comprises a crucial component of the gut-brain axis. The gut houses an extensive immune

system including the gut associated lymphoid tissue (GALT) which is constantly exposed to commensal microbes and metabolites. The GALT includes Peyer's patches, caecal patches and smaller lymphoid aggregates distributed throughout the GI tract. Similar to the BBB, mucosal barrier integrity is maintained via epithelial cell tight junctions, however in the gut additional mechanisms to regulate inflammatory responses include the presence of a protective mucus biofilm and antimicrobial proteins that are released from Paneth cells located within the epithelium at the base of crypts in the small intestine. Mucosal barrier integrity is also maintained via the replenishment of stem cells located deep within the crypts of the intestinal villi is regulated by enteric neural activity (Lundgren et al., 2011).

Understanding the bidirectional communication between gut microbes and the CNS is highly relevant to understanding biological drivers of altered neuroinflammation in ASD. In considering the implications of neuroinflammation in ASD, the relationship between the gastrointestinal tract, gut microbes and neuroinflammation in the CNS must be acknowledged. These interactions form the basis for current clinical trials employing fecal microbiota transplants to improve behavioural and GI symptoms in ASD patients (Kang et al., 2017). A critical question is whether ASD causes alterations in gut microbiota to impact immune function, or if excessive neuroinflammation regulates microbes to alter microbial population composition and function.

11. Conclusion

Altered glial structure and function, cytokine profiles and gastrointestinal immune dysfunction are evident in patients with ASD and relevant animal models. Moreover, there is strong evidence for nervous system interaction with immune pathways in autism. The characterisation of neuroinflammation to include the gut-brain axis is a burgeoning area of research promising to shed light on the underlying pathophysiology of ASD. Further investigation of glial and immune abnormalities in preclinical animal models displaying ASD-relevant behavioural phenotypes is needed to unravel biological mechanisms and identify potential treatment targets.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2019.04.037.

References

Abdallah, M.W., Larsen, N., Mortensen, E.L., Atladottir, H.O., Norgaard-Pedersen, B., Bonefeld-Jorgensen, E.C., et al., 2012b. Neonatal levels of cytokines and risk of autism spectrum disorders: an exploratory register-based historic birth cohort study utilizing the Danish Newborn Screening Biobank. J. Neuroimmunol. 252, 75–82.

Abdallah, M.W., Larsen, N., Grove, J., Norgaard-Pedersen, B., Thorsen, P., Mortensen, E.L., et al., 2012a. Amniotic fluid chemokines and autism spectrum disorders: an exploratory study utilizing a Danish Historic Birth Cohort. Brain Behav. Immun. 26, 170–176.

Abdallah, M.W., Larsen, N., Grove, J., Norgaard-Pedersen, B., Thorsen, P., Mortensen, E.L., et al., 2013. Amniotic fluid inflammatory cytokines: potential markers of immunologic dysfunction in autism spectrum disorders. World J. Biol. Psychiatry 14, 528–538.

Abdolhoseini, M., Walker, F., Johnson, S., 2016. Automated tracing of microglia using multilevel thresholding and minimum spanning trees. Conf. Proc. IEEE Eng. Med. Biol. Soc. 2016, 1208–1211.

Adams, K.L., Gallo, V., 2018. The diversity and disparity of the glial scar. Nat. Neurosci. 21, 9–15.

Ahlsen, G., Rosengren, L., Belfrage, M., Palm, A., Haglid, K., Hamberger, A., et al., 1993. Glial fibrillary acidic protein in the cerebrospinal fluid of children with autism and other neuropsychiatric disorders. Biol. Psychiatry 33, 734–743.

Akintunde, M.E., Rose, M., Krakowiak, P., Heuer, L., Ashwood, P., Hansen, R., et al., 2015. Increased production of IL-17 in children with autism spectrum disorders and co-morbid asthma. J. Neuroimmunol. 286, 33–41.

Al-Ayadhi, L.Y., 2005. Pro-inflammatory cytokines in autistic children in central Saudi Arabia. Neurosciences (Riyadh) 10, 155–158.

Al-Ayadhi, L.Y., Mostafa, G.A., 2012b. A lack of association between elevated serum levels of S100B protein and autoimmunity in autistic children. J. Neuroinflammation 9, 54.

Al-Ayadhi, L.Y., Mostafa, G.A., 2012a. Elevated serum levels of interleukin-17A in children with autism. J. Neuroinflammation 9, 158.

Anderson, C.M., Swanson, R.A., 2000. Astrocyte glutamate transport: review of properties, regulation, and physiological functions. Glia 32, 1–14.

Araque, A., Navarrete, M., 2010. Glial cells in neuronal network function. Philos. Trans. R. Soc. Lond. B Biol. Sci. 365, 2375–2381.

Asher, R.A., Morgenstern, D.A., Fidler, P.S., Adcock, K.H., Oohira, A., Braistead, J.E., et al., 2000. Neurocan is upregulated in injured brain and in cytokine-treated astrocytes. J. Neurosci. 20, 2427–2438.

Ashwood, P., Enstrom, A., Krakowiak, P., Hertz-Picciotto, I., Hansen, R., Croen, L.A., et al., 2008. Decreased transforming growth factor beta1 in autism: a potential link between immune dysregulation and impairment in clinical behavioral outcomes. J. Neuroimmunol. 204, 149–153.

Ashwood, P., Krakowiak, P., Hertz-Picciotto, I., Hansen, R., Pessah, I.N., Van de Water, J., 2011c. Associations of impaired behaviors with elevated plasma chemokines in autism spectrum disorders. J. Neuroimmunol. 232, 196–199.

Ashwood, P., Krakowiak, P., Hertz-Picciotto, I., Hansen, R., Pessah, I.N., Van de Water, J., 2011b. Altered T cell responses in children with autism. Brain Behav. Immun. 25, 840–849.

Ashwood, P., Krakowiak, P., Hertz-Picciotto, I., Hansen, R., Pessah, I., Van de Water, J., 2011a. Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. Brain Behav. Immun. 25, 40–45.

Atladottir, H.O., Thorsen, P., Ostergaard, L., Schendel, D.E., Lemcke, S., Abdallah, M., et al., 2010. Maternal infection requiring hospitalization during pregnancy and autism spectrum disorders. J. Autism Dev. Disord. 40, 1423–1430.

Balasingam, V., Tejada-Berges, T., Wright, E., Bouckova, R., Yong, V.W., 1994. Reactive astrogliosis in the neonatal mouse brain and its modulation by cytokines. J. Neurosci. 14, 846–856.

Banks, W.A., Ortiz, L., Plotkin, S.R., Kastin, A.J., 1991. Human interleukin (IL) 1 alpha, murine IL-1 alpha and murine IL-1 beta are transported from blood to brain in the mouse by a shared saturable mechanism. J. Pharmacol. Exp. Ther. 259, 988–996.

Banks, W.A., Kastin, A.J., Broadwell, R.D., 1995. Passage of cytokines across the blood-brain barrier. Neuroimmunomodulation 2, 241–248.

Basheer, S., Venkataswamy, M.M., Christopher, R., Van Amelsvoort, T., Srinath, S., Girimaji, S.C., et al., 2018. Immune aberrations in children with Autism Spectrum Disorder: a case-control study from a tertiary care neuropsychiatric hospital in India. Psychoneuroendocrinology 94, 162–167.

Bellinger, F.P., Madamba, S., Siggins, G.R., 1993. Interleukin 1 beta inhibits synaptic strength and long-term potentiation in the rat CA1 hippocampus. Brain Res. 628, 227–234.

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

Bessis, A., Bechade, C., Bernard, D., Roumier, A., 2007. Microglial control of neuronal death and synaptic properties. Glia 55, 233–238.

Bialas, A.R., Stevens, B., 2013. TGF-beta signaling regulates neuronal C1q expression and developmental synaptic refinement. Nat. Neurosci. 16, 1773–1782.

Biber, K., Neumann, H., Inoue, K., Boddeke, H.W., 2007. Neuronal 'On' and 'Off' signals control microglia. Trends Neurosci. 30, 596–602.

Brionne, T.C., Tesseur, I., Masliah, E., Wyss-Coray, T., 2003. Loss of TGF-β1 leads to increased neuronal cell death and microgliosis in mouse brain. Neuron 40, 1133–1145.

Budimirovic, D.B., Kaufmann, W.E., 2011. What can we learn about autism from studying fragile X syndrome? Dev. Neurosci. 33, 379–394.

Bush, T.G., Puvanachandra, N., Horner, C.H., Polito, A., Ostenfeld, T., Svendsen, C.N., et al., 1999. Leukocyte infiltration, neuronal degeneration, and neurite outgrowth after ablation of scar-forming, reactive astrocytes in adult transgenic mice. Neuron 23, 297–308.

Businaro, R., Corsi, M., Azzara, G., Di Raimo, T., Laviola, G., Romano, E., et al., 2016. Interleukin-18 modulation in autism spectrum disorders. J. Neuroinflammation 13, 2.

Butovsky, O., Jedrychowski, M.P., Moore, C.S., Cialic, R., Lanser, A.J., Gabriely, G., et al., 2014. Identification of a unique TGF-beta-dependent molecular and functional signature in microglia. Nat. Neurosci. 17, 131–143.

Capaldo, C.T., Nusrat, A., 2009. Cytokine regulation of tight junctions. Biochim. Biophys. Acta 1788, 864–871.

Careaga, M., Van de Water, J., Ashwood, P., 2010. Immune dysfunction in autism: a pathway to treatment. Neurotherapeutics 7, 283–292.

Cekanaviciute, E., Fathali, N., Doyle, K.P., Williams, A.M., Han, J., Buckwalter, M.S., 2014. Astrocytic transforming growth factor-beta signaling reduces subacute neuroinflammation after stroke in mice. Glia 62, 1227–1240.

Chez, M.G., Dowling, T., Patel, P.B., Khanna, P., Kominsky, M., 2007. Elevation of tumor necrosis factor-alpha in cerebrospinal fluid of autistic children. Pediatr. Neurol. 36, 361–365.

Christensen, J., Gronborg, T.K., Sorensen, M.J., Schendel, D., Parner, E.T., Pedersen, L.H., et al., 2013. Prenatal valproate exposure and risk of autism spectrum disorders and childhood autism. JAMA 309, 1696–1703.

Chung, W.S., Clarke, L.E., Wang, G.X., Stafford, B.K., Sher, A., Chakraborty, C., et al., 2013. Astrocytes mediate synapse elimination through MEGF10 and MERTK pathways. Nature 504, 394–400.

Chung, W.S., Allen, N.J., Eroglu, C., 2015. Astrocytes control synapse formation, function, and elimination. Cold Spring Harb. Perspect. Biol. 7, a020370.

Clarke, L.E., Barres, B.A., 2013. Emerging roles of astrocytes in neural circuit development. Nat. Rev. Neurosci. 14, 311–321.

Colonna, M., Butovsky, O., 2017. Microglia function in the central nervous system during health and neurodegeneration. Annu. Rev. Immunol. 35, 441–468.

Corthay, A., 2009. How do regulatory T cells work? Scand. J. Immunol. 70, 326–336.

Couper, K.N., Blount, D.G., Riley, E.M., 2008. IL-10: the master regulator of immunity to infection. J. Immunol. 180, 5771–5777.
Croonenberghs, J., Bosmans, E., Deboutte, D., Kenis, G., Maes, M., 2002. Activation of the inflammatory response system in autism. Neuropsychobiology 45, 1–6.
Cryan, J.F., Dinan, T.G., 2012. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. Nat. Rev. Neurosci. 13, 701–712.
Cunningham, A.J., Murray, C.A., O'Neill, L.A., Lynch, M.A., O'Connor, J.J., 1996. Interleukin-1 beta (IL-1 beta) and tumour necrosis factor (TNF) inhibit long-term potentiation in the rat dentate gyrus in vitro. Neurosci. Lett. 203, 17–20.
de Haas, A.H., van Weering, H.R., de Jong, E.K., Boddeke, H.W., Biber, K.P., 2007. Neuronal chemokines: versatile messengers in central nervous system cell interaction. Mol. Neurobiol. 36, 137–151.
de Magistris, L., Familiari, V., Pascotto, A., Sapone, A., Frolli, A., Iardino, P., et al., 2010. Alterations of the intestinal barrier in patients with autism spectrum disorders and in their first-degree relatives. J. Pediatr. Gastroenterol. Nutr. 51, 418–424.
de Theije, C.G., Wopereis, H., Ramadan, M., van Eijndthoven, T., Lambert, J., Knol, J., et al., 2014. Altered gut microbiota and activity in a murine model of autism spectrum disorders. Brain Behav. Immun. 37, 197–206.
Desbonnet, L., Clarke, G., Shanahan, F., Dinan, T.G., Cryan, J.F., 2014. Microbiota is essential for social development in the mouse. Mol. Psychiatry 19, 146–148.
Dheen, S.T., Kaur, C., Ling, E.A., 2007. Microglial activation and its implications in the brain diseases. Curr. Med. Chem. 14, 1189–1197.
Di Filippo, M., Chiasserini, D., Gardoni, F., Viviani, B., Tozzi, A., Giampa, C., et al., 2013. Effects of central and peripheral inflammation on hippocampal synaptic plasticity. Neurobiol. Dis. 52, 229–236.
Dinan, T.G., Cryan, J.F., 2017. The microbiome-gut-brain axis in health and disease. Gastroenterol. Clin. North Am. 46, 77–89.
Dinan, T.G., Stilling, R.M., Stanton, C., Cryan, J.F., 2015. Collective unconscious: how gut microbes shape human behavior. J. Psychiatr Res. 63, 1–9.
Diniz, L.P., Matias, I.C., Garcia, M.N., Gomes, F.C., 2014. Astrocytic control of neural circuit formation: highlights on TGF-beta signaling. Neurochem. Int. 78, 18–27.
Donzis, E.J., Tronson, N.C., 2014. Modulation of learning and memory by cytokines: signaling mechanisms and long term consequences. Neurobiol. Learn Mem. 115, 68–77.
Dubbelaar, M.L., Kracht, L., Eggen, B.J.L., Boddeke, E., 2018. The kaleidoscope of microglial phenotypes. Fron. Immunol. 9, 1753.
Edmonson, C., Ziats, M.N., Rennert, O.M., 2014. Altered glial marker expression in autistic post-mortem prefrontal cortex and cerebellum. Mol. Autism 5, 3.
Salah El Din El Wakkad A, & Tawheed Saleh M (2006) The Proinflammatory Cytokines in Children with Autism. vol. 9.
El Gohary, T.M., El Aziz, N.A., Darweesh, M., Sadaa, E.S., 2015. Plasma level of transforming growth factor β 1 in children with autism spectrum disorder. Egyptian J. Ear, Nose, Throat Allied Sci. 16, 69–73.
Emanuele, E., Orsi, P., Boso, M., Broglia, D., Brondino, N., Barale, F., et al., 2010. Low-grade endotoxemia in patients with severe autism. Neurosci. Lett. 471, 162–165.
Enstrom, A., Onore, C., Hertz-Picciotto, I., Hansen, R., Croen, L., Van de Water, J., et al., 2008. Detection of IL-17 and IL-23 in plasma samples of children with autism. Am. J. Biochem. Biotechnol. 4, 114–120.
Enstrom, A.M., Onore, C.E., Van de Water, J.A., Ashwood, P., 2010. Differential monocyte responses to TLR ligands in children with autism spectrum disorders. Brain Behav. Immun. 24, 64–71.
Erny, D., Hrabe de Angelis, A.L., Jaitin, D., Wieghofer, P., Staszewski, O., David, E., et al., 2015. Host microbiota constantly control maturation and function of microglia in the CNS. Nat. Neurosci. 18, 965–977.
Fernandez de Cossio, L., Guzman, A., van der Veldt, S., Luheshi, G.N., 2017. Prenatal infection leads to ASD-like behavior and altered synaptic pruning in the mouse offspring. Brain Behav. Immun. 63, 88–98.
Fields, R.D., Stevens-Graham, B., 2002. New insights into neuron-glia communication. Science 298, 556–562.
Filiano, A.J., Gadani, S.P., Kipnis, J., 2017. How and why do T cells and their derived cytokines affect the injured and healthy brain? Nat. Rev. Neurosci. 18, 375–384.
Fiorentino, M., Sapone, A., Senger, S., Camhi, S.S., Kadzielski, S.M., Buie, T.M., et al., 2016. Blood-brain barrier and intestinal epithelial barrier alterations in autism spectrum disorders. Mol. Autism 7, 49.
Gaffen, S.L., Jain, R., Garg, A.V., Cua, D.J., 2014. The IL-23-IL-17 immune axis: from mechanisms to therapeutic testing. Nat. Rev. Immunol. 14, 585–600.
Gao, Z., Zhu, Q., Zhang, Y., Zhao, Y., Cai, L., Shields, C.B., et al., 2013. Reciprocal modulation between microglia and astrocyte in reactive gliosis following the CNS injury. Mol. Neurobiol. 48, 690–701.
Gaykema, R.P., Goehler, L.E., Lyte, M., 2004. Brain response to cecal infection with Campylobacter jejuni: analysis with Fos immunohistochemistry. Brain Behav. Immun. 18, 238–245.
Ghaffari, M.A., Mousavinejad, E., Riahi, F., Mousavinejad, M., Afsharmanesh, M.R., 2016. Increased serum levels of tumor necrosis factor-alpha, resistin, and visfatin in the children with autism spectrum disorders: a case-control study. Neurol. Res. Int. 2016, 9060751.
Goshen, I., Kreisel, T., Ounallah-Saad, H., Renbaum, P., Zalzstein, Y., Ben-Hur, T., et al., 2007. A dual role for interleukin-1 in hippocampal-dependent memory processes. Psychoneuroendocrinology 32, 1106–1115.
Gruol, D.L., 2015. IL-6 regulation of synaptic function in the CNS. Neuropharmacology 96, 42–54.
Gupta, S., Aggarwal, S., Rashanravan, B., Lee, T., 1998. Th1- and Th2-like cytokines in CD4+ and CD8+ T cells in autism. J. Neuroimmunol. 85, 106–109.
Hanisch, U.K., Kettenmann, H., 2007. Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat. Neurosci. 10, 1387–1394.
Hashim, H., Abdelrahman, H., Mohammed, D., Karam, R., 2013. Association between plasma levels of transforming growth factor-β1, IL-23 and IL-17 and the severity of autism in Egyptian children. Res. Autism Spectrum Disord. 7, 199–204.
He, M., Dong, H., Huang, Y., Lu, S., Zhang, S., Qian, Y., et al., 2016. Astrocyte-derived CCL2 is associated with M1 activation and recruitment of cultured microglial cells. Cell Physiol. Biochem. 38, 859–870.
Hellstrom, I.C., Danik, M., Luheshi, G.N., Williams, S., 2005. Chronic LPS exposure produces changes in intrinsic membrane properties and a sustained IL-beta-dependent increase in GABAergic inhibition in hippocampal CA1 pyramidal neurons. Hippocampus 15, 656–664.
Heneka, M.T., Carson, M.J., El Khoury, J., Landreth, G.E., Brosseron, F., Feinstein, D.L., et al., 2015. Neuroinflammation in Alzheimer's disease. Lancet Neurol. 14, 388–405.
Heo, Y., Zhang, Y., Gao, D., Miller, V.M., Lawrence, D.A., 2011. Aberrant immune responses in a mouse with behavioral disorders. PLoS One 6, e20912.
Hinojosa, A.E., Garcia-Bueno, B., Leza, J.C., Madrigal, J.L., 2011. CCL2/MCP-1 modulation of microglial activation and proliferation. J. Neuroinflammation 8, 77.
Hsiao, E.Y., McBride, S.W., Chow, J., Mazmanian, S.K., Patterson, P.H., 2012. Modeling an autism risk factor in mice leads to permanent immune dysregulation. Proc. Natl. Acad. Sci. U.S.A. 109, 12776–12781.
Hsiao, E.Y., McBride, S.W., Hsien, S., Sharon, G., Hyde, E.R., McCue, T., et al., 2013. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell 155, 1451–1463.
Hu, C.C., Xu, X., Xiong, G.L., Xu, Q., Zhou, B.R., Li, C.Y., et al., 2018. Alterations in plasma cytokine levels in chinese children with autism spectrum disorder. Autism Res. 11, 989–999.
Hutsler, J.J., Zhang, H., 2010. Increased dendritic spine densities on cortical projection neurons in autism spectrum disorders. Brain Res. 1309, 83–94.
Just, M.A., Cherkassky, V.L., Keller, T.A., Minshew, N.J., 2004. Cortical activation and synchronization during sentence comprehension in high-functioning autism: evidence of underconnectivity. Brain 127, 1811–1821.
Just, M.A., Cherkassky, V.L., Keller, T.A., Kana, R.K., Minshew, N.J., 2007. Functional and anatomical cortical underconnectivity in autism: evidence from an FMRI study of an executive function task and corpus callosum morphometry. Cereb. Cortex 17, 951–961.
Jyonouchi, H., Sun, S., Le, H., 2001. Proinflammatory and regulatory cytokine production associated with innate and adaptive immune responses in children with autism spectrum disorders and developmental regression. J. Neuroimmunol. 120, 170–179.
Kang, D.W., Adams, J.B., Gregory, A.C., Borody, T., Chittick, L., Fasano, A., et al., 2017. Microbiota Transfer Therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. Microbiome 5, 10.
Kanner, A.A., Marchi, N., Fazio, V., Mayberg, M.R., Koltz, M.T., Siomin, V., et al., 2003. Serum S100beta: a noninvasive marker of blood-brain barrier function and brain lesions. Cancer 97, 2806–2813.
Karperien, A., Ahammer, H., Jelinek, H.F., 2013. Quantitating the subtleties of microglial morphology with fractal analysis. Front. Cell Neurosci. 7, 3.
Kettenmann, H., Kirchhoff, F., Verkhratsky, A., 2013. Microglia: new roles for the synaptic stripper. Neuron 77, 10–18.
Kierdorf, K., Prinz, M., 2013. Factors regulating microglia activation. Front. Cell Neurosci. 7, 44.
Kilpinen, H., Ylisaukko-Oja, T., Hennah, W., Palo, O.M., Varilo, T., Vanhala, R., et al., 2008. Association of DISC1 with autism and Asperger syndrome. Mol. Psychiatry 13, 187–196.
Kipnis, J., Cohen, H., Cardon, M., Ziv, Y., Schwartz, M., 2004. T cell deficiency leads to cognitive dysfunction: implications for therapeutic vaccination for schizophrenia and other psychiatric conditions. Proc. Natl. Acad. Sci. U.S.A. 101, 8180–8185.
Kirsten, T.B., Casarin, R.C., Bernardi, M.M., Felicio, L.F., 2018. Pioglitazone abolishes autistic-like behaviors via the IL-6 pathway. PLoS One 13, e0197060.
Kleinhans, N.M., Richards, T., Sterling, L., Stegbauer, K.C., Mahurin, R., Johnson, L.C., et al., 2008. Abnormal functional connectivity in autism spectrum disorders during face processing. Brain 131, 1000–1012.
Koeglsperger, T., Li, S., Brenneis, C., Saulnier, J.L., Mayo, L., Carrier, Y., et al., 2013. Impaired glutamate recycling and GluN2B-mediated neuronal calcium overload in mice lacking TGF-beta1 in the CNS. Glia 61, 985–1002.
Kondelkova, K., Vokurkova, D., Krejsek, J., Borska, L., Fiala, Z., Ctirad, A., 2010. Regulatory T cells (TREG) and their roles in immune system with respect to immunopathological disorders. Acta Medica (Hradec Kralove) 53, 73–77.
Kongsui, R., Beynon, S.B., Johnson, S.J., Walker, F.R., 2014. Quantitative assessment of microglial morphology and density reveals remarkable consistency in the distribution and morphology of cells within the healthy prefrontal cortex of the rat. J. Neuroinflammation 11, 182.
Korn, T., Bettelli, E., Oukka, M., Kuchroo, V.K., 2009. IL-17 and Th17 Cells. Annu. Rev. Immunol. 27, 485–517.
Koshino, H., Kana, R.K., Keller, T.A., Cherkassky, V.L., Minshew, N.J., Just, M.A., 2008. fMRI investigation of working memory for faces in autism: visual coding and underconnectivity with frontal areas. Cereb. Cortex 18, 289–300.
Krakowiak, P., Goines, P.E., Tancredi, D.J., Ashwood, P., Hansen, R.L., Hertz-Picciotto, I., et al., 2017. Neonatal cytokine profiles associated with autism spectrum disorder. Biol Psychiatry 81, 442–451.
Kreutzberg, G.W., 1995. Microglia, the first line of defence in brain pathologies. Arzneimittelforschung 45, 357–360.
Kreutzberg, G.W., 1996. Microglia: a sensor for pathological events in the CNS. Trends Neurosci. 19, 312–318.
Laing, K.J., Secombes, C.J., 2004. Chemokines. Dev. Comp. Immunol. 28, 443–460.
Laurence, J.A., Fatemi, S.H., 2005. Glial fibrillary acidic protein is elevated in superior frontal, parietal and cerebellar cortices of autistic subjects. Cerebellum 4, 206–210.
Lax, P., Limatola, C., Fucile, S., Trettel, F., Di Bartolomeo, S., Renzi, M., et al., 2002. Chemokine receptor CXCR2 regulates the functional properties of AMPA-type

glutamate receptor GluR1 in HEK cells. J. Neuroimmunol. 129, 66–73.
Lee, M., Krishnamurthy, J., Susi, A., Sullivan, C., Gorman, G.H., Hisle-Gorman, E., et al., 2018. Association of autism spectrum disorders and inflammatory bowel disease. J. Autism Dev. Disord. 48, 1523–1529.
Lenz, K.M., Nelson, L.H., 2018. Microglia and beyond: innate immune cells as regulators of brain development and behavioral function. Front. Immunol. 9, 698.
Letterio, J.J., Roberts, A.B., 1998. Regulation of immune responses by TGF-beta. Annu. Rev. Immunol. 16, 137–161.
Li, X., Chauhan, A., Sheikh, A.M., Patil, S., Chauhan, V., Li, X.M., et al., 2009. Elevated immune response in the brain of autistic patients. J. Neuroimmunol. 207, 111–116.
Li, Q., Han, Y., Dy, A.B.C., Hagerman, R.J., 2017. The gut microbiota and autism spectrum disorders. Front. Cell Neurosci. 11, 120.
Liang, J., Takeuchi, H., Doi, Y., Kawanokuchi, J., Sonobe, Y., Jin, S., et al., 2008. Excitatory amino acid transporter expression by astrocytes is neuroprotective against microglial excitotoxicity. Brain Res. 1210, 11-19.
Liddelow, S.A., Guttenplan, K.A., Clarke, L.E., Bennett, F.C., Bohlen, C.J., Schirmer, L., et al., 2017. Neurotoxic reactive astrocytes are induced by activated microglia. Nature 541, 481–487.
Liu, F., Horton-Sparks, K., Hull, V., Li, R.W., Martinez-Cerdeno, V., 2018. The valproic acid rat model of autism presents with gut bacterial dysbiosis similar to that in human autism. Mol. Autism 9, 61.
Lucchina, L., Depino, A.M., 2014. Altered peripheral and central inflammatory responses in a mouse model of autism. Autism Res. 7, 273–289.
Luna, R.A., Oezguen, N., Balderas, M., Venkatachalam, A., Runge, J.K., Versalovic, J., et al., 2017. Distinct microbiome-neuroimmune signatures correlate with functional abdominal pain in children with autism spectrum disorder. Cell Mol. Gastroenterol. Hepatol. 3, 218–230.
Lundgren, O., Jodal, M., Jansson, M., Ryberg, A.T., Svensson, L., 2011. Intestinal epithelial stem/progenitor cells are controlled by mucosal afferent nerves. PLoS One 6, e16295.
Lyte, M., Li, W., Opitz, N., Gaykema, R.P., Goehler, L.E., 2006. Induction of anxiety-like behavior in mice during the initial stages of infection with the agent of murine colonic hyperplasia Citrobacter rodentium. Physiol. Behav. 89, 350–357.
Malkova, N.V., Yu, C.Z., Hsiao, E.Y., Moore, M.J., Patterson, P.H., 2012. Maternal immune activation yields offspring displaying mouse versions of the three core symptoms of autism. Brain Behav. Immun. 26, 607–616.
Manzardo, A.M., Henkhaus, R., Dhillon, S., Butler, M.G., 2012. Plasma cytokine levels in children with autistic disorder and unrelated siblings. Int. J. Dev. Neurosci. 30, 121–127.
Marcaggi, P., Attwell, D., 2004. Role of glial amino acid transporters in synaptic transmission and brain energetics. Glia 47, 217–225.
Marchi, N., Rasmussen, P., Kapural, M., Fazio, V., Kight, K., Mayberg, M.R., et al., 2003. Peripheral markers of brain damage and blood-brain barrier dysfunction. Restor Neurol. Neurosci. 21, 109–121.
Martinez-Cerdeno, V., 2017. Dendrite and spine modifications in autism and related neurodevelopmental disorders in patients and animal models. Dev. Neurobiol. 77, 393–404.
Masi, A., Glozier, N., Dale, R., Guastella, A.J., 2017. The immune system, cytokines, and biomarkers in autism spectrum disorder. Neurosci. Bull. 33, 194–204.
McAfoose, J., Baune, B.T., 2009. Evidence for a cytokine model of cognitive function. Neurosci. Biobehav. Rev. 33, 355–366.
McElhanon, B.O., McCracken, C., Karpen, S., Sharp, W.G., 2014. Gastrointestinal symptoms in autism spectrum disorder: a meta-analysis. Pediatrics 133, 872–883.
Mittal, M., Siddiqui, M.R., Tran, K., Reddy, S.P., Malik, A.B., 2014. Reactive oxygen species in inflammation and tissue injury. Antioxid. Redox. Signal. 20, 1126–1167.
Molloy, C.A., Morrow, A.L., Meinzen-Derr, J., Schleifer, K., Dienger, K., Manning-Courtney, P., et al., 2006. Elevated cytokine levels in children with autism spectrum disorder. J. Neuroimmunol. 172, 198–205.
Morgan, J.T., Chana, G., Pardo, C.A., Achim, C., Semendeferi, K., Buckwalter, J., et al., 2010. Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. Biol. Psychiatry 68, 368–376.
Morgan, J.T., Chana, G., Abramson, I., Semendeferi, K., Courchesne, E., Everall, I.P., 2012. Abnormal microglial-neuronal spatial organization in the dorsolateral prefrontal cortex in autism. Brain Res. 1456, 72–81.
Mukaetova-Ladinska, E.B., Arnold, H., Jaros, E., Perry, R., Perry, E., 2004. Depletion of MAP2 expression and laminar cytoarchitectonic changes in dorsolateral prefrontal cortex in adult autistic individuals. Neuropathol. Appl. Neurobiol. 30, 615–623.
Najjar, S., Pearlman, D.M., Alper, K., Najjar, A., Devinsky, O., 2013. Neuroinflammation and psychiatric illness. J. Neuroinflammation 10, 43.
Nakajima, K., Kohsaka, S., 2004. Microglia: neuroprotective and neurotrophic cells in the central nervous system. Curr. Drug Targets Cardiovasc. Haematol. Disord. 4, 65–84.
Napoli, I., Neumann, H., 2009. Microglial clearance function in health and disease. Neuroscience 158, 1030–1038.
Napolioni, V., Ober-Reynolds, B., Szelinger, S., Corneveaux, J.J., Pawlowski, T., Ober-Reynolds, S., et al., 2013. Plasma cytokine profiling in sibling pairs discordant for autism spectrum disorder. J. Neuroinflammation 10, 38.
Okada, K., Hashimoto, K., Iwata, Y., Nakamura, K., Tsujii, M., Tsuchiya, K.J., et al., 2007. Decreased serum levels of transforming growth factor-beta1 in patients with autism. Prog. Neuropsychopharmacol. Biol. Psychiatry 31, 187–190.
Onore, C., Enstrom, A., Krakowiak, P., Hertz-Picciotto, I., Hansen, R., Van de Water, J., et al., 2009. Decreased cellular IL-23 but not IL-17 production in children with autism spectrum disorders. J. Neuroimmunol. 216, 126–129.
Onore, C., Careaga, M., Ashwood, P., 2012. The role of immune dysfunction in the pathophysiology of autism. Brain Behav. Immun. 26, 383–392.
Pacey, L.K., Guan, S., Tharmalingam, S., Thomsen, C., Hampson, D.R., 2015. Persistent astrocyte activation in the fragile X mouse cerebellum. Brain Behav. 5, e00400.
Pan, W., Zadina, J.E., Harlan, R.E., Weber, J.T., Banks, W.A., Kastin, A.J., 1997. Tumor necrosis factor-alpha: a neuromodulator in the CNS. Neurosci. Biobehav. Rev. 21, 603–613.
Paolicelli, R.C., 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, 1456–1458.
Pardo, C.A., Eberhart, C.G., 2007. The neurobiology of autism. Brain Pathol. 17, 434–447.
Pardo, C.A., Vargas, D.L., Zimmerman, A.W., 2005. Immunity, neuroglia and neuroinflammation in autism. Int. Rev. Psychiatry 17, 485–495.
Parker-Athill, E., Luo, D., Bailey, A., Giunta, B., Tian, J., Shytle, R.D., et al., 2009. Flavonoids, a prenatal prophylaxis via targeting JAK2/STAT3 signaling to oppose IL-6/MIA associated autism. J. Neuroimmunol. 217, 20–27.
Pekny, M., Wilhelmsson, U., Pekna, M., 2014. The dual role of astrocyte activation and reactive gliosis. Neurosci. Lett. 565, 30–38.
Perea, G., Navarrete, M., Araque, A., 2009. Tripartite synapses: astrocytes process and control synaptic information. Trends Neurosci. 32, 421–431.
Philips, T., Robberecht, W., 2011. Neuroinflammation in amyotrophic lateral sclerosis: role of glial activation in motor neuron disease. Lancet Neurol. 10, 253–263.
Placone, A.L., McGuiggan, P.M., Bergles, D.E., Guerrero-Cazares, H., Quinones-Hinojosa, A., Searson, P.C., 2015. Human astrocytes develop physiological morphology and remain quiescent in a novel 3D matrix. Biomaterials 42, 134–143.
Prieto, G.A., Cotman, C.W., 2017. Cytokines and cytokine networks target neurons to modulate long-term potentiation. Cytokine Growth Factor Rev. 34, 27–33.
Puma, C., Danik, M., Quirion, R., Ramon, F., Williams, S., 2001. The chemokine interleukin-8 acutely reduces Ca(2+) currents in identified cholinergic septal neurons expressing CXCR1 and CXCR2 receptor mRNAs. J. Neurochem. 78, 960–971.
Pun, P.B., Lu, J., Moochhala, S., 2009. Involvement of ROS in BBB dysfunction. Free Radic. Res. 43, 348–364.
Raymond, G.V., Bauman, M.L., Kemper, T.L., 1996. Hippocampus in autism: a Golgi analysis. Acta Neuropathol. 91, 117–119.
Reemst, K., Noctor, S.C., Lucassen, P.J., Hol, E.M., 2016. The indispensable roles of microglia and astrocytes during brain development. Front. Hum. Neurosci. 10, 566.
Ricci, S., Businaro, R., Ippoliti, F., Lo Vasco, V.R., Massoni, F., Onofri, E., et al., 2013. Altered cytokine and BDNF levels in autism spectrum disorder. Neurotox. Res. 24, 491–501.
Rock, R.B., Gekker, G., Hu, S., Sheng, W.S., Cheeran, M., Lokensgard, J.R., et al., 2004. Role of microglia in central nervous system infections. Clin. Microbiol. Rev. 17, 942–964 table of contents.
Rogers, J.T., Morganti, J.M., Bachstetter, A.D., Hudson, C.E., Peters, M.M., Grimmig, B.A., et al., 2011. CX3CR1 deficiency leads to impairment of hippocampal cognitive function and synaptic plasticity. J. Neurosci. 31, 16241–16250.
Rolls, A., Shechter, R., London, A., Segev, Y., Jacob-Hirsch, J., Amariglio, N., et al., 2008. Two faces of chondroitin sulfate proteoglycan in spinal cord repair: a role in microglia/macrophage activation. PLoS Med. 5, e171.
Rose, D., Ashwood, P., 2014. Potential cytokine biomarkers in autism spectrum disorders. Biomark Med. 8, 1171–1181.
Rosengren, L.E., Ahlsen, G., Belfrage, M., Gillberg, C., Haglid, K.G., Hamberger, A., 1992. A sensitive ELISA for glial fibrillary acidic protein: application in CSF of children. J. Neurosci. Methods 44, 113–119.
Roumier, A., Bechade, C., Poncer, J.C., Smalla, K.H., Tomasello, E., Vivier, E., et al., 2004. Impaired synaptic function in the microglial KARAP/DAP12-deficient mouse. J. Neurosci. 24, 11421–11428.
Roumier, A., Pascual, O., Bechade, C., Wakselman, S., Poncer, J.C., Real, E., et al., 2008. Prenatal activation of microglia induces delayed impairment of glutamatergic synaptic function. PLoS One 3, e2595.
Sawada, M., Kondo, N., Suzumura, A., Marunouchi, T., 1989. Production of tumor necrosis factor-alpha by microglia and astrocytes in culture. Brain Res. 491, 394–397.
Sgritta, M., Dooling, S.W., Buffington, S.A., Momin, E.N., Francis, M.B., Britton, R.A., et al., 2019. Mechanisms underlying microbial-mediated changes in social behavior in mouse models of autism spectrum disorder. Neuron 101 (246–259), e246.
Shen, Y., Ou, J., Liu, M., Shi, L., Li, Y., Xiao, L., et al., 2016. Altered plasma levels of chemokines in autism and their association with social behaviors. Psychiatry Res. 244, 300–305.
Shibuki, K., Gomi, H., Chen, L., Bao, S., Kim, J.J., Wakatsuki, H., et al., 1996. Deficient cerebellar long-term depression, impaired eyeblink conditioning, and normal motor coordination in GFAP mutant mice. Neuron 16, 587–599.
Shinozaki, Y., Shibata, K., Yoshida, K., Shigetomi, E., Gachet, C., Ikenaka, K., et al., 2017. Transformation of astrocytes to a neuroprotective phenotype by microglia via P2Y1 receptor downregulation. Cell Rep. 19, 1151–1164.
Shull, M.M., Ormsby, I., Kier, A.B., Pawlowski, S., Diebold, R.J., Yin, M., et al., 1992. Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. Nature 359, 693–699.
Singh, V.K., 1996. Plasma increase of interleukin-12 and interferon-gamma. Pathological significance autism. J. Neuroimmunol. 66, 143–145.
Sofroniew, M.V., Vinters, H.V., 2010. Astrocytes: biology and pathology. Acta Neuropathol. 119, 7–35.
Song, L., Pachter, J.S., 2004. Monocyte chemoattractant protein-1 alters expression of tight junction-associated proteins in brain microvascular endothelial cells. Microvasc. Res. 67, 78–89.
Stamatovic, S.M., Shakui, P., Keep, R.F., Moore, B.B., Kunkel, S.L., Van Rooijen, N., et al., 2005. Monocyte chemoattractant protein-1 regulation of blood-brain barrier permeability. J. Cereb. Blood Flow Metab. 25, 593–606.
Steinmetz, C.C., Turrigiano, G.G., 2010. Tumor necrosis factor-alpha signaling maintains the ability of cortical synapses to express synaptic scaling. J. Neurosci. 30, 14685–14690.
Supekar, K., Uddin, L.Q., Khouzam, A., Phillips, J., Gaillard, W.D., Kenworthy, L.E., et al.,

2013. Brain hyperconnectivity in children with autism and its links to social deficits. Cell Rep. 5, 738–747.
Suzuki, K., Matsuzaki, H., Iwata, K., Kameno, Y., Shimmura, C., Kawai, S., et al., 2011. Plasma cytokine profiles in subjects with high-functioning autism spectrum disorders. PLoS One 6, e20470.
Tabouy, L., Getselter, D., Ziv, O., Karpuj, M., Tabouy, T., Lukic, I., et al., 2018. Dysbiosis of microbiome and probiotic treatment in a genetic model of autism spectrum disorders. Brain Behav. Immun. 73, 310–319.
Takeshita, Y., Ransohoff, R.M., 2012. Inflammatory cell trafficking across the blood-brain barrier: chemokine regulation and in vitro models. Immunol. Rev. 248, 228–239.
Tancredi, V., D'Arcangelo, G., Grassi, F., Tarroni, P., Palmieri, G., Santoni, A., et al., 1992. Tumor necrosis factor alters synaptic transmission in rat hippocampal slices. Neurosci. Lett. 146, 176–178.
Tay, T.L., Savage, J.C., Hui, C.W., Bisht, K., Tremblay, M.E., 2017. Microglia across the lifespan: from origin to function in brain development, plasticity and cognition. J. Physiol. 595, 1929–1945.
Tetreault, N.A., Hakeem, A.Y., Jiang, S., Williams, B.A., Allman, E., Wold, B.J., et al., 2012. Microglia in the cerebral cortex in autism. J. Autism Dev. Disord. 42, 2569–2584.
Tonhajzerova, I., Ondrejka, I., Mestanik, M., Mikolka, P., Hrtanek, I., Mestanikova, A., et al., 2015. Inflammatory activity in autism spectrum disorder. Adv. Exp. Med. Biol. 861, 93–98.
Torres-Platas, S.G., Comeau, S., Rachalski, A., Bo, G.D., Cruceanu, C., Turecki, G., et al., 2014. Morphometric characterization of microglial phenotypes in human cerebral cortex. J. Neuroinflammation 11, 12.
Tsilioni, I., Taliou, A., Francis, K., Theoharides, T.C., 2015. Children with autism spectrum disorders, who improved with a luteolin-containing dietary formulation, show reduced serum levels of TNF and IL-6. Transl. Psychiatry 5, e647.
Turnbaugh, P.J., Ridaura, V.K., Faith, J.J., Rey, F.E., Knight, R., Gordon, J.I., 2009. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. Sci. Transl. Med. 1, 6ra14.
Vargas, D.L., Nascimbene, C., Krishnan, C., Zimmerman, A.W., Pardo, C.A., 2005. Neuroglial activation and neuroinflammation in the brain of patients with autism. Ann. Neurol. 57, 67–81.
Vilhardt, F., 2005. Microglia: phagocyte and glia cell. Int. J. Biochem. Cell Biol. 37, 17–21.
Wang, X., Wang, B.R., Zhang, X.J., Xu, Z., Ding, Y.Q., Ju, G., 2002. Evidences for vagus nerve in maintenance of immune balance and transmission of immune information from gut to brain in STM-infected rats. World J. Gastroenterol. 8, 540–545.
Wang, J., Zou, Q., Han, R., Li, Y., Wang, Y., 2017. Serum levels of Glial fibrillary acidic protein in Chinese children with autism spectrum disorders. Int. J. Dev. Neurosci. 57, 41–45.
Wei, H., Zou, H., Sheikh, A.M., Malik, M., Dobkin, C., Brown, W.T., et al., 2011. IL-6 is increased in the cerebellum of autistic brain and alters neural cell adhesion, migration and synaptic formation. J. Neuroinflammation 8, 52.
Wei, H., Chadman, K.K., McCloskey, D.P., Sheikh, A.M., Malik, M., Brown, W.T., et al., 2012. Brain IL-6 elevation causes neuronal circuitry imbalances and mediates autism-like behaviors. Biochim. Biophys. Acta 1822, 831–842.
Wei, H., Alberts, I., Li, X., 2013. Brain IL-6 and autism. Neuroscience 252, 320–325.
White, J.F., 2003. Intestinal pathophysiology in autism. Exp. Biol. Med. (Maywood) 228, 639–649.
Williams, R.S., Hauser, S.L., Purpura, D.P., DeLong, G.R., Swisher, C.N., 1980. Autism and mental retardation: neuropathologic studies performed in four retarded persons with autistic behavior. Arch. Neurol. 37, 749–753.
Wills, S., Cabanlit, M., Bennett, J., Ashwood, P., Amaral, D., Van de Water, J., 2007. Autoantibodies in autism spectrum disorders (ASD). Ann. N.Y. Acad. Sci. 1107, 79–91.
Xie, J., Huang, L., Li, X., Li, H., Zhou, Y., Zhu, H., et al., 2017. Immunological cytokine profiling identifies TNF-alpha as a key molecule dysregulated in autistic children. Oncotarget 8, 82390–82398.
Yirmiya, R., Goshen, I., 2011. Immune modulation of learning, memory, neural plasticity and neurogenesis. Brain Behav. Immun. 25, 181–213.
Zerbo, O., Yoshida, C., Grether, J.K., Van de Water, J., Ashwood, P., Delorenze, G.N., et al., 2014. Neonatal cytokines and chemokines and risk of Autism Spectrum Disorder: the Early Markers for Autism (EMA) study: a case-control study. J. Neuroinflammation 11, 113.
Zhang, D., Hu, X., Qian, L., O'Callaghan, J.P., Hong, J.S., 2010. Astrogliosis in CNS pathologies: is there a role for microglia? Mol. Neurobiol. 41, 232–241.
Zhou, L., Chong, M.M., Littman, D.R., 2009. Plasticity of CD4+ T cell lineage differentiation. Immunity 30, 646–655.
Zhou, Y., Tang, H., Liu, J., Dong, J., Xiong, H., 2011. Chemokine CCL2 modulation of neuronal excitability and synaptic transmission in rat hippocampal slices. J. Neurochem. 116, 406–414.
Zhu, C.B., Blakely, R.D., Hewlett, W.A., 2006. The proinflammatory cytokines interleukin-1beta and tumor necrosis factor-alpha activate serotonin transporters. Neuropsychopharmacology 31, 2121–2131.