# Immune mediators in the brain and peripheral tissues in autism spectrum disorder

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Abstract | Increasing evidence points to a central role for immune dysregulation in autism spectrum disorder (ASD). Several ASD risk genes encode components of the immune system and many maternal immune system-related risk factors — including autoimmunity, infection and fetal reactive antibodies — are associated with ASD. In addition, there is evidence of ongoing immune dysregulation in individuals with ASD and in animal models of this disorder. Recently, several molecular signalling pathways — including pathways downstream of cytokines, the receptor MET, major histocompatibility complex class I molecules, microglia and complement factors — have been identified that link immune activation to ASD phenotypes. Together, these findings indicate that the immune system is a point of convergence for multiple ASD-related genetic and environmental risk factors.

Autoimmune disorders
Disorders wherein the immune system attacks normal substances and tissues of the body.

Autism spectrum disorder (ASD) arises during the early years of life with a heterogeneous presentation and is diagnosed based on impairments in social skills and communication, repetitive behaviour, and narrow and intense interests[1]. The estimated prevalence of ASD has recently skyrocketed: in 1992, it was estimated that 1 in 500 children in the United States had ASD, but by 2007 this figure had been adjusted to 1 in 110, and current estimates have reached the alarming level of 1 in 68 children (and 1 in 42 boys) in the United States[2]. Although the wider diagnostic criteria for, and enhanced public awareness of, ASD have surely contributed to this increase, these factors cannot account for all — and in some estimates most — of this rise in prevalence[3]. This gap implies that one or more factors in our environment have increased the likelihood of children developing ASD. Consistent with this idea, recent reports have suggested that the environment may have a much larger role in causing ASD than had been initially proposed[4,5].

Although there are many diverse environmental factors that contribute to ASD[6], most of these converge on alterations in immune responses during prenatal or early postnatal development (FIG. 1). The immune system is designed to reflect environmental changes and to predict future changes as a defensive strategy. The genetic composition and initial programming of the immune system *in utero* and shortly after birth[7,8] determine how much environmental insult the immune system can buffer during the lifetime of each individual. This buffering is important not only for general health but also for neural processing owing to the pervasive and dynamic crosstalk that occurs between the immune and nervous systems. Indeed, immune status can have profound effects on brain development and cognition (BOX 1), and alterations in immune signalling can, in different contexts, induce beneficial, homeostatic or harmful effects.

Similar to ASD, childhood disorders of the immune system such as asthma, life-threatening food allergies and autoimmune disorders have reached epidemic levels over the past two decades[9,10]. As this time frame is too short for genetic changes at a population level to have had an appreciable impact on the prevalence of these conditions, these increases must have an environmental catalyst. It is hypothesized that the rise in childhood immune disorders reflects the exposure to an increasing number of environmental stressors during critical periods of development that results in disease expression in individuals with a vulnerable genetic background[11]. This multiple-hit model for immune disorders is also hypothesized in the aetiology of ASD[6,12]. Although accumulating evidence indicates that immune dysregulation increases the risk, and contributes to the pathophysiology, of ASD, this does not mean that immune responses to vaccines cause ASD. In fact, recent studies have debunked the myth of a link between ASD and early childhood vaccinations[13]. Despite the unequivocal and compelling research behind

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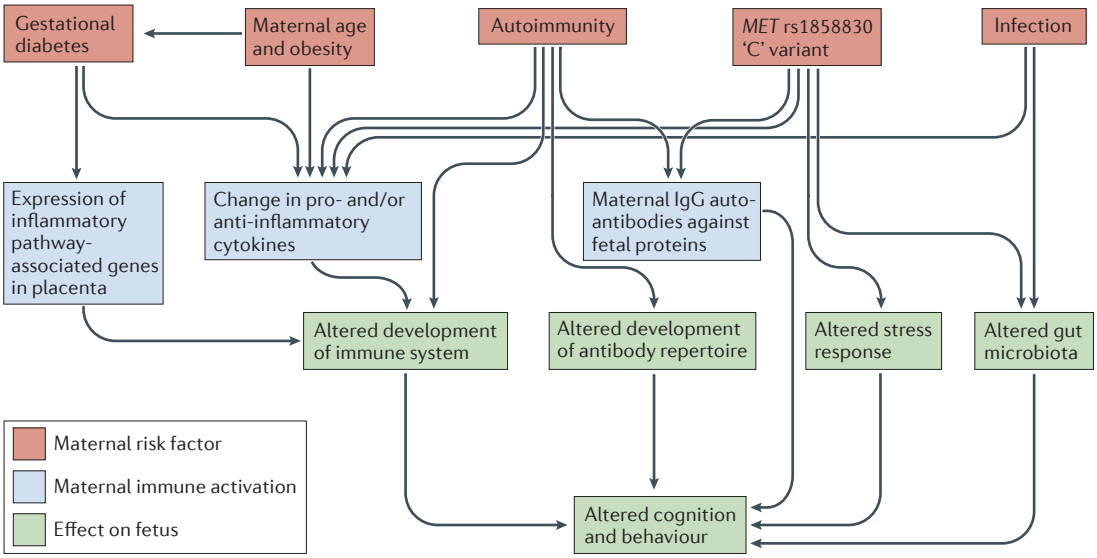



Figure 1 | **ASD risk factors during pregnancy converge on maternal immune system activation.** Maternal autoimmunity, infection during pregnancy, maternal age and obesity, gestational diabetes, and maternal *MET* variant rs1858830 'C' allele are all associated with a higher incidence of autism spectrum disorder (ASD). These risk factors (red boxes) cause maternal immune activation (MIA) (blue boxes), which manifests as changes in the maternal peripheral cytokine milieu, generation of immunoglobulin G (IgG) maternal autoantibodies that are reactive to fetal proteins, and activation of inflammatory pathway genes within the placenta. Based on findings in animal models, MIA is sufficient to induce long-lasting changes in brain development, the gut microbiota, and immune and endocrine systems, all of which can lead to altered cognition and behaviour in the developing fetus (green boxes).

the conclusion that vaccines do not cause ASD, society is increasingly at risk of preventable devastating diseases — including whooping cough, measles and tuberculosis — because of the unsubstantiated fear of vaccinations. For these reasons alone, it is crucial to disseminate and to improve our understanding of immune and environmental contributions to ASD.

In this Review, we provide an overview of the evidence that maternal and postnatal immune dysregulation play a part in the aetiology and pathophysiology of ASD. First, we examine evidence from genetic studies that indicates an association between ASD and mutations in immune genes. Next, we discuss the epidemiological, clinical and animal studies implicating prenatal immune system contributions to ASD, including autoimmunity, fetal reactive antibodies and maternal immune activation (MIA). Then, we review the evidence for chronic immune perturbations in individuals with ASD. Finally, we present the molecular mechanisms that might underlie the link between immune activation and ASD phenotypes, focusing on common molecular pathways that might be targeted by future, novel therapeutic interventions.

## Genetic studies

Early estimates of the heritability of ASD of approximately 90%[14,15], and even recently revised estimates of 38–54%, strongly suggest that genetic mutations are a major cause of ASD[4,16]. However, so far, no single gene has been identified that substantially increases the risk of developing ASD[17]. Instead, ASD seems to be associated with a large number of genetic mutations, including possibly hundreds of rare causal variants and more than 100 common variants of small effect[18,19]. Insight into the genetic pathways that are altered in ASD has come from studies of syndromic disorders with a high incidence of ASD, including fragile X syndrome, Rett syndrome, tuberous sclerosis, neurofibromatosis type 1 and PTEN (phosphatase and tensin homologue) macrocephaly. These syndromes are caused by mutations in single genes (fragile X mental retardation 1 (*FMR1*), methyl-CpG-binding protein 2 (*MECP2*), tuberous sclerosis 1 (*TSC1*) or *TSC2*, neurofibromin 1 (*NF1*) and *PTEN*, respectively) and account for 5–7% of ASD cases[20–22]. Recent reports have also highlighted the strong contribution of copy number variants to ASD, accounting for 7–20% of cases of this disorder[23–25].

Importantly, several ASD-associated genes encode components of the immune system (TABLE 1). One of the immune genes most associated with ASD is *MET*, which encodes a receptor that binds hepatocyte growth factor (HGF)[26]. A *MET* variant that includes a common G-to-C single-nucleotide polymorphism (SNP) in its promoter (the rs1858830 'C' allele) is associated with decreased MET signalling in the temporal lobe and ASD[27–29]. This *MET* variant leads to the disrupted expression of multiple downstream targets of the MET signalling cascade[28]. Post-mortem transcriptome analysis of individuals with ASD also shows reduced *MET* expression in the temporal lobe, suggesting that reduced *MET* levels may indeed cause ASD phenotypes[27,30,31]. Consistent with this idea, individuals with ASD and the *MET* 'C' allele exhibit reduced structural and functional connectivity in the temporoparietal lobes compared with both individuals with

Maternal immune activation
(MIA). An animal model of prenatal immune challenge generated by stimulating the maternal immune system with viral or bacterial mimics, live antigens or inflammatory cytokines.

Copy number variants
Deletions or duplications of chromosomal segments that lead to phenotypic diversity among individuals.

Single-nucleotide polymorphism
(SNP). The most common form of genetic variation due to nucleotide substitutions.

Box 1 | **Immune system influences on cognition and behaviour**

In the past 10 years, it has become increasingly clear that the immune status of an individual influences cognition and behaviour. In addition to mediating the response to disease, immune cells, especially T cells, have roles in many aspects of brain development and function[249,250]. This is perhaps best illustrated in mice with severe combined immunodeficiency (SCID) and nude mice, which are deprived of all lymphocytes and T cells, respectively. These animals exhibit impairments in hippocampal neurogenesis and learning and memory, as well as increased repetitive behaviours and anxiety[251,252]. Remarkably, replenishment of the immune system by adoptive transfer of wild-type splenocytes or by bone marrow reconstitution improves the learning ability of SCID and nude mice in several learning tasks and ameliorates repetitive behaviours[132,253,254], suggesting that the defects are not caused by lifelong immune deficiency but rather by ongoing depletion of immune cells. Interestingly, increased anxiety is not rescued by wild-type reconstitution, suggesting that anxiety has a developmental aetiology and is a lasting behavioural consequence of impaired immunity[252]. $CD4^+$ T cells mediate pro-cognitive effects[255] indirectly through effects at meningeal spaces rather than through infiltration into the CNS[256]. When mice are exposed to learning tasks, T cells home to the meninges and become activated, acquiring a T helper 2 ($T_H2$)-like phenotype (regarded as anti-inflammatory) and expressing high levels of interleukin-4 (IL-4), which cause myeloid cells in the meninges to become skewed to an M2 (also anti-inflammatory) phenotype[256]. Preventing this T cell migration to the meninges, or genetic deletion of *Il4*, results in a pro-inflammatory, M1 skewing of meningeal myeloid cells and deficits in learning and memory[256]. Conversely, reconstituting wild-type mice with T cells from *Il4*-knockout mice results in learning and memory deficits[132]. Thus, $CD4^+$ T cells, which clearly regulate brain immune status, normal cognition and emotional behaviour, may have important roles in causing and/or contributing to autism spectrum disorder and clearly represent an important potential therapeutic target for this condition.

ASD but without the *MET* variant and control individuals[31]. Moreover, structural MRI in individuals with ASD and the *MET* 'C' allele shows decreased cortical thickness in several brain regions that are associated with socio-communicative function[32]. Interestingly, MeCP2, which is implicated in Rett syndrome, regulates *MET* transcription, and the expression of *MET* is notably reduced in the temporal lobes of females with Rett syndrome in the absence of the *MET* 'C' allele[33], suggesting that reduced *MET* expression may contribute to the ASD-related pathophysiology in this syndrome.

Many of the ASD-related phenotypes that are associated with decreased MET signalling in the brain may result from deficits in the key functions that MET has in brain development (described below). However, MET could also cause these changes in neural circuitry and function indirectly through its ability to negatively regulate immune responsiveness[34,35] and gastrointestinal homeostasis[36,37]. Increased MET signalling ameliorates disease pathogenesis in several models of classic systemic inflammatory diseases, including multiple sclerosis and systemic lupus erythematosis (SLE)[38–42]. Moreover, during pregnancy, the *MET* 'C' allele is associated with a decrease in the production of the cytokine interleukin-10 (IL-10)[43], thus preventing the important gestational increase in IL-10 that is responsible for maternal immune suppression and tolerance to the developing fetus[44] (FIG. 1). The *MET* 'C' allele is also enriched in mothers with another immune-related risk factor for ASD — maternal antibodies that are reactive to fetal brain proteins[43] (discussed below). Finally, the *MET* 'C' allele may interact with environmental factors to increase the risk of ASD. Indeed, the *MET* 'C' allele and exposure to high levels of air pollution during prenatal development act synergistically to increase the risk of developing this disorder[45].

In addition to variants in *MET*, variants in the human leukocyte antigen (HLA) locus confer risk of neurodevelopmental disorders[46,47]. The HLA locus encodes the major histocompatibility complex (MHC) genes in humans, which are involved in diverse immune functions and comprise three classes. Although the limited scale of the genetic studies of MHC in relation to ASD does not allow any definitive associations to be made, variants in all three classes of MHC genes have been reported to enhance ASD risk: namely, the MHC class I (MHCI) *HLA-A2* haplotype, the MHCII *HLA-DRB1* alleles and the complement *C4B*-null allele in the MHCIII region[47]. As with many other ASD-related genes, the HLA associations are not specific to ASD; they are also associated with other neurodevelopmental disorders and autoimmune disorders, including rheumatoid arthritis and SLE[48], which occur at higher rates in the relatives of individuals with ASD[49]. The combination of a specific *HLA-DRB1*11* allele with a family history of autoimmune disorders increases the odds ratio for the association of this allele with ASD[50]. Similarly, ASD-associated deficiencies in a complement gene — *C4* — within the MHCIII region[51,52] are also among the strongest genetic risk factors for SLE[53], and individuals with idiopathic ASD exhibit a 34% decrease in plasma levels of C4b[54]. These ASD-linked deficits in complement are thought to lead to the activation of autoimmune responses directly, owing to the production of autoantibodies against excess cellular debris that is typically removed in a complement-dependent manner, and indirectly, through the chronic immune activation that is caused by recurrent and persistent bacterial infections[55].

Finally, mutations in genes that encode several members of the IL-1 cytokine receptor family are also associated with ASD. Two recent exome-sequencing studies of individuals with ASD found synonymous SNPs in the gene encoding the IL-1β decoy receptor IL-1 receptor type 2 (IL-1R2)[56,57]. The functional consequences of these SNPs have not yet been assessed, but synonymous SNPs can affect protein folding and mRNA splicing, stability and structure[58,59]. A rare ASD-associated mutation has also been identified in the gene encoding another IL-1 family receptor, IL-1 receptor accessory protein-like 1 (IL-1RAPL1)[60,61]. This mutation was originally identified in a screen for genes that are related to X-linked intellectual disability[62]. ASD-associated mutations, copy number variants and somatic mosaics have subsequently been discovered in this gene, most of which result in a loss of function of the encoded protein[63]. Together, these findings indicate that mutations in a wide array of immune genes contribute to ASD.

## Maternal immune contributions

***Autoimmune disorders.*** Although genetic mutations seem to contribute a sizeable amount of risk of ASD, recent estimates suggest that 50–60% of the risk of ASD is unaccounted for, which implies that environmental

Human leukocyte antigen
(HLA). The gene locus that encodes the human versions of three different classes of major histocompatibility complex proteins.

Complement
A system of plasma proteins that attack extracellular pathogens, assist in pathogen and cellular debris clearance by phagocytes and facilitate synaptic pruning in the brain.

Table 1 | **ASD-associated genes with roles in the immune system and CNS**

| Gene | CNS function of encoded protein | Immune function of encoded protein | Refs* |
|---|---|---|---|
| *CD99L2* | Unknown | Homophilic adhesion molecule that is involved in leukocyte extravasation | 46 |
| *JARID2* | Transcriptional repressor involved in neural tube fusion | Transcriptional repressor that regulates haematopoiesis | 46 |
| *TPO* | Necessary for proper brain development | Enzyme that produces thyroid hormones | 46 |
| *MET* | Mediates migration of neuronal precursors and excitatory synapse formation | Receptor for hepatocyte growth factor that promotes differentiation and proliferation of haematopoietic cells, and exerts broad anti-inflammatory effects | 26 |
| *MIF* | Unknown | Inflammatory cytokine that regulates innate immune response to bacterial pathogens | 55 |
| *PRKCB* | Implicated in circadian rhythms, and learning and memory | Serine/threonine-specific protein kinase that mediates B cell activation, T cell migration, antigen-presenting cell function and cytokine release | 55 |
| *HLA-A2* | Negatively regulates synapse formation and plasticity in the developing brain | MHC class I molecule that is expressed on all nucleated cells to identify them as 'self' to patrolling immune cells; regulates cellular immune responses to intracellular pathogens | 47 |
| *HLA-DRB1* | Unknown | MHC class II molecule that is expressed on antigen-presenting cells (dendritic cells, monocytes, macrophages and microglia); initiates cellular immune responses to extracellular pathogens | 47 |
| *C4B* | May be involved in complement-mediated synaptic pruning | Complement cascade protein that is involved in clearing pathogens and cellular debris | 55 |
| *IL1RAPL1* | Involved in synapse formation through trans-synaptic adhesion interactions | Interleukin-1 family receptor with unknown immune function | 60 |
| *EIF4E* | Promotes translation of many ASD-associated genes with synaptic roles | Part of a multisubunit complex downstream of mTOR; initiates cap-dependent translation | 20 |
| *FMR1* ( | Regulates translation of many ASD-associated genes with synaptic roles | RNA-binding protein that is involved in translational control; forms a complex with eIF4E to suppress translation | 20 |
| *NF1* | Negatively regulates RAS-mediated neurotransmitter and neurotrophin signalling | Tumour suppressor and GTPase-activating protein that negatively regulates the *RAS* oncogene signal transduction pathway (the RAS pathway feeds into the mTOR pathway, and RAS mediates cytokine and growth factor signalling in the immune system) | 21 |
| *PTEN* | Regulates neuronal metabolic processes and the mTOR pathway | Tumour suppressor that acts as a phosphatase that inhibits PI3K signalling (the PI3K pathway also integrates cytokine, neurotransmitter and growth factor signalling) and is an upstream regulator of the mTOR pathway | 20 |
| *TSC1* and *TSC2* | Negatively regulates the mTOR pathway; induces neuroprotective autophagy in response to glucose deprivation and stroke | Tumour suppressors that, when in complex with each other, have GTPase activity and negatively regulate the mTOR pathway | 20 |

ASD, autism spectrum disorder; *C4B*, complement component 4B; *CD99L2*, CD99 molecule-like 2; *EIF4E*, eukaryotic translation initiation factor 4E; *FMR1*, fragile X mental retardation 1; HLA, human leukocyte antigen; *HLA-A2*, MHC class I, A; *HLA-DRB1*, MHC class II DR beta 1; *IL1RAPL1*, interleukin-1 receptor accessory protein-like 1; *JARID2*, Jumonji AT-rich interactive domain 2; MHC, major histocompatibility complex; *MIF*, macrophage migration inhibitory factor; mTOR, mammalian target of rapamycin; *NF1*, neurofibromin 1; *PRKCB*, protein kinase C beta; *PTEN*, phosphatase and tensin homologue; *TPO*, thyroid peroxidase; *TSC*, tuberous sclerosis. *Citations are reviews; for an updated list of references for genetic associations, see the Simons Foundation Autism Research Initiative (SFARI) Gene database.

factors or gene–environment interactions contribute substantially to the risk of this disorder[4,16]. In addition to mutations in immune genes, autoimmune disorders, allergies and asthma are strongly associated with the families of children with ASD[49]. Estimates indicate that there is up to a 50% increase in the odds of an ASD diagnosis among children who have a parent who has had an autoimmune disorder[49,64]. Although maternal immune disorders such as rheumatoid arthritis, SLE and type 1 diabetes account for the largest proportion of this increased risk of ASD in offspring, paternal immune disorders and a general familial history of immune disorders in the absence of maternal immune conditions also seem to contribute, suggesting that there is a heritable component for this autoimmune association[49,65]. Indeed, autoimmune disorders are over-represented in the population of individuals with ASD[66]. However, such disorders also increase the risk of intellectual disability and several other neurodevelopmental disorders, suggesting that immune disorders generally increase the vulnerability of the developing brain to developmental defects[67]. The strong contribution of maternal immune status to ASD risk could involve genetic factors and environmental influences on the developing fetus. Similar to the situation caused by the *MET* 'C' allele, mothers with autoimmune disorders fail to develop an anti-inflammatory immune system profile that is typical during pregnancy[68] (FIG. 1). Although the relative contributions of these genetic and environmental factors are yet to be determined, animal models show a causal link between the activation of the maternal immune system and altered neurodevelopment (see below).

***Fetal-brain-reactive antibodies.*** The association of maternal autoimmune disorders with ASD in offspring may be mediated by the passive transfer to the fetus of maternal immunoglobulin G (IgG) antibodies that exhibit reactivity to self-proteins in the mother or child. In typical development, passive immunity protects the fetus and early neonate from infection until the child's adaptive immune system has matured. These fetal maternal IgG antibodies could theoretically enter the fetal brain during early gestation because the blood–brain barrier (BBB) is not mature until the postnatal period[69]. Bolstering this hypothesis, women with rheumatoid arthritis or SLE are more than four times as likely as women without these disorders (10.5% versus 2.6%) to harbour peripheral antibodies with reactivity to neurons from the brains of fetal and adult mice[70]. Moreover, 53% of mothers of a child with ASD who test positive for anti-brain antibodies also have anti-nuclear antibodies — an indicator of latent autoimmunity — compared with 13.4% of mothers of a child with ASD who do not test positive for anti-brain antibodies[70]. Thus, autoimmunity in pregnant women, even at a clinically undetectable level, may be associated with the production of maternal antibodies that can reach the fetal brain and potentially perturb fetal brain development.

A functional role for such maternal autoantibodies has recently been demonstrated by several groups. Injecting NMDA receptor (NMDAR)-specific autoantibodies derived from the blood of patients with SLE into a pregnant mouse alters brain development and impairs cognitive behaviour in the offspring[71]. Antibody clones that recognize the NR2A and NR2B NMDAR subunits seem to be a major cause of these behavioural changes. Interestingly, although 30–60% of individuals with SLE have NMDAR-specific autoantibodies, each antibody clone has unique physiological effects. Some antibodies have potent co-agonist properties, leading to an increased calcium influx through NMDARs and eventually neuronal death[72], whereas other clones with slightly different epitopes are not pathogenic and still others cross-react with C1q, a component of the complement cascade[73]. Remarkably, the effects of these NMDAR-specific antibodies are sex specific in mice: they cause death through apoptosis of NR2A-expressing neurons, which are enriched in the brainstem of female fetuses but not male fetuses[74]. A female-specific vulnerability to these antibodies could result in increased rates of resorption of female fetuses and lead to an increase in the proportion of male offspring, similar to the male skewing seen in ASD. In addition, female fetuses with these antibodies that are carried to term would be expected to show more profound deficits. This prediction may fit with the recurrent observation that a subset of girls with ASD shows more social communication impairments and lower cognitive abilities than boys with matched low IQs[75]. Collectively, these results suggest mechanisms whereby maternal autoimmuntity can impair brain development in offspring in a sex-specific manner.

Fetal-brain-reactive antibodies have also been found in approximately 11% of mothers of children with ASD in the absence of any evidence of autoimmunity. These antibodies recognize fetal brain proteins of 37 kDa, 39 kDa and 73 kDa, and may confer specific pathophysiology (such as abnormal brain enlargement, self-injurious behaviour and greater language deficits) depending on the antibody clone[76]. Several studies suggest that these maternal IgG antibodies are ASD specific, as these antibodies did not detect proteins of these sizes in mothers of unaffected children[76]. However, it is currently unknown whether these ASD-specific maternal IgG antibodies are sufficient to cause ASD. Additional studies are needed to determine the risk of developing ASD in subsequent children born to mothers harbouring ASD-specific antibodies and, therefore, whether these banding patterns could be used as an ASD biomarker.

Seven proteins — lactate dehydrogenase A (LDHA), LDHB, Y-box-binding protein 1 (YBX1), guanine deaminase (cypin), stress-induced phosphoprotein 1 (STIP1), collapsin response mediator protein 1 (CRMP1; also known as DRP1) and CRMP2 (also known as DRP2) — have recently been reported to be targeted by fetal-brain-reactive antibodies. These seven proteins are expressed in the developing brain, where they have roles in growth cone motility, dendritic morphology, cellular stress and metabolism, and/or transcription regulation[77]. Disrupting the function of any of these proteins can alter brain development, thereby contributing to ASD pathogenesis. Indeed, injecting pregnant non-human primates with maternal IgG antibodies from mothers of

Maternal immunoglobulin G (IgG) antibodies
IgG antibodies that pass through the placenta during the third trimester and enter fetal circulation, where they persist at high titre levels for several months after birth.

Blood–brain barrier
(BBB). A selectively permeable network of endothelial cells, pericytes and astrocytes separating the circulating blood from the brain extracellular fluid. The BBB begins to form in the first trimester and is fully formed by birth in humans. Infection, disease and certain drugs can increase the permeability of the BBB.

Fetal-brain-reactive antibodies
Maternally derived immunoglobulin G antibodies that can cross the placenta and bind to fetal brain proteins.

ASD children induces ASD-like behavioural changes in offspring, including reduced reciprocal interactions and inappropriate approach behaviours[78,79]. In addition, these offspring have larger brains — including larger frontal lobes — which may be consistent with the aberrant white matter tracts found in a subset of young children with ASD[79–82]. Similar species-specific aberrant social behaviours in offspring are induced when pregnant mice are injected with human IgG-ASD[83]. Moreover, a single low-dose intraventricular injection of IgG-ASD at mid-gestation in mice causes increases in repetitive behaviours and alterations in social approach behaviour[84]. Thus, the presence of maternally derived brain-directed autoantibodies in early development is associated with a higher incidence of ASD and specific pathophysiology. Research is ongoing to determine whether these fetal antibodies exert their functions by acting directly on their protein targets in the brain and to determine which targets are causal for specific ASD phenotypes.

***Maternal infection.*** In addition to maternal antibodies, acute immune activation that is caused by maternal infection during specific periods of gestation contributes to ASD risk in offspring[85]. Maternal infection was first associated with ASD following the observation that ASD incidence increased from 0.05% to 8–13% in children of mothers that were exposed to the 1964 rubella pandemic during pregnancy[86–88]. Numerous single-case studies have also associated ASD with various parasitic, bacterial and viral prenatal infections, including toxoplasmosis, syphilis, varicella, cytomegalovirus, mumps and herpes simplex virus infection[85]. This diversity of infectious agents suggests that general immune activation during gestation, rather than a specific immune disorder or virus, underlies the link with ASD. Consistent with this idea of heightened immune activity and infection, several recent studies have reported increased levels of pro-inflammatory cytokines in the amniotic fluid of mothers of children with ASD[89,90].

Definitive evidence that maternal infection during pregnancy is a risk factor for ASD in offspring was obtained through more recent studies of the Danish health registry[91]. Data from more than one million children born between 1980 and 2005 revealed an almost threefold increase in the rate of ASD diagnosis in children born to mothers who were hospitalized for viral infection in the first trimester and in children of women who experienced an episode of fever lasting 1 week or more before gestational week 32 (REFS 91,92). These data point to a specific temporal window of immune activation during the first trimester of fetal development, and the association with a specific duration of fever suggests that an immune activation threshold must be surpassed to confer risk of developing ASD. It is important to note that most maternal infections that fall within this time-window and are above this threshold do not lead to ASD in offspring, suggesting that both the immune status of the mother and the immunogenetic background of the developing child may be critical factors in determining outcome. In support of this hypothesis, a recent study using a mouse model of MIA found that the degree of maternal immune response (as measured by weight loss and tumour necrosis factor (TNF) serum levels) to prenatal immune challenge with polyinosinic–polycytidylic acid (poly(I:C)), a viral mimic, is positively associated with the severity of sensorimotor gating deficits in the offspring[93]. Finally, similar to maternal autoimmune disorders, maternal infection during pregnancy has been associated with other developmental disorders, including schizophrenia and mood disorders[94]. It has been proposed that the specific combination of gestational week, type of immune activation (viral, bacterial or chronic) and the duration or intensity of activation may determine which disorder manifests in offspring[12].

Studies using several animal models of maternal infection during pregnancy support the association of MIA with ASD phenotypes. These models include influenza infection, viral and bacterial mimics (poly(I:C) and lipopolysaccharide, respectively) and specific cytokines, such as IL-2 and IL-6. These studies support the idea that the timing of MIA and the type of antigen that is used for immune challenge can lead to overlapping but distinct phenotypes, including behavioural outcomes and transcriptome signatures in the developing brains of offspring[95]. Poly(I:C) injection at mid-gestation in mice and non-human primates generates offspring that display all (in mice) or some (in non-human primates) of the three core behavioural symptoms of ASD[96–98]. Both mouse and non-human primate MIA offspring also exhibit deficiencies in sensorimotor gating and increased anxiety; these co-morbidities are also observed in a subset of individuals with ASD. In addition, mouse MIA offspring show localized aberrations in Purkinje cells[99], similar to the localized deficits in cerebellar Purkinje cells that have been reported in many post-mortem ASD cases[100]. These MIA-induced ASD-like behaviours and neuropathologies in offspring seem to be caused by altered levels of maternal cytokines, including IL-2, IL-6 and IL-10. Both a single injection of IL-6 at mid-gestation or low-dose IL-2 injections daily between gestational days 12 and 16 in mice are sufficient to induce ASD-like behavioural changes and neuropathologies in offspring, including deficits in sensorimotor gating, increased anxiety and stereotypical behaviour, aberrant social interactions, and increased serum levels of pro-inflammatory cytokines[96,101]. Maternal cytokines also seem to be necessary for the ASD-like phenotypes in offspring because poly(I:C) injections at mid-gestation on an *Il6*-knockout background, co-administration of an IL-6 function-blocking antibody and poly(I:C), or overexpression of the anti-inflammatory cytokine IL-10 by macrophages prevent the MIA-induced changes in gene expression and behaviour[96,102]. Despite several considerations in interpreting the findings from animal models of ASD (BOX 2), these results provide strong support for the involvement of MIA in the development of ASD in offspring.

## Chronic immune changes in ASD

***Peripheral changes.*** In addition to immune dysregulation in families, especially in mothers, there is ample evidence of ongoing immune dysfunction in the peripheral immune system and the brain of individuals with ASD[103].

Polyinosinic–polycytidylic acid
(Poly(I:C)). Mismatched double-stranded RNA that acts as a viral mimic.

Box 2 | **Considerations for interpreting results from animal models of ASD**

Despite the excitement surrounding the relevance of the maternal immune activation (MIA) model to autism spectrum disorder (ASD), there are several caveats that must be considered in interpreting results from this model. First, it is important to note that MIA models a single environmental risk factor amid a sea of implicated genetic and environmental susceptibilities for ASD and therefore should not be expected to capture all of the diverse phenotypes across the autism spectrum. Second, MIA is a shared environmental risk factor for a wide range of neuropsychiatric and degenerative disorders that manifest at distinct time points during life. Therefore, MIA models also should not be expected to express pathophysiology exclusive to ASD. It is important to note that these caveats are not exclusive to the MIA models, but rather are also applicable to monogenetic animal models of ASD. Similar to some of the MIA models, most of the genetic ASD models fail to recapitulate the full pathophysiology observed in individuals with ASD. In some instances, genetic models either show no overt pathology or demonstrate behaviours that are opposite to those characterizing ASD[257,258]. Moreover, many of the genes that were initially thought to be exclusively linked to ASD have turned out to be shared risk factors for other neuropsychiatric disorders, particularly schizophrenia. Rather than undermining their relevance to ASD, the caveats to preclinical ASD animal models could be embraced and used experimentally to test hypotheses and develop molecular models for the cause of different forms of ASD and other disorders. These models provide a reductive platform from which we can build more complex models, such as pairing MIA with specific genetic backgrounds or later-life immune insults. For example, pairing a low dose of polyinosinic:polycytidylic acid at gestation day 9 with chronic mild stress at adolescence unmasked schizophrenia-like behaviours and biomarkers in mice[259]. In the future, similar pairings of MIA with other ASD risk factors may parse the phenotypic heterogeneity of ASD into subtypes of this condition, as well as other disorders, reflecting specific combinations of genetic and environmental insults during particular developmental periods of susceptibility.

Similar to their relatives, individuals with ASD have an increased incidence of autoimmune disorders, allergies and asthma[66,104]. A subset of individuals with ASD also have autoantibodies that are reactive to CNS self-proteins, including serotonin receptors[105], glial fibrillary acidic protein[105], myelin basic protein[106–108], and unidentified targets in the basal ganglia, prefrontal cortex, cingulate gyrus and cerebellum[105,106,108–113]. Because unaffected siblings of people with ASD have similar autoantibody profiles[108,114], brain-directed autoantibodies may not be generally predictive of ASD and may instead represent secondary autoimmune processes or evidence of a previous CNS injury. Nevertheless, a recent study found an association between specific autoantibodies that recognize cerebellar targets of 45 kDa and 65 kDa and ASD diagnosis, impaired behavioural scores and lower cognitive and adaptive function[115]. Interestingly, the maternally derived anti-brain autoantibodies discussed above have a much higher degree of association with ASD diagnosis and specific pathophysiology than patients' own autoantibodies. This discrepancy may represent a critical difference in the timing of exposure, with insults during gestation having greater pathogenic impact than later-stage insults owing to the limited potential for exposure after the BBB is formed and brain architecture is more developed.

In addition to the presence of autoantibodies, many reports have identified changes in cytokine levels in the blood of individuals with ASD who are over 2 years of age. These changes include increases in the levels of IL-1β, IL-6, IL-8, IL-12p40 (also known as IL-12β) and granulocyte–macrophage colony-stimulating factor (GM-CSF), which are generally considered to be pro-inflammatory cytokines, and decreases in the levels of IL-10 and transforming growth factor-β (TGFβ), which are generally considered to be anti-inflammatory cytokines[116–121]. The lower levels of TGFβ in these children with ASD are associated with less adaptive behaviours and worse behavioural symptoms[119]. Increases in the levels of IL-1β, IL-6, IL-8 and IL-12p40 are specifically associated with a regressive form of ASD and more impaired stereotypical behaviours, and increases in the levels of the chemokines C-C motif chemokine 2 (CCL2; also known as MCP1), CCL5 and eotaxin are associated with higher aberrant behaviour scores and more impaired development[121]. These changes in peripheral cytokine levels may be developmentally regulated, as measurements at earlier ages differ in both the cytokines altered and the direction of their change in ASD. For example, the levels of several cytokines were decreased (GM-CSF, interferon-γ (IFNγ), IL-2, IL-4 and IL-6) in neonatal blood samples taken from individuals who were later diagnosed with ASD in a large recent longitudinal study using the Danish Newborn Screening Biobank[122]. Importantly, similar to the situation for peripheral autoantibodies and ASD, peripheral cytokine profiles in individuals with ASD and their unaffected siblings are similar[123], suggesting that these changes alone may not be sufficient to cause ASD.

In addition to altered cytokine profiles, evidence exists for impaired immune cell function and responsiveness following immune challenge in ASD. Natural killer cells from individuals with ASD are defective in their normal function of lysing infected cells when challenged[124,125]. Monocytes isolated from individuals with ASD also exhibit impaired responses to challenge: they secrete excess pro-inflammatory cytokines following challenge with ligands for Toll-like receptor 4 (TLR4; a receptor that is responsive to bacterial pathogens) but show reduced production of these same cytokines when challenged with ligands for TLR9 (a receptor that is responsive to viral pathogens)[125,126]. Similarly, circulating levels of $CD4^+$ T cell populations are low, biased towards an anti-inflammatory (T helper 2 ($T_H2$)) profile and exhibit a dysfunctional response to stimulation in individuals with ASD[127–131]. Studies trying to associate particular cellular immunophenotypes with symptom severity have produced seemingly contradictory results. For example, T cell skewing to a $T_H2$ phenotype (which is considered to be anti-inflammatory and is found in a subset of the ASD population) has been associated with better cognitive and adaptive behaviour[127,132], but in another study increased levels of the classical $T_H2$ cytokine IL-4 have been associated with greater impairments in non-verbal communication[120]. Despite these ambiguities, sufficient evidence indicates that general abnormalities in peripheral immunity are a common feature in the population of individuals with ASD.

Studies in mice have provided further support for an association between peripheral immune changes and ASD. The offspring of MIA mice exhibit peripheral immune abnormalities. For example, T cells from adult MIA offspring secrete excess pro-inflammatory cytokines when challenged and show a bias towards

$T_H1$ and $T_H17$ phenotypes[101,133,134]. In addition, myeloid cells from these animals are increased in number and produce higher levels of IL-12p40 and CCL3, which is consistent with a pro-inflammatory immune profile[133,135]. Collectively, these findings suggest that MIA induces an irregular immune phenotype that persists into adulthood in rodents, as in ASD, but is distinct in nature from the T cell response profile in ASD. However, comparing results from animal studies using a single model (MIA) with ASD in humans resulting from a wide range of aetiologies may be misleading. Moreover, cross-species comparisons from the current literature are not informative because of the often limited number of cytokines assayed in human samples, the wide range of ages, co-morbidities and therapies within the patient population, and the challenge of comparing postnatal ages between rodents and humans. Nevertheless, these studies do clearly show that an environmental risk factor for ASD causes long-lasting immune dysregulation that is associated with ASD-like phenotypes in rodents. Importantly, future studies with larger numbers of individuals with ASD are needed to determine whether there is a consistent peripheral cytokine signature that is diagnostic for ASD or even negatively associated with ASD expression in unaffected siblings.

***Immune changes in the CNS.*** It is often assumed that changes in cytokine levels in the blood from individuals with ASD reflect changes in cytokine levels in the brain. The findings of some studies are consistent with this idea, reporting increases in GM-CSF, IL-6, IL-8, TNF and IFNγ levels in the frontal cortex, and increases in IL-6, TGFβ and CCL2 levels in the anterior cingulate gyrus and cerebellum[116,136,137]. As further evidence of potential neuroinflammation, microglia in the dorsolateral prefrontal cortex exhibit increased MHCII expression (a marker of activation), an activated morphology (amoeboid shape) and increased density[138,139]. However, other results contradict these findings: some studies have shown decreases or no change in sensitive markers of CNS immune activation — including quinolinic acid, neopterin and biopterin — in the cerebrospinal fluid of individuals with ASD[140–142]. Similarly, increases in the levels of soluble TNF receptors that blunt the inflammatory response are present in the cerebrospinal fluid and serum from individuals with ASD[142]. Future studies defining the roles for ASD-associated changes in cytokines as mediators of neuroinflammation, as dynamic adaptive responses to peripheral changes or simply as growth factors or neuromodulators will be crucial to explain these contradictory findings.

In mouse models of MIA, offspring with ASD-like behaviours and neuropathology also exhibit long-lasting changes in cytokine levels in the brain. The levels of a broad range of cytokines are increased in the fetal brain hours after poly(I:C) injection in pregnant mice[143,144], and many of these cytokines remain chronically altered in the brains of offspring throughout postnatal development and into adulthood[145]. Although cytokine levels are generally increased at birth and in the adult brain, levels of many cytokines are decreased in frontal cortical regions during peak periods of synaptogenesis and plasticity[145]. These changes do not correlate with changes in serum cytokine levels and are not accompanied by changes in BBB permeability or immune cell infiltration into the brain parenchyma. Thus, these results do not fit the classical definition of neural inflammation and suggest caution in interpreting changes in cytokines levels at one time point as indicative of an inflammatory process[146] (BOX 3). Understanding how a discrete event of immune activation during pregnancy causes ongoing and dynamic dysregulation of immune molecules in the brains of offspring is one of the most important areas for future research in this field.

In addition to altered cytokine levels, the brains of individuals with ASD also show changes in the expression of genes encoding proteins that were initially discovered in the immune system. A weighted gene co-expression network analysis comparing the expression of more than 30,000 genes in post-mortem autistic and control brains revealed two distinct networks — each comprising more than 400 genes — that are disrupted in the brains of individuals with ASD[30]. One of the networks of genes is associated with synaptic function and is downregulated in ASD, whereas the other module contains immune-related genes and is upregulated in ASD. Taken together, these results indicate ongoing dysregulation of the immune system and altered expression of immune molecules in the CNS in ASD and in mouse models of MIA. How these changes relate to the pathophysiology observed in these individuals and animal models remains unclear. As the immune system is primarily involved in tissue repair and homeostatic processes, these alterations could represent compensatory responses to dysfunctional network activity and cellular stress. Studies addressing the temporal dynamics of these central alterations with age and whether they are linked to the ongoing and dynamic immune changes in the periphery are important areas for future research.

***A role for the microbiota?*** Adding to the complexity of the neural–immune axis is the recent focus on the gut as an important nexus for nervous–immune–endocrine system interactions. The initial colonization of the gut is dominated by maternal microbiota during birth, and this formative colonization assists in priming the developing immune system and directs immune homeostasis[147–149]. Children with ASD have excessive levels of *Clostridium* spp. and *Desulfovibrio* spp. in their gut microbiome[150–152], and this imbalance could influence peripheral immune responses and potentially contribute to the observed abnormalities in immune cell composition and function in these individuals. In fact, recent studies suggest that gut microbiota may act as a 'tuning fork' for the immune system. In particular, the composition of gut microbiota strongly influences the T cell repertoire, which is defined as the various subtypes — each with their own effector properties — and myriad T cell receptor clones within these subtypes[153,154]. Certain microbiota signatures in the gut inhibit the differentiation of brain-supportive T cell populations, and administration of *Bacteroides fragilis* restores the proper balance of T cell populations in mice[155].

Gut microbiota
A diverse set of microorganisms that inhabit the gut and shape host immune function.

The idea that therapies directed at altering the gut microbiota may regulate immune function and rescue ASD-related phenotypes has received support from animal models of ASD. Offspring from the poly(I:C) mouse model of MIA exhibit gastrointestinal changes that are also found in some humans with ASD[156,157], including increased intestinal permeability and abnormal intestinal cytokine profiles[158]. These MIA offspring also have abnormal microbiota signatures, most significantly in increased levels of *Clostridium* spp., paralleling findings in humans with ASD[151,159]. Remarkably, oral treatment with *B. fragilis* restores intestinal permeability and serum cytokine levels, rectifies microbiota imbalances, ameliorates stereotypical and anxiety-like behaviour, improves sensorimotor gating, and increases the number and duration of ultrasonic vocalizations in these mice[158]. Interestingly, *B. fragilis* has no effect on the deficits in sociability in these offspring. Future studies will determine whether social behaviours are resistant to probiotic therapy, whether *B. fragilis* also reduces the underlying neuropathology and whether it does so through normalizing immune dysregulation in offspring.

Box 3 | **Detecting neuroinflammation**

Inflammation in the body is a protective, organized and adaptive response to invading pathogens[260] that is rigorously defined by four hallmarks: increases in pro-inflammatory cytokine levels, activation of macrophages, recruitment of leukocytes to sites of inflammation and local tissue damage. Inflammation begins with an abrupt rise in the levels of pro-inflammatory cytokines, followed by a gradual rise in the levels of anti-inflammatory cytokines, which limits damage to secondary tissues. Classically, neuroinflammation occurs when the nervous system is exposed to infection or trauma that is accompanied by breaches in the blood–brain barrier (BBB). Under these conditions, microglia and astrocytes adopt a reactive phenotype (gliosis), and proliferate and perpetuate cellular and molecular responses that are aimed at removing infected or damaged tissue. Prolonged gliosis can recruit peripheral leukocytes, amplify the initial tissue damage and thereby cause neurodegeneration in the surrounding healthy tissue.

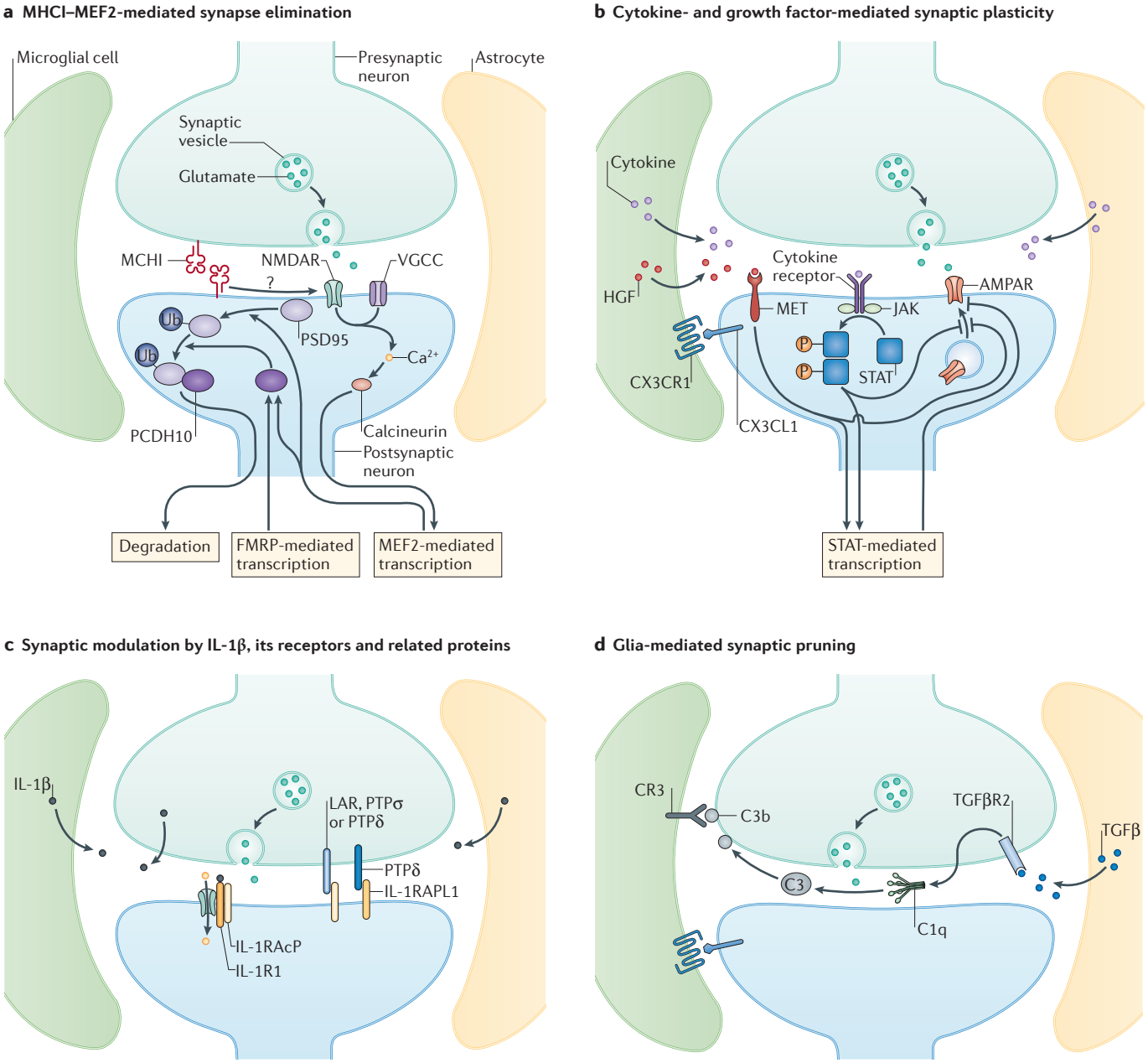
In the past 10 years, since our ability to measure cytokine levels and microglial morphology in the brain has become routine, the definition of neuroinflammation has grown increasingly murky to the point that the presence of any single hallmark of classical inflammation is now sufficient to define a disease as being 'inflammatory'. Reports of increased levels of pro-inflammatory cytokines in the post-mortem brains of individuals with autism spectrum disorder (ASD), in particular, have led to the hypothesis that chronic neuroinflammation plays a part in ASD pathogenesis. However, numerous studies cited as supporting this hypothesis have assessed only a handful of pro-inflammatory cytokines (typically interleukin-1β (IL-1β), IL-6, tumour necrosis factor (TNF) and interferon-γ (IFNγ)) without reporting any other hallmark of neuroinflammation[120,136,142]. Individual cytokines function as part of a larger homeostatic network of both pro- and anti-inflammatory, as well as regulatory, cytokines in which each factor can influence the synthesis and action of the other factors[261]. Thus, the impact of these cytokine networks on immune cells and tissues cannot be inferred from examining the levels of individual cytokines[262]. Determining whether inflammatory conditions predominate requires assessing (at the very least) the levels of a wide range of cytokines as well as the accompanying expected cellular signs of inflammation, such as microglial activation. However, despite the increasing numbers of studies measuring microglial activation, this classification remains subjective and represents a range of morphologies and states that change with developmental age and are only beginning to be understood.

Defining neurological and psychiatric diseases as inflammatory requires even more rigorous assessment within the brain because immune molecules and cells in the CNS are involved in physiological processes that can be mistaken for pathogenesis[263,264]. Currently, there is no consensus in the ASD field as to which criteria must be met to satisfy the label 'neuroinflammation', despite the widespread assumption that any inflammatory process is detrimental and leads to degeneration. Given the abundance of immune signalling that occurs under physiological conditions in the developing and mature brain, some of which is described in this Review, choosing a definitive set of criteria that constitutes a pathological state will prove to be challenging[265]. Nevertheless, it is particularly important in the case of ASD to define those immune mediators that serve adaptive roles and those that are pathological to better understand the mechanisms underlying this disorder and to tap into the exciting potential of targeting those functions for the future development of new neuroimmune therapies to treat ASD.

## Potential mechanisms

As our understanding and appreciation of neural–immune crosstalk continue to grow, there is an increasing focus on determining how immune dysregulation might alter brain connectivity and function to contribute to ASD-like phenotypes. In the past 10 years, immune molecules on neurons or glia in the brain have been demonstrated to regulate every stage of brain development and function[160,161]. The body repurposes immune cells, immune molecules and their receptors for widely divergent tasks in both region-specific and developmental-stage-specific manners. Alterations in the expression of these immune molecules in the brain, by genetic mutations or as a result of environmental risk factors, can lead to transient and/or lasting changes in brain development and function.

***Cytokines.*** Findings from both epidemiological studies and animal models indicate that cytokine imbalances can disturb fetal development and/or chronically impair brain function[162]. Cytokines and their receptors are expressed by neurons and glia throughout development and regulate a diverse array of physiological processes in brain development, plasticity and function[160]. For example, IL-1β and its receptors have important roles in a wide range of processes, including neurogenesis and synapse formation and plasticity, from early prenatal CNS development to postnatal development and adulthood[163–169]. Moreover, a mouse model mimicking the ASD-associated null mutation in *IL1RAPL1* exhibits reduced cortical synapse density, an enhanced ratio of excitation to inhibition in the amygdala and deficits in associative memory[169,170]. These findings are similar to those found in *Mecp2*-null mice, a model of Rett syndrome[170,171]. IL-1β functions within a tight homeostatic range; deviations above or below physiological levels lead to impairments in long-term potentiation and synaptic plasticity — two key molecular processes thought to underlie learning and memory[172]. Several other pro-inflammatory cytokines, including TNF, have similar diverse roles in early brain development and postnatal synaptic plasticity[173,174]. IFNγ also negatively regulates homeostatic processes during experience-dependent plasticity in the visual system[175]. Collectively, these findings suggest that increases or decreases in cytokine levels owing to genetic mutations or downstream of MIA could have deleterious effects on brain development and function independent of any role in inflammation (BOX 3; FIG. 2).




What mechanisms downstream from cytokines affect brain development? In the periphery, numerous cytokines signal through the Janus kinase–signal transducer and activation of transcription (JAK–STAT) pathway, which comprises three mammalian JAK and seven STAT proteins[176]. Receptor binding activates the kinase function of JAK, which recruits and phosphorylates STAT. Upon STAT dimerization, the complex translocates to the nucleus, where it regulates the transcription of genes involved in cell growth, differentiation and function, and immune genes including those encoding MHCI molecules. Although it is unknown whether the JAK–STAT pathway works in similar ways in the brain, recent reports suggest that JAK–STAT signalling is important for normal brain functions and that cytokines regulate this signalling in neurons. For example, STAT1 is upregulated in the visual cortex following long-term monocular deprivation, where it regulates AMPA receptor surface expression and synaptic function[175,177]. Moreover, IFNγ enhances STAT1 expression, which reduces visual cortical plasticity[175]. STAT1 is also implicated in spatial learning[178], and STAT1 and STAT3 have roles in hippocampal plasticity[179,180]. Although JAK–STAT signalling in the brain has just begun to be explored, it may represent an important therapeutic target for pathogenic cytokine-mediated alterations in brain development and function, such as those described above for ASD.

***MET.*** In addition to regulating immunity, MET has essential roles in the brain throughout development. *MET* is expressed throughout the neocortex, hippocampus[181],

Figure 2 | **Immune molecules at glutamatergic synapses.** At the 'quad-partite' synapse[218], pre- and postsynaptic neurons, astrocytes and microglia communicate using immune mediators, many of which are altered in individuals with autism spectrum disorder (ASD). Each panel represents molecular pathways that are used by immune molecules at the synapse to regulate synapse formation and/or plasticity. **a** | Major histocompatibility complex class I (MHCI) molecules are located at synapses, where they act through calcineurin to activate myocyte-specific enhancer factor 2 (MEF2) transcription factors to negatively regulate synapse strength and density. MHCI-dependent activation of MEF2 requires calcium influx through NMDA receptors (NMDARs) and voltage-gated calcium channels (VGCCs), although the molecules linking increases in MHCI expression and increases in NMDAR and VGCC conductance remain unknown (denoted by the question mark). MEF2 can act in concert with fragile X mental retardation protein 1 (FMRP) to stimulate the ubiquitylation of postsynaptic density protein 95 (PSD95) and increased association with protocadherin 10 (PCDH10), which then chaperones PSD95 to proteasomes for degradation. **b** | In general, cytokines released by astrocytes, microglia and/or neurons bind to their specific receptors and activate Janus kinase–signal transducer and activation of transcription (JAK–STAT) signalling at the synapse and alters transcription, leading to the negative regulation of AMPA receptor (AMPAR) expression, either through inhibiting new insertion or increasing internalization. Growth factors, especially hepatocyte growth factor (HGF), are also thought to be secreted from glial cells into the synaptic cleft, where they bind to and activate MET, which negatively regulates AMPAR expression, possibly through STAT-mediated transcription. Chemokines are also presented by neurons and bind to receptors on glial cells, depicted here for the interaction between CX3C chemokine receptor 1 (CX3CR1) on microglia and its ligand, CX3CL1, secreted by neurons or expressed in a tethered form on the neuronal cell surface. CX3CR1–CX3CL1 signalling is required for the migration of sufficient numbers of microglia into the brain in early development and, at older ages, for synaptic plasticity under physiological conditions. **c** | Interleukin-1β (IL-1β) exerts distinct effects at the synapse. Binding of IL-1β to IL-1 receptor type 1 (IL-1R1) recruits the IL-1 receptor accessory protein (IL-1RAcP), which increases NMDAR signalling. Unbound IL-1RAcP acts as a trans-synaptic adhesion molecule through its interactions with presynaptic protein tyrosine phosphatase-σ (PTPσ), PTPδ and leukocyte common antigen related (LAR). IL-1R accessory protein-like receptor 1 (IL-1RAPL1) also acts as a synaptic organizer by binding to presynaptic PTPδ. These trans-synaptic interactions exert multiple effects on synapse formation and plasticity, as described in the main text. **d** | Astrocyte-secreted transforming growth factor-β (TGFβ) binds to neuronal TGFβ receptor 2 (TGFβR2) (placed here presynaptically owing to findings at the neuromuscular junction), which induces neuronal secretion of the complement protein C1q. C1q initiates the complement cascade, leading to cleavage of C3 into C3b, which binds to synaptic surfaces. Microglial expressed complement receptor 3 (CR3) recognizes tagged synapses and initiates synaptic pruning at a subset of synapses. CX3CR1–CX3CL1 is also required for microglia-mediated synaptic pruning in early development, and spine elimination and formation in mature circuits. Although currently unknown, local neuron–microglia signalling through CX3CR1–CX3CL1 may serve as an instructive signal for complement-mediated synaptic pruning.

cerebellum[182] and brainstem[183], where it is enriched in excitatory pre- and postsynaptic compartments[184]. MET signalling induced by HGF increases the levels and clustering of synaptic proteins[185,186], increases the number of dendritic spines[187], modulates hippocampal synaptic function[186,188] and enhances hippocampal long-term potentiation[189]. Moreover, decreases in *MET* expression confer local hyperconnectivity, which is a putative hallmark of ASD pathophysiology[190]. Consistent with these results, reduced *MET* expression, as seen in ASD, alters key neurodevelopmental processes and is associated with structural and functional alterations in ASD[26]. Further investigation is needed to determine whether targeting downstream MET signalling modulates circuit activity and ameliorates the impairments in socio-communicative function associated with decreased *MET* expression[31].

***MHC class I molecules.*** MHCI molecules have been implicated in ASD through genetic associations (described above) and as downstream effectors of MIA[191]. MHCI molecules are found on all nucleated cells in the body, where they mediate the adaptive and innate immune responses. They are also present in neurons and glia throughout the CNS of many mammalian species[192,193]. In cortical pyramidal neurons, MHCI is present both pre- and postsynaptically at glutamatergic synapses, where its surface expression is tightly modulated by activity, therefore allowing it to regulate synaptic plasticity[192,194–200].

During CNS development, MHCI controls axonal and dendritic outgrowth, negatively regulates the initial establishment of cortical connections and promotes synapse elimination during activity-dependent refinement of connections in the developing visual system[191,193]. Interestingly, poly(I:C)-induced MIA in mice causes a dramatic increase in the expression of MHCI molecules on cortical neurons from newborn offspring. Dissociated neurons from MIA offspring also exhibit a profound deficit in their ability to form glutamatergic synapses. Remarkably, normalizing the levels of MHCI in cultured MIA neurons prevents the MIA-induced decrease in synapse density[191]. Thus, changes in MHCI signalling may be a common mechanism through which mutations in MHC genes and exposure to maternal infection could alter the establishment of connectivity in the developing brains of offspring. Whether changes in MHCI levels act downstream of mutations in other immune or non-immune ASD-linked genes, use convergent ASD-linked signalling hubs at the glutamatergic synapse or contribute to ASD-related behaviours are active areas of investigation.

***MEF2 transcription factors.*** Although myocyte-specific enhancer factor 2 (MEF2) transcription factors are not classical immune molecules, they seem to mediate the effects of immune dysregulation on synaptic connectivity. These transcription factors are also associated with ASD risk through genetic mutations and act as central molecular hubs downstream of several other ASD risk factors. MEF2 transcription factors regulate gene expression in an activity-dependent manner, affecting the expression of many proteins that regulate synaptic plasticity and function during neural development[201,202].

Interestingly, *MEF2C* haploinsufficiency syndrome is a recently discovered neurodevelopmental disorder that is characterized by autism-like behaviours, intellectual disability, high rates of epilepsy and abnormal movements[203]. MEF2 negatively regulates the establishment of hippocampal connections[202] and mediates the effects of MHCI on cortical connectivity in normal brain development and the MIA-induced deficit in synapse formation in newborn neurons[191]. In the genome-wide transcriptional profiling study of ASD[30] that was mentioned previously, the MEF2 splicing factor ataxin 2-binding protein 1 (A2BP1; also known as RBFOX1) — previously implicated in ASD[204,205] — was identified as a central hub within the network of downregulated genes associated with synaptic function. Moreover, in an integrative functional genomic analysis of ASD-associated genes, two co-expressed networks

upregulated during early fetal development and during late fetal–early postnatal development showed binding site enrichment for two isoforms of MEF2 (MEF2A and MEF2C)[206] that are predicted to drive the transcriptional co-regulation of both processes. In addition, MEF2 interacts with fragile X mental retardation protein 1 (FMRP; encoded by *FMR1* in humans) to regulate glutamatergic synaptic function[207], and this interaction controls the expression of protocadherin 10 (*Pcdh10*), which is another ASD-linked gene and is necessary for MEF2-mediated activity-dependent synapse elimination[208]. Together, these findings suggest that ASD that is associated with immune dysregulation during gestation, as well as idiopathic and syndromic forms of ASD, may converge on a molecular pathway with MEF2 as a hub[209]. Identifying new therapeutic targets within the signalling cascades upstream and downstream of MEF2 should be a priority for ASD research owing to their potential efficacy in treating a wide range of disorders on the autism spectrum.

***Microglia and complement.*** Microglia and complement may — similar to cytokines, MHCI molecules and MEF2 — also mediate the effects of both environmental and genetic ASD risk factors. Activated microglia are present in increased numbers and with an altered distribution in post-mortem brain tissue, especially the prefrontal cortex, in a subset of ASD cases[116,138,210,211]. Although microglia are best known for their role in clearing debris following injury, they have recently been shown to have important roles in the normal brain[212]. During development, microglia phagocytose debris from naturally occurring cell death, secrete trophic factors such as insulin-like growth factor 1 (IGF1) and TGFβ, regulate neurogenesis through phagocytosis of neural precursor cells and participate in activity-dependent elimination of synaptic connections[213–215]. Deficits in microglia, such as those seen in the CX3C chemokine receptor 1 (*Cx3cr1*)-knockout mice, lead to increased densities of immature synapses in the cerebral cortex, deficits in functional connectivity across brain regions and ASD-like behaviours[216,217].

Components of the complement cascade may mediate some of these microglial functions. In the peripheral immune system, the complement pathway mediates clearance of cellular debris and increases the degree of antibody binding to circulating bacteria and infected cells, thereby enhancing their destruction. Intriguingly, the complement *C4* gene lies within the MHC region, and deficiencies in C4 are associated with ASD[51,129,218]. Complement deficiency is also strongly linked to autoimmune disorders, especially SLE[219]. In the brain, the complement protein C1q is secreted by neurons[220]. Although many of the details of the complement cascade in the brain are unknown, C1q typically forms complexes with other C1 proteins to activate the C3 convertase, which cleaves C3 into opsonizing fragments in the periphery[221]. One of these fragments, C3b, is thought to tag weak synapses in the CNS. These tagged synapses are subsequently pruned through phagocytosis by microglia expressing complement receptor 3 (CR3)[222]. Thus, early immune insults have the potential to diminish or enhance microglia-mediated synaptic pruning. Astrocyte-dependent secretion of TGFβ regulates C1q expression and deposition in the lateral geniculate nucleus[223]. Because TGFβ levels are increased in the cerebrospinal fluid and in post-mortem brain tissue from individuals with ASD[116], it is possible that these increases in TGFβ levels in ASD brains could cause and/or reflect compensatory or pathology-inducing alterations in synaptic pruning. Importantly, microglia, similar to their macrophage relatives in the periphery, can be primed early in development[224]. Depending on the developmental trigger, altered priming can impair or enhance reactions to subsequent immune challenges[225,226]. Whether, and how, this immune priming contributes to ASD phenotypes remains an important area for future research.

Phagocytosis
The engulfment of extracellular pathogens or cellular debris by certain immune cells, including microglia.

## Perspective

***A common pathway?*** The results reviewed here suggest that many of the diverse immune contributions to ASD — including dysregulated signalling through MET, cytokines, MHCI molecules and microglia–complement — share the downstream effect of regulating synapse formation and elimination, thereby controlling synaptic function and plasticity in the developing and mature brain. Although research into the molecular mechanisms used by these immune ASD risk factors is in its infancy, one common signalling hub — MEF2 — has already been identified (FIG. 3). Importantly, this hub also mediates the effects of mutations in *MEF2* and *FMR1* in contributing to ASD-related phenotypes.

In addition to converging on MEF2 signalling, it is likely that many immune risk factors will converge on the most common intracellular signalling hub for ASD-risk genes — mammalian target of rapamycin (mTOR). Numerous studies of monogenic forms of ASD have implicated the mTOR pathway in ASD[227]. mTOR is a serine/threonine protein kinase that controls many aspects of neural development as well as the function and plasticity of the mature brain[228,229]. This pathway integrates signals from growth factors and hormones, neurotransmitters, cytokines and stress mediators, and involves crosstalk between two intracellular signalling cascades — RAS–ERK and PI3K–AKT — that are essential for immune and neuronal function. In the immune system, mTOR integrates signals from the immune microenvironment to direct immune cell metabolism, differentiation and function, and is important for mounting an adaptive immune response[230]. Alterations in mTOR signalling seem to underlie several common monogenic forms of ASD, including fragile X syndrome, tuberous sclerosis, Rett syndrome, PTEN macrocephaly and neurofibromatosis type 1, and syndromes with autistic-like features, such as *MEF2C* haploinsufficiency and 9q34.3 deletion syndrome. Interestingly, IGF1, which ameliorates some behavioural abnormalities and neuropathologies of Rett syndrome and 22q13 deletion syndrome and is secreted by microglia, activates mTOR[231].

Although most of the immune risk factors for ASD discussed above have not yet been directly studied for their effects on mTOR, there is some evidence to date that is consistent with the hypothesis that immune dysregulation may converge on this central signalling

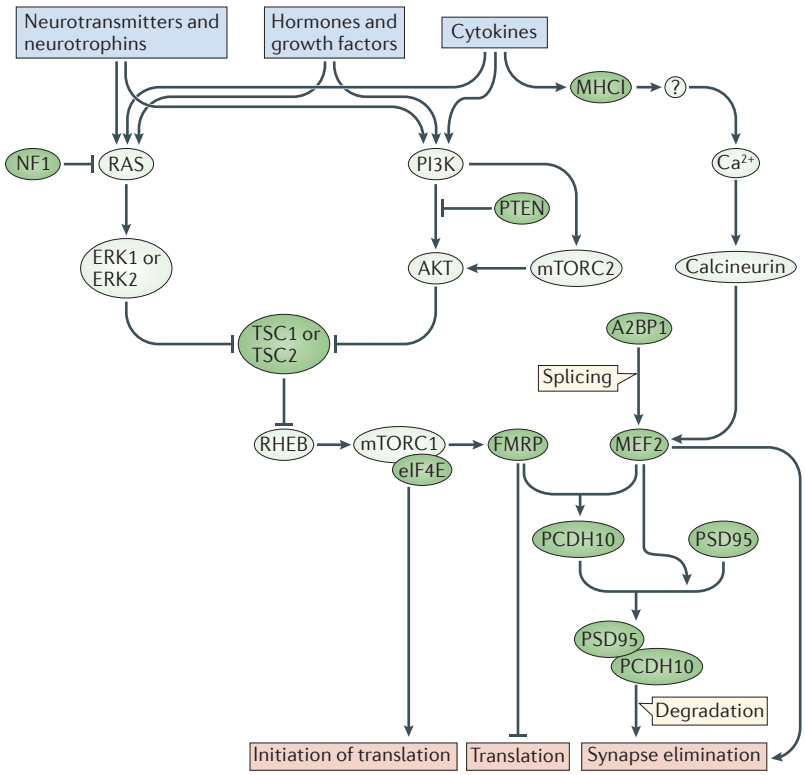



Figure 3 | **Synaptic immune signalling converges on mTOR.** Immune and neuronal receptor signalling activates various intracellular molecular pathways that feed into the mammalian target of rapamycin (mTOR) pathway and activate myocyte-specific enhancer factor 2 (MEF2)-dependent transcriptional regulation. These central signalling pathways may become dysregulated through genetic mutations or environmental exposures that are associated with autism spectrum disorder (ASD) and thereby alter neural development and function. mTOR activity regulates numerous processes, including protein synthesis, mitochondrial function, lipid synthesis, cell growth and proliferation, synaptic plasticity, neurogenesis, neuronal cell death, ion channel expression and cytoskeletal dynamics. Importantly, mTOR complex 1 (mTORC1) regulates the synthesis of glutamatergic receptors and protein products, including SH3 and multiple ankyrin repeat domains protein (SHANK), synapse-associated protein 90/postsynaptic density protein 95 (SAP90/PSD95)-associated protein (SAPAP), neuroligins, and AMPA and NMDA receptor subunits, many of which are genetically associated with ASD. Mutations in the genes that cause most of the syndromic forms of ASD — fragile X mental retardation 1 (*FMR1*), neurofibromin 1 (*NF1*), phosphatase and tensin homologue (*PTEN*), tuberous sclerosis 1 (*TCS1*) and *TCS2* — disrupt components of the mTOR signalling pathway. MEF2 is also implicated in ASD. *MEF2C* haploinsufficiency syndrome is characterized by ASD-like behaviours, perhaps through the function of MEF2 in regulating transcription during synapse formation and elimination. It is also likely that MEF2 regulates the expression of cytokine receptors in a positive or negative feedback loop, as the promoters of some cytokine receptors (such as interleukin-1 receptor accessory protein-like 1 (*IL1RAPL1*)) contain a MEF2-binding motif. MEF2 is also a target of the splicing regulator ataxin 2-binding protein 1 (*A2BP1*) — the central gene in a synaptic module identified in a transcriptome analysis of brain tissue from individuals with ASD. The protein products of genes that have been associated with ASD are shown in dark green. eIF4E, eukaryotic translation initiation factor, 4E; FMRP, fragile X mental retardation protein 1; MHCI, major histocompatibility complex class I; PCDH10, protocadherin 10.

pathway. For example, MET translates extracellular signals into mTOR activation; thus, the ASD-related *MET* 'C' variant is predicted to lead to hypoactive mTOR signalling[232]. Moreover, many cytokine receptors found at the synapse typically activate mTOR signalling in the periphery, potentially causing phenotypes similar to monogenic forms of ASD. Although it is unknown whether mTOR is activated in response to cytokines in the brain, the signalling components for mTOR are present in neurons and are activated by a growing list of growth factors, guidance molecules and neurotransmitters[228]. mTOR is therefore a focal point to integrate immune signalling in the brain, changes in cytokine levels due to MIA, postnatal environmental insults or mutations in immune genes, and ongoing immune dysregulation throughout life. Determining whether, and how, the immune contributions discussed in this Review converge on this common mTOR pathway will be crucial for determining how pervasive mTOR signalling is across the diverse forms of ASD and for the development of new therapeutics that target mTOR in the future.

***Therapeutics.*** One of the most exciting implications of the discovery that immune dysregulation may contribute to ASD is the possibility that agents targeting immune function could alleviate some of the symptoms associated with this spectrum of disorders. One of the most effective, albeit drastic, therapeutic approaches for restoring immune function is bone marrow transplantation. Remarkably, transplantation to reconstitute the brain with wild-type microglia reverses somatic phenotypes, neuropathological changes and behavioural abnormalities in two mouse models of ASD — the *Mecp2*$^{-/y}$ model of Rett syndrome[233] and the MIA model[133]. Full immune system reconstitution from control animals also rescues peripheral immune abnormalities in MIA offspring, including deficits in regulatory T cells and a disproportionate increase in CD4$^{+}$ memory T cells with an inflammatory profile previously implicated in learning and memory deficits[132,133] (BOX 3). On the basis of these animal studies, clinical trials using strategies to reconstitute immune cells in individuals with ASD have been initiated[234,235].

Another class of potential therapies targeting immune function is anti-inflammatory or immunosuppressive agents. To date, therapies in this category have focused on decreasing the assumed ongoing inflammation in the periphery and brain of individuals with ASD. Consistent with this idea, minocycline, a broad-spectrum antibiotic that has immunosuppressive properties, corrects synaptic abnormalities, heightened anxiety and social deficits in *Fmr1*-knockout mice[236,237]. In a randomized double-blind, placebo-controlled trial in children and adolescents with fragile X syndrome, 3 months of minocycline treatment improved anxiety and mood-related behaviours[238]. Although antibiotic treatment may also improve symptoms in idiopathic forms of ASD[239–241], ASD with regressive features may be resistant to minocycline therapy[242]. The exact immunomodulatory mechanisms of minocycline are unknown; however, it may exert its effects through a combination of suppressing cytokine signalling, enhancing neurotrophic factor secretion and suppressing mTOR signalling[243]. Minocycline could relieve ASD-related phenotypes directly through altering neural function or indirectly through ameliorating ongoing immune dysfunction or through regulating the intestinal microbiota[241].

Despite their potential, treatments focused on preventing or ameliorating neuroinflammation in ASD should be considered with caution because the immune dysregulation associated with ASD may not always be pro-inflammatory. As discussed above, mouse models of MIA exhibit decreased levels and function of many types of immune molecules in the brains of offspring during postnatal development, which is the opposite of the widely assumed inflammatory processes present in ASD[145]. Moreover, many of the immune molecules associated with ASD play important parts in brain development, function and plasticity. Thus, altered levels of these immune molecules in ASD may alter brain development through defects in their normal function in addition to, or even rather than, causing inflammation. Finally, it is important to consider the possibility that limited periods of neuroinflammation could be an adaptive response to allow the brain to cope with ASD-related deficits[146]. Studies in humans indicate that fever improves many ASD symptoms, especially deficits in communication and increases in repetitive behaviours[244–246]. Although these effects have not been directly tested in animal models, several studies suggest that fever temporarily corrects chronic mitochondrial dysfunction — a frequently observed co-morbidity seen in ASD — through alterations in purinergic signalling[247,248]. The mechanisms underlying these paradoxical effects of fever are important areas of ongoing research, as they may well reveal new therapeutic targets for ASD. If inflammation is indeed adaptive, as implied by these reports, then treating individuals with anti-inflammatory agents could have unintended detrimental consequences. Nevertheless, despite the possible complexity in their roles in ASD, immune molecules provide a new and important set of targets for ASD drug discovery in the future.

## Note added in proof

It is important to note that a recent study failed to find any benefit from wild-type microglia transplantation in three rodent models of Rett syndrome[266], including the model used in the original study[233]. The reason for these divergent findings is unclear, but it will be essential to understand, as it will affect the potential therapeutic value of bone marrow or stem cell transplantation in individuals with Rett syndrome.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**Competing interests statement**
The authors declare no competing interests.

**FURTHER INFORMATION**
Simons Foundation Autism Research Initiative (SFARI) Gene: https://gene.sfari.org

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