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Autoimmunity in autism

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Abstract

Autism spectrum disorders is a heterogenous group of neurodevelopmental disorders, the etiology or etiologies of which remain unknown. Increasing evidence of autoimmune phenomena in individuals with autism could represent the presence of altered or inappropriate immune responses in this disorder, and this immune system dysfunction may represent novel targets for treatment. Furthermore, in recent studies, antibodies directed against the fetal brain have been detected in some mothers of children with autism; these antibodies have the ability to alter behavioral outcomes in the offspring of animal models. A better understanding of the involvement of the immune response in early brain development, with respect to autism, may have important therapeutic implications.

Keywords

Autism spectrum disorder; autoantibody; autoimmunity; immune; inflammation; neuroinflammation

Introduction

Autism spectrum disorder (ASD) is a group of heterogeneous, neurodevelopmental disorders that include autistic disorder, pervasive developmental disorder-not otherwise specified (PDD-NOS) and Asperger syndrome, which, along with Rett syndrome and childhood disintegrative disorder, comprise the pervasive developmental disorders [1]. ASD typically is diagnosed within the first 36 months of life and is characterized by impairments in social reciprocity, verbal and nonverbal communication, and repetitive and stereotypical behaviors [1]. The prevalence of ASD is increasing, with some estimates as high as 8 cases per 1000 births [2,3].

The exact etiology of ASD remains unknown; however, due to the heterogeneity of symptoms within ASD, such as the pattern of symptom onset, severity of disabilities, epilepsy, sleep problems, gastrointestinal disturbances, and deficits in IQ, distinct

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phenotypes may exist within ASD and may represent separate etiologies or combinations of susceptibility and environmental factors [4–6]. As with most complex disorders, it is likely that interactions between multiple causative factors, including genetics, epigenetics, pre- and postnatal environments, exist that may contribute to neurodevelopment changes and may lead to features that are characteristic of ASD. As such, the factors contributing to the development of ASD may vary between individuals. Recent research has highlighted a potential role for the immune system in ASD, including immunogenetic abnormalities and the inappropriate response of the immune system to environmental challenges. Evidence of abnormal immune activation has included repeated observations of increased autoreactive antibodies in children with ASD and their mothers [7–29]. In this review, the complex relationship that may exist between autoimmunity and ASD, and the possible treatment implications for ASD are discussed.

Evidence of immune system abnormalities in autism spectrum disorder

Genetics and the immune system—Multiple genetic association studies have implicated different genes with the development of ASD, several of which are involved with the function of the immune system. The HLA alleles A2, DR4 and DR11 have been linked with susceptibility to ASD [24–27], as well as decreased lymphocyte responsiveness [25]. Within the HLA class III region, there is a complement C4B null allele, resulting from duplications of C4A, that confers a relative risk of 4.3 for the development of ASD [28,30,31]. In addition, several studies have implicated the *MET* (met proto-oncogene) tyrosine kinase pathway with ASD [32–34]. Signaling via the MET receptor is necessary for immune regulation in APCs, including dendritic cells and monocytes [35]. Similarly, the serine and threonine kinase C gene *PRKCB1,* which is involved in both B-cell activation and neuronal function, has been linked to ASD in some studies [36,37], but not in others [38,39], perhaps reflective of population differences. Peripheral blood RNA expression studies demonstrated an upregulation of genes involved in innate immune activation through the NK pathway in individuals with ASD [40], a finding that was confirmed in functional studies using NK-cells [41–43]. Functional polymorphisms of macrophage inhibitory factor (MIF), which has several effects on innate and adaptive immune responses, have also been associated with individuals with ASD, but has not been detected in unaffected siblings [29]. Interestingly, increased sera concentrations of MIF correlated with worsening behavioral assessments in individuals with ASD compared with their unaffected siblings [29]. Moreover, genes that can affect immune responses, such as PTEN (which may be involved in T regulatory cell development) and reelin, have been associated with ASD [24–29,33,44–46]. An epidemiological study in Portugal identified mitochondrial respiratory-chain disease in 4.2% of children with ASD [47]; conditions such as mitochondrial respiratory-chain disease could have widespread effects on many body systems, including the activity of the immune system. Overall, these studies suggest that multiple susceptibility genes related to innate immune activation and/or the loss of adaptive immune regulation may be involved in the etiology of ASD.

Evidence of inflammatory cytokine responses in ASD

Neuroimmune interactions begin early in development, and the health of one system is contingent on the health of the other. Both systems share common signaling molecules and

receptors, such as cytokines and neuropeptides/neurotransmitters. Aberrant responses from immune cells present in the CNS, including microglial cells in the brain, have been implicated in neuronal cell death [48–51], which is partly mediated through the actions of inflammatory cytokines and neuropeptides. The inflammatory cytokines IL-1, IL-6 and TNFα can directly affect the CNS, can alter neurodevelopment and, subsequently, may have an impact on behavior [52–54]. For example, neuropoietic cytokines, such as IL-6, can have direct effects on neurons and glia *in vitro,* including changes in proliferation, survival, death, neurite outgrowth and gene expression [54,55]; while *in vivo,* the peripheral administration of IL-6 to gestating mice has been demonstrated to affect behavior and neurodevelopment of offspring [10]. In addition, cytokines such as TNFα have been linked to oligodendrocyte toxicity, as well as aberrant neurite growth [56–58]. While a cytokine-mediated mechanism has yet to be conclusively established in neurodevelopmental disorders such as ASD or schizophrenia, evidence of adverse immunological functioning in these disorders suggests that neuroimmune interactions may be altered and may affect neurodevelopment and early brain development [59–62].

In studies of post-mortem brain specimens, evidence of elevated cytokine responses, including IL-6 and TNFα, has been observed in the brains of individuals with ASD compared with controls [63,64]. These cytokines appear to have been derived from microglial and astroglial cells of the innate immune system [64]. In the temporal cortex of the brain, transcriptome expression patterns from individuals with ASD also demonstrated that enhanced immune responses occurred in the brains of individuals with ASD compared with controls, and were consistent with increased autoimmune responses and increased inflammatory cytokine signaling [34]. Moreover, several researchers have suggested that there is an increased presence of proinflammatory cytokines and a skewing of the ratio of Th1-associated cytokines compared with Th2-associated cytokines in the brain and periphery of individuals with ASD (Table 1) [9,11,12,65–68]. Increased IFNγ has been detected in brain tissue, CSF, plasma, serum, cultured PBMCs and cultured NK-cells from individuals with ASD [11,41,63,64,66,67]. However, a separate study failed to demonstrate any differences in plasma IFNγ levels [69], and another study demonstrated decreased IFNγ responses to phorbol myristate acetate (PMA)/ionomycin stimulation in PBMCs in individuals with ASD compared with controls [65]. These discrepancies may be due to the patient population, for example, whether individuals had a full autism diagnosis or were classified on the broader ASD spectrum, patient age and gender, as well as the analytical techniques, power of the statistical analysis and tissue/ specimens used. Another factor that often complicates studies of ASD has been the use of siblings of children with ASD as controls. Importantly, as autism is highly heritable, similar findings in analyses of the immune system in individuals with ASD compared with their siblings may reflect that there are shared immune susceptibilities that are not the result of brain pathology or neurodevelopmental changes. While most studies have demonstrated increased proinflammatory cytokine production in individuals with ASD [9,11,12,65–68], some studies have reported a decreased production of regulatory cytokines, including IL-10 and TGFβ1, in individuals with ASD [9,12,68,70,71]. As both IL-10 and TGFβ1 have a role in regulating the immune response, these data suggest that immune regulation may be impaired

in ASD; this impaired immune regulation could lead to a breakdown in immunological tolerance.

The potential role of perinatal or chronic infection in ASD

Environmental factors, such as congenital rubella infection, have previously been reported to be associated with ASD [72,73]. Perinatal *Haemophilus influenzae* and CMV infections have been associated with ASD through significant damage to the immature brain [74]. Associations between ASD diagnosis and increased ear infections [75], antibiotic use [75] and infections in the first 30 days of life (and lower rates compared with controls thereafter) [76] have also been observed. However, many of these association studies have not been replicated and, to date, no infectious agent has been definitively causally linked with ASD. Furthermore, because many different infectious agents have been implicated in ASD, one possible hypothesis is that an inappropriate immune response or immune dysfunction in response to a variety of possible infectious agents, which occurs at critical periods in neurodevelopment, may have more of an impact on neurodevelopment than any one specific causative agent.

Evidence of a role for autoimmunity in ASD

Brain autoantibodies in individuals with ASD: Evidence of a loss of tolerance that could lead to autoimmunity in ASD includes the demonstration of antibody reactivity to several regions of the brain, such as the human frontal cortex [15,77], temporal cortex [78], cerebellum [15,21,79], hypothalamus and thalamus [19], and brain endothelial cells [18], as well as the presence of anti-nuclear antibodies [78] (Table 2). Antibodies with reactivity to the caudate nucleus and cerebellum in rats, but not toward the hippocampus or brainstem, have also been described [80]. As several different CNS targets have been observed in individuals with ASD, these data may suggest that there are multiple mechanisms whereby CNS reactivity has developed in ASD. In addition, it is unclear whether these antibodies are produced as a primary event that may affect neurodevelopment or as a result of early brain inflammation. Moreover, the increased presence of antibodies against CNS targets does not address the possible function these antibodies have, specifically whether the antibodies block cellular function, act as agonists or antagonists on receptor systems, or bind to self-protein and result in tissue damage. Previous disparate reports of autoantibody reactivity to myelin basic protein (MBP) in ASD underscore the difficulty in accessing autoreactive immune responses in a highly heterogenous disorder. Singh and colleagues reported that sera samples from approximately 58% of children with ASD (≤ 10 years old), compared with 9% of controls ($p \leq 0.0001$), reacted to MBP in a protein-immunoblotting assay [14], a finding that was replicated in further studies by these researchers [81,82], and was independently demonstrated in studies by Connolly and colleagues [18] and by Vojdani and colleagues [83]. In the studies by Singh and colleagues, Western immunoblotting was used to confirm MBP reactivity; this assay was conducted by separating MBP on a reducing (denaturing) gel, transferring the MBP to a nitrocellulose membrane, and incubating the denatured protein in the presence of sera with a detergent. The reactivity of the sera to the denatured MBP then was confirmed using secondary anti-human Ig antibody. One advantage of this method is that only reactivity to the protein of interest (eg, MBP) was identified; however, false positives due to the use of non-human MBP or poor blocking may have occurred.

Vojdani and colleagues [83] and Connolly and colleagues [18] used ELISA to determine MBP reactivity in order to limit reactivity to intact non-denatured protein; in addition, individuals with multiple sclerosis, who produce antibodies to MBP during active phases of disease, were included as disease controls in the study by Vojdani and colleagues [83]. In contrast, Silva and colleagues examined plasma reactivity to homogenized human brain and human MBP protein by Western immunoblotting in children with ASD (n = 171) and pediatric controls (n = 54), and were able to identify antibody reactivity to a protein at approximately 20 kDa; this reactivity could be used to discriminate between children with ASD and controls ($p = 0.00046$, Mann-Whitney *U*-test) [84]. Although the putative antigen was a similar molecular weight to MBP, it was not an MBP isoform [84]. The brain extracts and MBP proteins were separated on the same denaturing gels, which minimizes inter-assay variability. One limiting factor of this study is that reactivity to whole protein was not determined. Furthermore, Libbey and colleagues reported similar titers of antibodies against MBP, as assessed by ELISA, in the plasma of individuals with ASD (either individuals with autism symptoms that appeared early in life or those that met normal developmental milestones, but then underwent regression and developed ASD) when compared with controls [85]. Moreover, it was demonstrated that MBP-reactive antibodies positively correlated with increased age, therefore age matching is critical in these experiments, and disparate ages of cases compared with controls could have affected previously published reports [85]. Similarly, contradictory results have been reported from examinations of sera for the presence of autoantibodies against glial fibrillary acidic protein (GFAP) in individuals with autism and individuals with Tourette syndrome, compared with controls [86]. There are, however, some findings that suggest antibodies to neurofilaments and brain-derived neurotrophic factor may be increased in individuals with ASD compared with controls (Table 2) [18,20,79,83], although these findings have not been replicated. Overall, these data suggest that antibodies reactive against CNS and brain proteins exist in individuals with ASD; however, the exact antigen to which these antibodies bind has not been identified. These data also suggest that not all individuals with ASD have the same autoantibody repertoire, and that antibody specificity may vary among individuals with ASD.

Predisposition to autoimmunity in families with children with ASD: Several studies have demonstrated increased familial histories of autoimmune diseases including type I diabetes, rheumatoid arthritis (RA), hypothyroidism, psoriasis and systemic lupus erythematosus in first degree relatives of individuals with ASD [87–91]. Interestingly, 70% of children that had a developmental regression associated with ASD diagnosis had a 1st or 2nd degree relative with an autoimmune disease, compared with 55% of children who had an early onset of ASD symptoms [89]. Of the familial autoimmune disorders linked to ASD, an RA diagnosis was observed in greater than one-third of relatives (34 to 46%) [87], and an increased frequency of hyperthyroidism has also been reported (36% in families with an individual with ASD compared with 14% in control families studied) [92]. In addition, a maternal diagnosis of psoriasis in the 4 years surrounding pregnancy, or allergy/asthma in the second trimester of pregnancy conferred a 2-fold increased risk of ASD diagnosis in offspring [88]. These data suggest that inappropriate maternal immune responses may alter the course of neurodevelopment. It should be noted, however, that many of these reports rely

on medical chart reviews or interviews, which can underreport or overreport incidences, respectively, and as such the role of familial autoimmunity in the etiology of ASD requires further investigation.

The presence of maternal anti-fetal antibodies: The breakdown of maternal tolerance to the fetal brain and the generation of antibodies directed toward fetal brain tissue has been detected in mothers of children with ASD (Table 3) [13,16,22,23,93,94]. Braunschweig and colleagues identified the presence of antibodies that were reactive to human fetal brain proteins that were 37 kDa and 73 kDa in molecular weight in 12% of mothers of children with autism [16]. Another study by Croen and colleagues detected the presence of antibodies to human fetal brain proteins of 39 kDa and 73 kDa in mothers of children with autism [23]. Interestingly, the pattern of 37 kDa and 73 kDa staining was more prevalent in children who had autism symptom regression [16], and the 39 kDa and 73 kDa pattern was present more often in children with early onset autism symptoms [23]. In both studies, the specificity of the antibody staining patterns was high, compared with mothers of typically developing children or mothers of children with developmental delay, who did not demonstrate these antibody staining patterns. Notably, Singer and colleagues also reported that sera from mothers of children with ASD was reactive to a 73 kDa embryonic brain protein from rats [13], and to proteins of approximately 36 kDa and 39 kDa derived from human fetal brains at 17 weeks of gestation [13]. At present, the significance of these bands is difficult to ascertain without the identification of moieties to which these bands correspond, which could potentially be ubiquitous, tissue specific, or only expressed during specific development stages. In addition, with the exception of the 73 kDa bands, which were also identified in children with ASD [15], the bands found in the sera of mothers of children with ASD do not correspond to those of their children. To explore the possible pathogenic roles of these antibodies, the effect of these individual antibodies on developing fetuses must be investigated in animal models.

Maternal brain-reactive antibodies and models of neurodevelopment: Two studies have investigated the effect of transferring antibodies from mothers of children with ASD to pregnant animals to determine the mechanism of influence of the antibodies on neurodevelopment [93,95]. The first study transferred sera demonstrated to contain neuronal specific antibodies from a mother of three children, one of whom had autism and another with a language disorder, into pregnant mice by intraperitoneal injection. The offspring of the mice injected with sera from mothers of children with ASD were tested using a battery of behavioral assessments, and demonstrated several deficits in social behavior and motor skills [93]. In addition, examinations of brain specimens from these offspring demonstrated evidence of cerebellar abnormalities [93]. The offspring of mice injected with sera from mothers of healthy, typically developing children did not demonstrate any abnormalities in behaviors or neuropathology [93].

In the second study, human IgG was isolated from mothers of children with ASD or mothers of typically developing children and injected into rhesus macaques [95]. Purified IgG was pooled from the sera of mothers of children with ASD (n = 12) that had demonstrated IgG reactivity to fetal brain extract and intravenously injected into rhesus macaques (n = 4) at

gestation days 27, 41 and 55. Similarly, pooled IgG from mothers (n = 7) of typically developing children who had no evidence of reactivity to fetal brain extract were used as controls and injected into pregnant rhesus monkeys (n = 4) [95]. The offspring from both groups were observed, and behavioral testing was conducted from 1 month to 1 year of age. Following weaning, animals exposed to IgG from mothers of children with ASD displayed more social irregularities, including less social behavior and increased whole body stereotypic behaviors, including increased activity, pacing and back-flipping, compared with animals exposed to IgG from mothers of typically developing children, who did not demonstrate these behaviors [95]. These data suggest that the transfer of IgG from mothers of children with ASD can lead to abnormalities in behavioral outcomes. Also, antibodies to neuronal proteins that are contained within the IgG fraction may likely be responsible for such changes; however, further experiments are needed to confirm these findings. In addition, as the antibodies were not selected based on specific reactivity, it is difficult to determine what these antibodies may be reactive to and whether it is one antibody or a combination of several antibodies with different specificities. Moreover, it is unclear whether these antibodies cause neuronal damage, or inhibit functions that lead to behavioral changes.

Therapeutics targeting the immune response in ASD—Pharmaceutical treatments for ASD currently focus on behavioral function, including irritability and stereotypies; however, it is unknown whether these medications significantly affect the immune system. SSRIs and risperidone are common clinical interventions for managing behavior in ASD; these treatments have been reviewed in previous publications (see references [96–98]). While risperidone is the only FDA-approved treatment for irritability in ASD, typical and atypical antipsychotics, including clozapine and olanzapine, as well as psychotropics, such as divalproex sodium and lithium, have also been used to treat other behavioral aspects of ASD [96]. The immunological effects of several of these pharmaceuticals have been evaluated in another neurodevelopmental disorder, schizophrenia, in which improved symptoms correlated with reductions in proinflammatory cytokines, and support the potential involvement of cytokine abnormalities in behavioral symptoms [99–102]. These findings raise the possibility that in some individuals with ASD, behavior may be altered in part through changes of the immune response, as well as through direct or indirect effects of drugs on the immune response, such as through serotonin receptors on immune cells. It is also possible that the immune profile of a patient prior to treatment may predict responsiveness to specific pharmaceutical interventions. There have been no studies conducted to date specifically examining this relationship in autism. Furthermore, there have been few placebo-controlled clinical trials of anti-inflammatory or immunomodulatory agents to evaluate whether these drugs beneficially alter behavioral responses in individuals with ASD. Thus far, contradictory evidence has been reported in the limited pilot clinical trials undertaken for immunomodulatory therapies, such as steroid [103–105] or IVIG treatment [106–109], most likely due to patient selection and the highly heterogeneous nature of ASD. Future studies that either demonstrate immune or autoimmune dysfunction in ASD are warranted and may help to identify patient groups that would benefit from certain treatments. In contrast to conventional pharmaceutical treatments, recent studies concluded that more than half of children with ASD were undergoing complementary and alternative medicinal (CAM) therapy to treat specific ASD symptoms [110–114], with many

treatments aimed at addressing immune dysfunctions. While there is little or conflicting evidence to support many CAM therapeutics, these therapies remain popular within the autism community.

Conclusion

A large amount of evidence supports widespread immune dysregulation in individuals with ASD. Immune dysfunction, increased cytokine production, decreased regulation and the presence of brain reactive antibodies suggest that autoimmune responses may occur, or have previously occurred, in individuals with ASD. Moreover, researchers have demonstrated that purified IgG containing fetal brain-reactive antibodies from the mothers of children with ASD can induce abnormalities in behavioral symptoms in animal models. These findings suggest that neural reactive antibodies may be causative rather than secondary to abnormal brain development in ASD. Taken together, these results suggest that therapeutic immunomodulation, which can lead to more balanced immune responses, may be beneficial. Future therapeutic strategies targeting the immune abnormalities observed in ASD may be warranted. However, it must be noted that the exact role of any immune dysfunction in ASD and whether this dysfunction has a critical role in the core features of ASD or contributes to secondary features, such as epilepsy, sleep disturbances or gastrointestinal symptoms, is not fully understood. Further research into the nature of the immune dysfunction in ASD and the specific targets of CNS/brain-reactive antibodies in individuals with ASD is needed. Such studies will enhance the understanding of the underlying mechanisms of both immune anomalies and altered neurodevelopment in the etiology of ASD, and may reveal targets for future therapeutic interventions.

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•• of outstanding interest

• of special interest

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Table 1.

A summary of studies of cytokine levels in individuals with autism spectrum disorder.

Cytokine/ chemokine tested	Change detected in cytokine/chemokine levels in patients with ASD	Tissue source	Method	Reference
Th1-associated cytokines				
IFNγ	Increased	CSF, brain tissue, whole blood, plasma and cultured PBMCs	Luminex and ELISA	[11,63,64,66,67]
IFNγ	No change	Plasma	ELISA	[69]
IFNγ + T-cells	Decreased	Cultured PBMCs	Flow cytometry	[65]
IL-12	Increased	Plasma	ELISA	[11]
IL-2	Increased	Serum and cultured PBMCs	ELISA	[8,66]
IL-2	No Change	Brain tissue	Luminex	[63]
IL-2 + T-cells	Decreased	Cultured PBMCs	Flow cytometry	[65]
Acute inflammatory cytokines				
IL-1β	Increased	Brain tissue and stimulated PBMCs	Luminex and ELISA	[63,115]
IL-1β	No change	Plasma	ELISA	[69]
IL-6	Increased	CSF, brain tissue and cultured PBMCs	Luminex and ELISA	[63,64,67,115]
IL-6	No change	Plasma, serum and cultured PBMCs	ELISA and flow cytometry	[7,11,65]
TNFα	Increased	Brain tissue and cultured PBMCs	Luminex and ELISA	[63,67,115]
TNFα	No change	Plasma	ELISA	[11,69]
TNFRI	Increased	Stimulated PBMCs	ELISA	[115]
TNFRII	Increased	Stimulated PBMCs and serum	ELISA	[7,115]
IL-8	Increased	CSF and brain tissue	Luminex	[63,64]
MCP-1	Increased	CSF and brain tissue	Luminex and ELISA	[64]
MIP-1β	Increased	CSF	Luminex	[64]
TGFβ	Increased	Brain tissue	Luminex and ELISA	[64]
TGFβ	Decreased	Plasma	ELISA	[70,71]
GM-CSF	Increased	Brain tissue	Luminex	[63]
Th2-associated cytokines				
IL-4	Increased	Cultured PBMCs	ELISA and flow cytometry	[66,65]
IL-4	No change	Brain tissue	Luminex	[63]

Cytokine/ chemokine tested	Change detected in cytokine/chemokine levels in patients with ASD	Tissue source	Method	Reference
IL-5	Increased	Cultured PBMCs	ELISA	[63,66]
IL-5	No change	Brain tissue	Luminex	[63]
IL-10	No change	Brain tissue and cultured PBMCs	Luminex, ELISA and flow cytometry	[63,65,66]
IL-13	Increased	Cultured PBMCs	ELISA	[66]

Four studies were excluded from this table because children with autism spectrum disorder (**ASD**) that were recruited in these excluded studies experienced gastrointestinal symptoms, which may confound cytokine results [9,12,68,116].

MCP-1 monocyte chemotactic protein 1, **MIP-1β** macrophage inflammatory protein 1β, **TNFRI** TNF receptor 1, **TNFRII** TNF receptor II

Table 2.

Studies investigating the presence of autoreactive antibodies in individuals with ASD.

Sample tested	Positive or negative finding	Findings	Reference
Brain			
Whole brain extract	Positive	Samples from individuals with ASD reacted to a 20 kDa protein.	[84]
Cerebral cortex	Positive	18% of samples from individuals with ASD vs 0% controls had antibodies against rat cerebral cortex.	[80]
Prefrontal cortex	Positive	Samples from individuals with ASD reacted to a 100 kDa protein.	[15]
	Negative	No reactivity to frontal cortex proteins was detected.	[77,79]
Temporal cortex	Positive	27% of samples from individuals with ASD vs 2% controls had antibodies against proteins in the temporal cortex.	[78]
Brodmann's area 10	Positive	Increased density of a 160 kDa protein band in samples from individuals with ASD.	[15]
Caudate nucleus	Positive	49% individuals with ASD vs 0% controls reacted to bands at 49, 115 and 160 kDa from rat caudate nucleus.	[80]
	Positive	Reactivity towards 100 kDa in ASD compared with controls.	[15]
Cingulate gyrus	Positive	Increased density of a 73 kDa protein band in samples from individuals with ASD.	[15]
Putamen	Positive	Increased density of a 100 kDa protein band in samples from individuals with ASD was detected.	[15]
Thalamus	Positive	29% of samples from individuals with ASD vs 8% of controls reacted to a 52 kDa protein.	[19]
Hypothalamus	Positive	37% of samples from individuals with ASD vs 11% of controls reacted with 42, 46 and 48 kDa proteins.	[19]
	Positive	30% of samples from individuals with ASD vs 11% controls reacted to a 52 kDa protein.	[19]
Cerebellum	Positive	9% of samples from individuals with ASD vs 0% of controls reacted to rat cerebellum proteins.	[80]
	Positive	Samples from individuals with ASD reacted with a 73 kDa protein.	[15]
	Positive	Reactivity to cerebellum proteins in 88% of samples from individuals with ASD vs 23% controls.	[79]
	Positive	21% of samples from individuals with ASD vs 2% controls reacted to an approximately 52 kDa protein.	[21]
CNS proteins			
MBP	Positive	35% of samples from individuals with ASD vs 5% controls reacted to MBP and other ligands simulaneously[1].	[83]
	Positive	58% of samples from individuals with ASD vs 9% controls reacted with MBP.	[14]
	Positive	60% of samples from individuals with ASD vs 0% controls reacted with MBP.	[81]
	Positive	64% of samples from individuals with ASD vs 0% controls reacted with MBP.	[82]
	Positive	Increased levels of MBP autoantibodies in individuals with ASD vs controls ($p < 0.001$).	[18]
	Negative	No reactivity to myelin basic protein was detected.	[84]

Sample tested	Positive or negative finding	Findings	Reference
Neurofilaments	Positive	Anti-neurofilament IgG detected in 41% of samples from individuals with ASD vs 7% controls; IgM detected in 53% ASD vs 22% controls.	[79]
	Positive	35% of samples from individuals with ASD vs 5% of controls reacted to neurofilament protein [1].	[83]
	Positive	57% of samples from individuals with ASD vs 0% of controls reacted to neurofilament protein.	[82]
	Positive	55% of samples from individuals with ASD vs 27% of controls reacted to neurofilament protein.	[20]
Glial fibrillary acidic protein (GFAP)	Positive	32% of samples from individuals with ASD vs 9% of controls reacted to GFAP.	[20]
	Negative	No reactivity to GFAP was detected.	[86]
Brain-derived neurotrophic factor (BDNF)	Positive	Increased BDNF autoantibodies present in individuals with ASD vs healthy controls ($p < 0.001$).	[18]
Other			
Anti-nuclear antibodies (ANAs)	Positive	20% of samples from individuals with ASD vs 3% of controls had ANAs; levels correlated to disease severity.	[117]
	Positive	27% of samples from individuals with ASD vs 0% of controls had ANAs.	[78]
	Negative	No ANAs or laminin antibodies were detected.	[118]
Hsps	Positive	Hsp90 autoantibodies detected in 19% of samples from individuals with ASD vs 0% of controls.	[119]
	Positive	Hsp60 autoantibody levels in individuals with ASD were similar to those in autoimmune adults.	[120]
Tubulin	Positive	Autoantibodies detected in 35% of samples from individuals with ASD vs 5% of controls [1].	[83]
Metallothionein-1	Positive	Autoantibodies detected in 35% of samples from individuals with ASD and their families vs 9% of controls and their families; no difference between individuals with ASD and their family members was observed.	[121]
	Negative	No reactivity to metallothionein-1 was detected.	[122]

Increased self-reactive antibodies have been detected in individuals with autism spectrum disorder (**ASD**) compared with controls, using human brain extract unless otherwise indicated.

[1]35% of ASD and 5% of control individuals had simultaneous IgG reactivity to myelin basic protein (**MBP**), myelin-associated glycoprotein, neurofilament protein, ganglioside, sulfatide, α,β-crystallin, chondroitin sulfate, myelin oligodendrocyte glycoprotein and tubulin.

Table 3.

Studies investigating the presence of maternal fetal-reactive antibodies in ASD.

Maternal fetal-reactive antibodies			
Study investigators (year)	**Target of maternal antibodies**	**Tissue source**	**Reference**
Warren *et al*(1990)	~ 54% (6/11) of sera from MAC reacted to lymphocytes from children with ASD.	Peripheral blood from children	[94]
Dalton *et al* (2003)	Sera from a mother of two children with ASD reacted against murine cerebellar Purkinje cells and brainstem neurons.	Mouse postnatal day 1 and adult brain	[93]
Zimmerman *et al* (2007)	(i) Of 11 MAC, sera samples from 5 (45%) MAC reacted to a 30 kDa protein band. The remaining 6 (55%) MAC reacted to a > 250 kDa protein band compared with 0% (0/10) of MTDC. (ii) Reactivity to gestational day 18 fetal brain proteins, but not postnatal or adult rat brain proteins, was detected in sera from MAC, children with autism, and children with other developmental disorders.	Fetal, postnatal and adult rat brain extracts	[22]
Croen *et al* (2008)	(i) Reactivity to a 39 kDa protein in 7% (6/84) of samples from MAC compared with 2% (3/152) of MTDC was detected. (ii) Reactivity to a 73 kDa protein in 13% of samples from MAC and 7% of MTDC was detected. (iii) Reactivity to both proteins was detected in 37.5% (3/84) of samples from MAC with early onset ASD, but both bands were not present in samples from mothers with children with developmental regression ASD. (iv) Reactivity to a 37 kDa protein was detected in MAC compared with MTDC, but these results were not significant.	Human fetal brain extract	[23]
Braunschweig *et al* (2008)	37 kDa and 73 kDa protein bands were detected in 11.5% (7/61) of samples from MAC compared with 0% (0/40) MTDC in fetal brain extracts, but not adult brain extracts; these findings were associated with developmental regression ASD.	Human fetal and adult brain extract	[16]
Singer *et al* (2008)	(i) Reactivity at 36 and 39 kDa in human fetal brain extract. (ii) Greater band specificity in 100 MAC compared with 100 MTDC at 73 kDa to rat embryo, but not in adult rat, adult human or human fetal brain. (iii) Reactivity to a 100 kDa protein toward adult human brain region samples.	Adult and fetal rat and human brain extracts	[13]
Transfer of human maternal fetal-reactive antibodies to pregnant animals			
Animal model	**Results**		**Reference**
Mice	Decreased exploratory behavior and decreased ability to orientate was observed.		[93]
Rhesus macaques	Whole body stereotypies, pacing during mother-preference test and increased nonsocial activity were observed.		[95]

ASD autism spectrum disorders, **MAC** mothers of children with ASD, **MTDC** mothers of typically developing children