Immune dysfunction and neuroinflammation in autism spectrum disorder

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Autism spectrum disorder (ASD) is a complex heterogeneous neurodevelopmental disorder with a complex pathogenesis. Many studies over the last four decades have recognized altered immune responses among individuals diagnosed with ASD. The purpose of this critical and comprehensive review is to examine the hypothesis that immune dysfunction is frequently present in those with ASD. It was found that often individuals diagnosed with ASD have alterations in immune cells such as T cells, B cells, monocytes, natural killer cells, and dendritic cells. Also, many individuals diagnosed with ASD have alterations in immunoglobulins and increased autoantibodies. Finally, a significant portion of individuals diagnosed with ASD have elevated peripheral cytokines and chemokines and associated neuroinflammation. In conclusion, immune dysregulation and inflammation are important components in the diagnosis and treatment of ASD.

Key words: autism, cytokines, innate immunity, neuroinflammation

INTRODUCTION

Autism spectrum disorder (ASD) is considered a complex and heterogeneous neurologic disorder, showing features of core abnormalities in social relationship and communication, repetitive behavior and deficits in verbal and non-verbal interaction, with stereotyped behaviors and interests and even visual dysfunction (APA 2013, Wu et al. 2015, Bakroon and Lakshminarayanan 2016). The pathogenesis of ASD is complex and controversial, and both nutritional and immune causes have been recently associated with this complex pathology (Hsiao 2013, Endreffy et al. 2016, Fujiwara et al. 2016, Zerbo et al. 2016). Controversial issues arose from attempting to distinguishing between children's and adults ASD, and particularly Asperger syndrome with high functioning autism in adults, where language delays may be used to distinguish the two pathologies, as the poor performance on language tests also challenges the assumption that early language development in Asperger syndrome is essentially normal (Howlin 2003, Baron-Cohem and Wheelwright 2004). Immune system and neuroinflammation appear to play a fundamental role in ASD development, despite some concern about whether it causes ASD onset or regulates ASD pathogenesis and symptomatology. Immunity plays a pivotal role in the neurodevelopment of central and peripheral nervous systems, regulating neuronal proliferation, synapse formation, and plasticity, along with removing apoptotic neurons, but also actively participating in many neurological activities (De Jong et al. 2011, Wang et al. 2014, Jašarević et al. 2015, Young et al. 2016). Several studies over the last four decades have recognized altered immune responses in individuals diagnosed with ASD (Hsiao 2013, Noriega and Savelkoul 2014, Estes and McAllister 2015). Many people with ASD frequently display infections, environmental and food allergies, asthma, seizures, unexplained skin rashes, and persistent intestinal yeast infections (Sakamoto et al. 2015, Li and Zhou 2016). Also, signs of immune tolerance loss, e.g., allergies, over-reaction to vaccines/infections and undiagnosed autoimmune disorders appeared to be more commonly present in individuals diagnosed with ASD (Kern et al. 2014, Ruggeri et al. 2014, Wang et al. 2014). The involvement of the immune system in the neurobiological pattern of ASD currently is a topic of

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intensive research. The purpose of the present review is to explore the state-of-art research in this field and explore the hypothesis that ASD individuals frequently also have a significant immune dysfunction.

Alterations in immune cells in ASD

T cells

Potential immune cells involved in ASD, in different model species, are listed and detailed in Table I. Recent studies reported that children with ASD may have several different immune phenotypes and sub-phenotypes that correlate with increasingly severe behavioral impairments (Careaga et al. 2015). The causative relationship between ASD and immune function impairment has yet far to be fully elucidated. T cells play a significant role in adaptive immunity, particularly because of their distinctive feature dealing with immunological T-cell memory. Anomalies in T cell function were reported in ASD (Jyonouchi et al. 2005, Ashwood et al. 2011b, Bailey et al. 2012, Onore

et al. 2012, Mead and Ashwood 2015) but no causative issues were further outlined. Previous reports identified a reduced percentage of CD4+ T-helper cells, lower number of T cells (CD2+ cells) and lower percentages and number of total lymphocytes in ASD subjects compared to controls (Warren et al. 1990, Yonk et al. 1990, Ashwood et al. 2011b, Lopez-Cacho et al. 2016). Furthermore, the CD4+ T-cell subpopulations showed a decrease in CD4RA+ T cells, which are responsible for inducing suppressor T cells (Yonk et al. 1990, Ahmad et al. 2016). In a recent twin-model with ASD, CD4+ depression was assessed by a dysregulation in the production of interferon-gamma (IFN-γ) and interleukin-17 (IL-17), associated with other neurological comorbidities (Magid-Bernstein et al. 2015). A decrease in CD4+ T-cell appears to be a common finding in ASD. Several studies in the past reported abnormalities in T-lymphocytes in about 35% of ASD patients with decreased numbers of CD4+ T-cells and T-helper cells (CD4+CD8), altered the CD4/CD8 ratios, and an increased number of suppressor T-cells (CD4+CD8+) (Gupta et al. 1996, 1998). In support of this, a double-blind study reported a significant reduction of autistic symptoms in 56% of

Table I. Overview of the studies to date detailing a relationship between ASD and immune function

IMMUNE CELLS TYPE/SUBTYPE		EXPERIMENTAL SETTING		EVIDENCE	REFERENCES
T-cell	Th17	Mouse model	↑	Targeting Th17 allows pregnant mothers to be protected from viral infections causing ASD in offspring	Choi et al. 2016
		Cell from subjects	↑	Production of IL-17 in cells from children with ASD	Akintunde et al. 2015
	CD4+	Blood-ASD	↓	Depression of peripheral CD4+ Dysregulation Th1/Th2/Th17	Yonk et al. 1990, Ahmad et al. 2016
	CD8+	Blood-ASD	↓	Altered peripheral levels of CD20+ B-cells, T(CD8+), CD4+CDw29+, CD2+, CD4+CD45RA+ T cells	Yonk et al. 1990
	Colonic C8 cells and gut γδT-cells	Gut specimen	↑	Increase in cell infiltration-Altered gut immunity	Furlano et al. 2001
	CD4+CD25high regulatory T cells	Children with ASD	↓	Decreased blood levels	Mostafa et al. 2010
B-cell	Response to estrogens and DHT	ASD subjects	↓ ↑	Decreased response to hormones, with less growth depression and less mitochondrial upregulation Mitochondrial defects	Sharpe et al. 2013a, 2013b
Granulocytes	Neutrophils	Newborns	↑	Chemokine MCP-1	Zerbo et al. 2014
			↓	Chemokines RANTES, MIP-1α	
Natural killer cells (NK)	CD57+CD3-	Children with ASD	↓	Decreased levels in peripheral blood samples	Siniscalco et al. 2016
	NK, NKT	59 adult patients with ASD	↑	CD25 expression	López-Cacho et al. 2016
Monocytes	Monocytes, neopterin	31 ASD children	↑	Increased blood levels	Sweeten et al. 2003
Innate cells	Innate immunity	ASD children	↓ ↑	Dysregulated innate immunity	Jyonouchi et al. 2005
	Cytokine levels	ASD subjects	↓ ↑	Dysregulated cytokines (TNF-α, IL-8, IL-9, IL-13, IL-15)	Pecorelli et al. 2016

ASD children, following treatment with the potent opiate antagonist naltrexone. The drug increased the number of T-helper inducers and reduced the number of T-cytotoxic suppressors, resulting in a normalization of the CD4/CD8 ratio (Scifo et al. 1996). Those studies were, however, not further continued in the following years, probably because of new markers and interests in the immunological spectrum of ASD. Recent attempts have also tried to develop an animal model of autism-risk stressing on the immune disorder pattern (Hsiao et al. 2012). The offspring of mothers in mouse models, which underwent a maternal immune activation (MIA), showed altered immune profiles. The immunological changes included a deficit in CD4+, TCRβ(+) Foxp3(+) CD25(+) T regulatory cells, increased CD4-mediated production of IL6 and IL-17 and increased levels of peripheral Gr-(1)+ cells with abnormalities in the myeloid hematopoietic lineage. When irradiated mice were re-populated with immune cells from MIA mice, they were not able to restore the immune phenotype, providing evidence supportive of the existence of an individual's peripheral context affecting the long-time reprogramming of immune function. However, behaviorally abnormal MIA-derived offspring, which exhibited stereotyped and repetitive attitudes and anxiety-like behaviors and were previously irradiated, when transplanted with control or MIA-derived immune populations, restored a normal behavioral and neurological pattern, showing a fundamental relationship between ASD and immune dysregulation (Hsiao et al. 2012).

From an immunological point of view, CD4+ lymphocyte helper-cell activities are fundamentally divided into T-helper 1 (Th1) (cell-mediated immunity), and Th2 (humoral immunity) subsets. Th1 is the first-line of defense system primarily against viral, fungi, and protozoa, while Th2 helps the B-cells to produce antibodies, although this paradigm has been recently updated (Moore et al. 2001, Rossignol 2007, Hirahara et al. 2016). Current immunology has deeply revised these concepts, by introducing further CD4+ populations, most of which with regulatory functions. In this perspective, the role of T-cell response in ASD has come to the spotlight because of impairments observed in either Th1, Th2 or Th17 phenotype content, immune dysregulation in their relationship with innate and acquired immunity and the B-cell repertoire and expression of major cytokines, e.g. IL-1β, TNF-α, IL-2, IL-4, IL-13, IL-17 and IL-10 (Moore et al. 2001, Molloy et al. 2006, Rossignol 2007, Li et al. 2009, Estes and McAllister 2016). Furthermore, a number of abnormal immune adaptive responses have been reported in ASD patients. Previous studies revealed activation of the Th1 system with increased production of IL-12 and interferon when compared to controls (Stubbs 1995, Singh 1996), and was recently confirmed (Ross et al. 2013). Alterations in the level of pro-inflammatory cytokines such as IL-12,

IL-1, IL-6, TNF-α, IL-23 and the brain-derived neurotrophic factor (BDNF) in ASD was recently reported (Ricci et al. 2013). ASD children express increased IFNγ and IL-1 receptor antagonist (IL-1ra), resulting in a Th1 skewing (Croonenberghs et al. 2002a, Goines et al. 2011). Also, in ASD patients, compared to controls, an increase in the production of markers of cell-mediated immunity were observed, together with augmented T-cell production of TNFα and decreased IL-10 production was recently reported (Rossignol 2007, Ashwood et al. 2011b). Many studies indicated that ASD children had an evident shift in the CD4+-cell population from Th1 cells toward Th2 cells (Gupta et al. 1996, 1998, Ashwood et al. 2011b). This shift might enhance susceptibility to chronic viral infections in ASD (Croonenberghs et al. 2002b). Past reports showed that ASD children have an increased production of immunoglobulin E (IgE) and IL-4 producing CD4+ T-cells, and lower levels of IL-2 producing CD4+ T-cells compared to controls (Gupta et al. 1996, 1998), and this was recently confirmed (Rossignol 2007, Theoharides 2013).

Gut epithelial dysfunction and gut microbiome impairment were also reported in ASD, due to disorder in the T-cell mediated immune response (Furlano et al. 2001, Luna et al. 2016). Moreover, past reports showed that about 5% of ASD patients have IgA deficiency, and 30–40% have low serum IgA levels (Gupta et al. 1996, Wasilewska et al. 2012). ASD patients often display an enhanced sensitivity to gluten, with increased anti-gliadin antibodies compared to healthy individuals (Lau et al. 2013). This suggests a disorder in the mucosal immunity in ASD (Ashwood et al. 2004). This was even confirmed for anti-transglutaminase autoantibodies (Rosenspire et al. 2011). In ASD, particularly in children with regressive ASD, a shift in the immunoglobulin composition in serum, with low-normal IgA and CD23-expressing B-cells, was observed (Wasilewska et al. 2012).

Mucosal immunity should also affect regulatory T cells (Kinoshita and Takeda 2014). The activity of Th17 cells in mucosal immunity has also been associated with the role exerted by vitamin D in this context and in mucosal immune tolerance (Chirumbolo 2015). This would suggest a role for vitamin D in ASD (Mostafa and Al-Ayadhi 2012, Cannell and Grant 2013, Saad et al. 2015). At least, as observed in mouse models, an association of impaired Th17 function and ASD was reported (Choi et al. 2016). Children with ASD express an increased amount of IL-17 and IL-17a, with co-morbid asthma (Al-Ayadhi and Mostafa 2012, Akintunde et al. 2015). Therefore the role of IL-17 in ASD appears to be of significant interest. Finally, an autistic "endophenotype" able to produce antibodies reactive to brain proteins (Connolly et al. 2006, Cabanlit et al. 2007), seems to have a typical immunological pattern, characterized by 1) the existence and increase of immune cells producing proinflammatory cytokines and IL-10;

2) an increase in CD8+ naïve (CD45RA+/CCR7+) T lymphocytes and CD8+ effector memory (CD45RA-/CCR7-) cells and also 3) a decrease in CD4+ terminally differentiated (CD45RA-/CCR7+) (Saresella et al. 2009). These data also support significant alterations in the adaptive immune responses in children diagnosed with ASD.

B cells

Children diagnosed with ASD in comparison to controls were reported to either have an increased absolute number of B cells per volume of blood of about 20 to 25% (Ashwood et al. 2011a), or had no change (Heuer et al. 2012). Although when comparing the two studies, patients differed in median age and the experimental approaches were quite different. A recent survey reported that variability in single nucleotide polymorphism (SNPs) in the CD157 or bone marrow stromal cell antigen-1 (BST-1), particularly the CT genotype in rs10001565, is frequently associated with subjects suffering from ASD as compared to controls, and, thus demonstrates the possibility of an impaired B-cell population in ASD (Yokoyama et al. 2015). It is intriguing to note that both CD38 and CD157 modulate the innate and adaptive immune response in T and B cells (Malavasi et al. 2006). Probably, in subjects with ASD, a mitochondrial dysfunction in B-cells may explain why B lymphocytes in ASD exhibit a differential immune response to estrogens, dihydrotestosterone, and hormone disrupters, which were associated with ASD onset (Sharpe et al. 2013a). B-cell sensitivity to external stimuli has also been reported for thimerosal (Sharpe et al. 2013b). Individuals with ASD display a reduced number of B-cells and an increased amount of NK cells (Bressler et al. 2012). This evidence may suggest that the reduction in immunoglobulin levels observed in ASD may be a consequence of B-cell depletion or impairment, but recent findings seem to contradict this opinion (Hauer et al. 2012).

Peripheral monocluate cells

Monocytes differentiate into macrophages upon migration into the surrounding tissues. Macrophages, in turn, phagocytize pathogens and present antigens to lymphocytes (Berdowska and Zwirska-Korczala 2001, Sweeten et al. 2003). When stimulated by IFNγ, monocytes and macrophages produce increased amounts of neopterin. High levels of neopterin could serve as an indicator of monocyte/macrophage activation, as well as activation of T cells and cell-mediated immunity (Berdowska and Zwirska-Korczala 2001, Zhao et al. 2015). Previous studies in ASD revealed a somewhat higher number of absolute monocyte counts and a significantly higher percentage of monocytes, in relation to total leukocytes in ASD as compared to healthy controls (Denny et al. 1996, Sweeten et al. 2003). Furthermore, augmented expression of activation markers on these monocytes suggested that these cells were in an activated state (Ashwood et al. 2011a). A fundamental question that should arise from ASD research is the impact of neuroimmune alterations in ASD (Gottfried et al. 2015, Siniscalco 2015). Immunological impairment in innate immunity was observed in ASD. Peripheral blood monocytes were found to increase the release inflammatory cytokines such as IL-1β, TNF-α, and IL-6 in response to TLR2 and TLR4 stimulus and, particularly, IL-6 and IL-10 (Jyonouchi et al. 2005, Manzardo et al. 2012, Jyonouchi et al. 2014). Increased production of IL-6 and IL-1β was associated with increased impairment of social behaviors in individuals diagnosed with ASD was observed (Enstrom et al. 2009). Innate immunity, therefore, plays a fundamental role in ASD development. Innate immunity-related cytokines and chemokines are altered in ASD compared with controls, and suggest these molecules may be used as ASD risk immune markers (Zerbo et al. 2014, Masi et al. 2015, Xu et al. 2015). Alternative mRNA splicing disorder in ASD may explain some impairment in monocyte/macrophage and natural killer cells (Stamova et al. 2013).

All these observations suggest that impairment in innate immunity may have a role in ASD, but are not necessarily causative. The immunological profile of ASD subjects regarding innate immunity may be somewhat more controversial. Despite a pro-inflammatory hallmark in ASD, recent reports showed that ASD individuals in comparison to controls have a cytokine pattern of peripheral monocyte/macrophages producing reduced pro-inflammatory cytokines following activation of TLR7/8 (IL-6, IL-23), TLR2/6 (IL-6), TLR4 (IL-12p40), without stimuli (IL-1ß, IL-6, and TNF-α) after challenging with T cell mitogens (IFN-γ, IL-17, and IL-12p40), and with antigens from the genus *Candida* (IL-10 or IL-12p40) (Jyonouchi et al. 2012). Recent evidence showed that the endocannabinoid receptor, which is involved in a system able to modulate the immune response via the cannabinoid receptor type 2(CB2R), and while is highly expressed in macrophages and microglia, its function is impaired in ASD patients (Siniscalco et al. 2014). Many soluble factors, besides leukocyte receptors, are fundamental markers in ASD. For example, brain IL-6 is considered a major neuroimmune mediator in ASD onset and development (Wei et al. 2013). Macrophage activity is also altered in ASD. As another example, several ligands of CCR4, such as macrophage-derived chemokines (MDC) or CCL22 and thymus and activated/regulated chemokine (TARC) or CCL17, were reported to be elevated in ASD children as compared to controls (Al Ayadhi and Mostafa 2013). It was recently reported for macrophage migration inhibitory factor (MIF) that

there was a genetic association between a functional polymorphism in the genetic promoter for MIF and ASD. As a consequence, it was suggested that MIF may play an important functional role in ASD pathogenesis (Grigorenko et al. 2008).

Natural killer (NK) cells

Ashwood and others (2011a) reported that the absolute numbers of NK cells were approximately 40% higher in children diagnosed with ASD compared to controls. These data are in agreement with Enstrom and colleagues (2010) who reported greater numbers of NK cells and increased gene expression of NK cell-related cellular receptors and effector molecules in children diagnosed with ASD in comparison to controls (Enstrom et al. 2010). However, Vojdani and others (2008) have reported 45% of the ASD children had a decreased responsiveness of NK cells to *in-vitro* stimulation (Vojdani et al. 2008). The increase in NK cell numbers may, therefore, reveal a compensatory mechanism to increase cell numbers to make up for NK cell function deficiencies (Ashwood et al. 2011a). The number of CD57+CD3-lymphocytes appeared to fall below the clinically accepted range in ASD children (Siniscalco et al. 2016). Moreover, children with ASD have activating KIR/HLA complexes (aKIR/HLA), where KIR is the killer cell immune globulin-like receptor, expressed on NK cells, i.e., in utero these immune interactions may promote immune activation in ASD (Guerini et al. 2014). Abnormalities in NK cells in ASD may predispose individuals to the development of autoimmunity and/ or adverse neuroimmune interactions during critical periods of development (Enstrom et al. 2010). The presence of autoantibodies towards central nervous system (CNS) proteins is a common finding in ASD and may reflect an ongoing inflammatory and or autoimmune process in individuals with ASD that could be started by abnormal NK cell activation (Ashwood et al. 2006). In this circumstance, the expansion of NK cell numbers may result from intensified immune/autoimmune responses probably mediated through the increased production of homeostatic and growth factors such as cytokines (Ashwood et al. 2011a).

Dendritic cells

Dendritic cells actively participate in innate immunity and also modulate the adaptive immune response and immune tolerance. They are phagocytic cells, expressing several innate pattern-recognition receptors (PPRs), which are targeted by pathogen-associated molecular pattern molecules (PAMPs) on microbes or damage-associated molecular pattern molecules of endogenous tissues (Steinman 2007, Breece et al. 2013).

After binding these ligands/antigens, dendritic cells undertake maturation steps that increase mobility for migration, produce chemokines to convert other immune cells, and co-stimulatory molecules for priming of naïve T cells or stimulation of effector T cells and secrete large quantities of cytokines that control neighboring immune cells (Banchereau et al. 2000, Ueno et al. 2007, Breece et al. 2013). Myeloid dendritic cells frequency is augmented in ASD (Breece et al. 2013). Both myeloid dendritic cells, with phenotype defined as Lin-1⁻BDCA1⁺CD11c⁺ and Lin-1⁻BDCA3⁺CD123⁻) and plasmacytoid dendritic cells, namely (Lin-1⁻BDCA2⁺CD123⁺ or Lin-1⁻BDCA4⁺CD11c⁻) are impaired in ASD, where plasmacytoid phenotypes seem to be associated with developmental regression in ASD and an increase in the amygdala volume (Breece et al. 2013). Moreover, dendritic cells play a significant role in inducing both central and peripheral tolerance. Dendritic cells in the periphery continuously capture and present low dose non-immunogenic antigens to T cells with limited or absent co-stimulation to maintain tolerance either by deletion, the induction of unresponsiveness or generation of adaptive T regulatory cells (Steinman 2007). Breece and others (2013) found that there are increased circulating frequencies of blood myeloid dendritic cells in young children diagnosed with ASD. These increased frequencies were associated with an increased volume of the amygdalas and increased repetitive behaviors in patients with ASD (Breece et al. 2013).

Alterations in immunoglobulins in ASD

Previous studies have reported changes in the levels of immunoglobulins among individuals diagnosed with ASD. However, these reports are controversial, and no clear consensus has been reached. IgG and IgM are either increased (Croonenberghs et al. 2002b) or decreased (Gupta et al. 1996, Heuer et al. 2008) in ASD individuals compared to healthy controls. The supposed presence in the serum of anti-neuronal antibodies including anti-glutamic acid decarboxylase antibodies (anti-GAD), has been recently dismissed (Bayram et al. 2016). Plasma markers in ASD were assessed and revealed serum IgG antibodies to casein, egg whites, egg yolks, and peanuts (Esparham et al. 2015). Many ASD subjects showed polymorphism in the glutathione scavenging system, namely the absence of glutathione S-transferase (GSTM) at the 1p13.3 location, and heterozygosity for the glutathione S-transferase I105V (GSTP1), while most of ASD patients exhibited genetic polymorphism of the mitochondrial gene superoxide dismutase A16V (SOD2) (Esparham et al. 2015). Therefore, nutritional, environmental and immunological factors should play a significant role in ASD pathogenesis. Heuer and others (2008) reported low levels of immunoglobulin

(IgG and IgM) in children diagnosed with ASD, which also correlated with increased behavioral severity of autistic symptoms on the *Aberrant Behavior Checklist* (ABC) (Heuer et al. 2008). However, in comparing the previous studies, the differences are attributed to different sample sizes, the median age of patients with ASD, variations in types of control samples, and different experimental approaches (Heuer et al. 2008). Even alterations in maternal immune globulins and the presence of autoantibodies may cause ASD in the offspring (Poleataev et al. 2014, Brown et al. 2015). The presence of autoantibodies is also reported in ASD children (Elamin and Al-Ayadhi 2014, Mostafa et al. 2014). Further research is needed to enhance our comprehension of dysregulated antibodies in ASD.

Peripheral cytokines and chemokines in ASD

Both cytokines and chemokines are small proteins secreted by immune or other somatic cells with hormone-like, informative activity. They have the ability to induce modifications in neighboring responsive cells. Some cytokines are considered pro-inflammatory and can be induced during an immune response to recruit cells of the immune system to the site of infection (Wang et al. 2014). Individuals diagnosed with ASD have been observed to have altered cytokine profiles compared to healthy controls, and to be in a chronic state of cytokine induction (Ashwood et al. 2011c, 2011d, Wang et al. 2014). Patients diagnosed with ASD in comparison to controls have a diminished Th2 anti-inflammatory response and an increased Th1 pro-inflammatory cytokine response. This includes an enhanced innate and adaptive immune reaction through the Th1 pathway and suggests that localized brain inflammation and autoimmune dysfunction may be involved in ASD (Ashwood et al. 2011c, 2011d, Wang et al. 2014). More specifically, Ashwood and others (2011c, 2011d) reported elevated cytokines including IL-1β, IL-6, IL-8 and IL-12p40, MCP-1, RANTES and eotaxin in children diagnosed with ASD in comparison to controls (Ashwood et al. 2011c, 2011d). It was recently confirmed that persons with ASD in comparison to controls have cytokine functional impairment (Goines and Ashwood 2013, Krakowiak et al. 2015, Masi et al. 2015, Pecorelli et al. 2016). In previous studies, elevated inflammatory mediators were associated with more abnormal behaviors in individuals with ASD (Ashwood et al. 2011c, 2011d). It was also reported in other studies that increased levels of inflammatory cytokines in plasma of children diagnosed with ASD in comparison to controls were observed for IFNγ (Singh 1996), MIF (Grigorenko et al. 2008), platelet-derived growth factor-BB (PDGF-BB) (Kajizuka et al. 2010), and dysregulation of IL-18 (Businaro et al.

2016). The immune dysregulation of cells should also affect the function and production of cytokines.

Autoantibodies

Autoantibodies were reported to numerous and varied targets in ASD. They may point to cellular damage involved in increasing inflammation, revealing antigens otherwise hidden and/or epitope spreading (Onore et al. 2012). Elevated serum autoantibodies to many neuron-specific antigens and cross-reactive peptides have been found in ASD children including antibodies directed against cerebellar Purkinje cells and other neural proteins (Onore et al. 2012, Wang et al. 2014), but some of the findings have been inconsistent between studies. Multiple studies reported an elevated number of autoantibodies that react against the brain and central nervous system in children diagnosed with ASD when compared to healthy controls (Cabanlit et al. 2007, Wills et al. 2007). It was not only brain-specific IgGs autoantibodies occurred at a high frequency among children diagnosed with ASD in comparison to controls (Cabanlit et al. 2007, Elamin and Al-Ayadhi 2014), but also anti-neuron antibodies that were associated with more severe cognitive and behavioral profiles in children diagnosed with ASD (Piras et al. 2014). Interestingly, brain autoantibodies are also associated with lower serum docosahexaenoic acids (DHA) levels in ASD in comparison to controls (Mostafa et al. 2015) and higher blood mercury (BHg) levels (Mostafa and Refai 2007, Mostafa and Al-Ayadhi 2015, Mostafa et al. 2016a). High Hg levels in children with ASD may relate to increased exposure to Hg, but can also be a consequence of a decreased ability to excrete Hg, leading to a higher body-burden (Khaled et al. 2016). Neuroinflammation, with increased levels of neurokinin A, is seen in some children with ASD and is evidenced by elevated BHg levels. In fact, a recent study found a positive relationship between the Childhood Autism Rating Scale (CARS) scores and the levels of serum neurokinin A and BHg (Mostafa et al. 2016a). Furthermore, Pb induces neuroinflammation and autoimmunity in ASD (Mostafa et al. 2016b). A recent study suggests that increased levels of BPb in some children with ASD may trigger the production of serum anti-ribosomal P antibodies (Mostafa et al. 2016b). It has been reported that ASD children often have a lower zinc (Zn)-to-copper (Cu) ratio in blood compared to healthy controls (Faber et al. 2009, Li et al. 2014, Crăciun et al. 2016) and children with other neurological disorders (Macedoni-Lukšič et al. 2015). It has been suggested that metallothionein (MT) dysfunction may occur as a cause of Hg accumulation in children with ASD, and the same dysfunction may also lead to Zn deficiency (Bjørklund 2013). Zinc plays a major role in the immune system, and Zn deficient subjects may experience increased susceptibility to a variety of

pathogens. Both trace elements (or heavy metals) and autoantibodies may be causes of neuroinflammation.

Many researchers suggested the presence of maternal autoantibodies that can have a detrimental effect on fetal brain development during pregnancy. Maternal pathogenic autoantibodies can reach the fetus, affecting the fetal brain tissue (Zimmerman et al. 2007, Croen et al. 2008). Therefore, it is possible that brain-specific maternal autoantibodies might have an impact in some neurological developmental disorders. Also, children diagnosed with ASD in comparison to controls more frequently have a family history of autoimmune disorders, such as type 1 diabetes and ulcerative colitis (Zimmerman et al. 2007, Croen et al. 2008, Wang et al. 2014).

Neuroinflammation and neurobiology in ASD

What are the implications of the immune dysregulated function observed in ASD? Is immune dysregulated function a primary cause or rather an ancillary source of the etiopathogenesis of ASD?

ASD is one of an increasing number of neurological disorders in both children and adults that are found to involve a neuroinflammatory process, which can occur in the absence of overt leukocyte infiltration. One of the main indicators of neuroinflammation in ASD is microglial activation. Microglia are the primary immune cells of the CNS and are very similar to peripheral macrophages. Furthermore, it is reported that microglia defend the brain by clearing cellular debris and dead neurons from the nervous tissue through phagocytosis. However, when microglial activation is exaggerated and sustained, this can lead to collateral damage or damage of healthy tissue (Rodriguez and Kern 2011). For example, excessive and sustained microglial activation can result in the loss of healthy synaptic connections. Exaggerated and sustained microglial activation is consistently found in ASD (Vargas et al. 2005). Inflammation in postmortem brain specimens of individuals diagnosed with ASD has been observed, specifically, in the cerebellum, anterior cingular gyrus and the mid-frontal regions of the brain (Vargas et al. 2005). Neuroglial activation and presence of increased levels of inflammatory cytokines such as IFN-γ, IL-1β, IL-6, TNF-α and chemokines CCL-2 were found in the brain tissue of individuals diagnosed with ASD. Also, accumulation of macrophages chemoattractant protein-1 (MCP-1) and inflammatory cells (microglia and astroglia) around the blood vessels were reported, and suggest an inflammation of the blood vessels. Also, an increased pro-inflammatory cytokine profile in the cerebral spinal fluid (CSF) was reported in living ASD children (Vargas et al. 2005, Morgan et al. 2010). The cerebellum, in particular, showed the most prominent histological changes and microglial

activation in ASD. This emphasizes the role of microglia in the neuroinflammation pattern of ASD. Moreover, some of the cerebellar tissues had an accumulation of perivascular macrophages and monocytes and deposition of complement membrane attack complexes (Vargas et al. 2005). Activation of microglial cells and perivascular macrophages was measured by increased MHC II expression and were observed in the cortical regions, white matter, and cerebellum of patients diagnosed with ASD in comparison to controls. This microglial and astroglial activation in the cerebellum was associated with degenerating Purkinje cells, granule cells, and axons (Vargas et al. 2005). Altered microglial profiles found in post-mortem brain samples from ASD patients in comparison to controls showed an increase in average microglial somal volume in white matter and an increase in microglial density in gray matter (Morgan et al. 2010). Also, postmortem temporal cortex samples from ASD and general population controls were assessed for transcriptome differences and it was observed that samples from ASD individuals showed increases in expression of immune-related genes (Garbett et al. 2008). It was also previously revealed that individuals diagnosed with ASD in comparison to controls have an inflammatory response presenting as an increased lymphocytes and proinflammatory cytokines, including TNF-α and IFN-γ and less of the anti-inflammatory cytokine IL-10 (Torrente et al. 2002, Ashwood et al. 2004, Nakagawa and Chiba 2016). As a result, among individuals diagnosed with ASD, inflammation can become chronic.

Recent findings suggested that imbalances in the neuro-immune function in ASD are signaling an ongoing neuroinflammation process. Some researchers have suggested a possible allergic response from the brain as a cause of ASD (Theoharides 2013, Theoharides et al. 2016), where mast cell activation is involved (Theoharides et al. 2012) and possibly neurotensin and other stress-related hormones, such as corticotrophin releasing hormone (CRH), play a significant role (Tsilioni et al. 2014). Besides the neuroinflammatory perspective, ASD has been associated with deep functional disorders in neuronal function. For example, altered neural connectivity in inhibitory and excitatory cortical circuits based fundamentally on functional non-invasive studies showing atypical synchronization and connectivity patterns between cortical areas was revealed in children and adults with ASD in comparison to controls (Zikopoulos and Barbas 2013). Furthermore, investigators have suggested that changes in prefrontal axons and their network disruption may cause ASD (Zikopoulos and Barbas 2010). It is rather difficult, at present, to know how immunophenotype differences, altered immune function, and immune dysregulation may interact or contribute to other potential pathomechanisms of ASD such as atypical interneuron connectivity or mirror neuron defects, which could be related to autoimmune

processes or immune imbalances in the brain. It is tempting to speculate that astrocyte function may be the bottleneck of a possible suggestive answer to this question (Bianco-Suarez et al. 2016). The neurobiological pattern of ASD has recently introduced the role of mirror neurons (Schulte-Rüther et al. 2016, Linkovski et al. 2016, Saffin and Tohid 2016). Mirror neurons exert direct action that impact the occipital cortex, cingulate cortex, insula and dorsal prefrontal cortex in ASD (Yang and Hoffmann 2015). Future research should elucidate if these disorders may come either from neuroinflammation events or dysregulation in the astrocyte-synapse interaction.

CONCLUSIONS

ASD is a complex heterogeneous neurodevelopmental disorder. Immune dysregulation and inflammation are important components of ASD and are significant to diagnosis and treatment of the disorder. Many animal models can provide insights into the role of neuroinflammation in ASD onset and pathogenesis (Zatkova et al. 2016, Codagnone et al. 2015) and certainly the use of a mouse model system such as the mGluR5 knockout support the role of neuroinflammation in cognitive disorders related to ASD (Zantomio et al. 2015). The dysfunctional immunophenotype seen in subjects with ASD may play a pivotal role in ASD pathogenesis and, thus, may be a therapeutic target of future interest. It is possible that understanding the role of mirror neurons in the dorsal prefrontal cortex may be important to neurodevelopment, but it is also possible that there is an important role for astrocytes in neurodevelopment. It was recently revealed that dysregulation of some micro-RNAs, for example, miR-146a, miR-221, and miR-656, in skin fibroblasts are associated with the onset of ASD (Nguyen et al. 2016). This evidence might indirectly support the role of reconstitution of damaged tissue in the pathogenesis of ADS, as occurs in epilepsy (Kim et al. 2016). Glia could also exert a significant role in ASD, an immune injury or a chemical insults. Future research should improve our knowledge about the role, if causative or consequential, of the immune impairment observed in ASD, which could potentially allow for targeted therapeutics. However, to date, it has not been elucidated whether immune disorders are causative of ASD or if ASD causes immune dysfunction. Genetic polymorphism exists, which, could drive immune tolerance or exacerbate immune responses towards the development of ASD. The aberrations of cytokine immune response, neuroinflammation in the CSF and brain tissue, and peripheral immune abnormalities in both the innate and adaptive responses among persons with ASD suggest that they could be causative of the disorder or a comorbidity of the disorder. The use of

the described immune abnormalities in this review as biomarkers should further help in the early identification and treatment of ASD. Biomarkers or miRNAs will also be important to further understand the potentials links between immune dysregulation and resulting changes in neural connectivity and behavior. More research is needed to explain the mechanisms of immune dysfunction and neuroinflammation in ASD, and how these aberrations affect the neurological and behavioral changes in ASD.

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