Individuals with ASD demonstrate an aberrant immune response in central nervous system (CNS), peripheral blood, and the gastrointestinal tract. Overactivation within microglia and astrocytes was proved in post-mortem brain biopsies and biomarker studies. Increased autoimmunity mainly due to maternal anti-fetal brain antibodies crossing the placenta during pregnancy was found. Inflammatory cytokines concentration was found to be altered in serum, placenta and cerebral spinal fluid in children with autism and their relatives. Moreover, skewed production of immunoglobulins or B- and T-cell dysfunction confirmed that humoral and cellular response is altered in individuals with ASD. Additionally, the presence of gastritis, lymphoid nodular hyperplasia, colonic lymphoid nodular hyperplasia, eosinophilic infiltration and others confirmed that these inflammatory alterations affect the digestive system as well.

The immune and CNS communicate extensively. While lymphoid organs are hardwired by autonomic nervous system and neuroendocrine hormones serve as a regulator of cytokines balance, the immune homeostasis is critical for proper neurodevelopment and thereby behavior. These brain-to-immune interactions may be responsible developing immune-mediated diseases. Cytokine proteins impact directly on neurons activity. They are involved in neurodevelopment, as well in prenatal as in postnatal period. There is also a body of evidence linking cytokines to higher order neurological functions, including cognition and memory. Therefore, disruption within cytokine homeostasis may be responsible for a variety of neurological consequences relevant to ASD.
Interleukin 1β (IL-1B) is present in the nervous system during neurogenesis, migration, differentiation, synapse formation, plasticity, and responses to injury. It was proved that permanent expression of IL-1B in hippocampus impairs spatial memory. The protein was found to be involved in neural progenitor cell proliferation in particular CNS regions which contribute to region-specific growth in autistic brains. IL-1B is responsible for the formation of excitatory synapses. Moreover, this protein has been associated with altering sleep patterns and together with other cytokines responsible for so called sickness behaviour characterized by lethargy, depression, anxiety, and difficulties in ability to focus and express social skills.

Interleukin 6 (IL-6) is involved in neuronal precursors self-renewing, neuronal migrating and promoting cell survival. The cytokine was found to be responsible for regulating neurite outgrowth, as well. The protein and its receptors are expressed in brain of healthy and disease states, however a low expression is needed for proper neurodevelopment. It was proved that permanent IL-6 overexpression results in reducing expression of glutamate receptors and L-type calcium channels. IL-6 also promotes the predominance of excitatory to inhibitory synapses and the development of proper recognition memory.

Interleukin 4 (IL-4) being expressed in the brain is responsible for promoting oligodendrogenesis of neuronal progenitor cells. The cytokine was also found to be involved in regulating progenitor cell proliferation and differentiation and synapse formation, particularly of GABAergic type. IL-4 also plays a neuroprotective role and promotes the development of higher order cognitive processes and the absence of the protein is associated with loss of T-cell function in CNS.

Interferon gamma (IFN-γ) was found to be responsible for neuronal differentiation of neural progenitor cells. The protein serves as a regulator of dendritic morphology and synapses formation and activity. It was proved that overexpressing IFN-γ leads to elevated concentration of major histocompatibility complex I (MHCI) proteins in the brain and these proteins have been lately found to be expressed in CNS to regulate synaptic scaling.

Tumor necrosis factor-α (TNF-α) may induce cell death on neurons and prune synapses. Transforming growth factor β (TGF-B1) acts as a regulator of neuronal migration, neural cells survival and synapse formation. The lack of the expression of the protein results in altered CNS development as extracellular matrix is disintegrated and deficits in glutamatergic and GABAergic synapses function occur leading to seizures, motor incoordination and severe behavioral abnormalities.

In the light of these facts, the aim of this report was to review the literature on the links between the immune-related factors and developing ASD.

Materials and Methods

Available articles from PubMed and Google Scholar were analyzed using time descriptors: 1996-2015 and key words: autism spectrum disorder, cytokines, immune system, immunity.

In order to present the analyzed issues in the clearest and most comprehensive way, the article is divided into the following subsections:

- Altered immune function in ASD patients in prenatal period
- Altered immune function in postnatal period
  - Neuroinflammation
  - Cytokine and chemokine profile
  - Immunoglobulin levels
  - Cellular response
  - Gastrointestinal malfunctions
  - Sensitivity toward environmental toxicants

Results

Altered Immune Function in Asd Patients in Prenatal Period

Growing evidence indicates that maternal autoantibodies influence harmfully to fetus brain development. These are IgG class immunoglobulins providing passive immunity to the fetus as they are able to transplacental transmission, nevertheless pathogenic autoantibodies are devoid of those advantageous properties. In animal studies it was shown that they alter socially the behavior of the offspring. Moreover, it was proved that specific neural antibody are induced during gestation and the offspring demonstrates histological abnormalities in the brain and cognitive declines in later life.

The direct properties of these molecules, either in terms of pathogenicity or targets of antibodies, have not been fully identified. Nevertheless in 2008, Braunschweig et al characterized those molecules and showed significantly higher autoreactivity toward a protein at 37 and 73kDa in mothers of ASD children. The researchers
have now identified 7 specific target antigens: lactate dehydrogenase A and B (LDH), cypin, stress-induced phosphoprotein 1 (STIP1), collapsin response mediator phosphoproteins 1 and 2 (CRMP1, CRMP2), and Y-box-binding protein and have coined the term “maternal autoantibody-related,” or MAR, autism for these cases. Exclusive reactivity to specific antigen combinations was noted in 23% of mothers of children with ASD and in only 1% of mothers of normally developing children. The putative antigens are thought to be present on GABAergic interneurons in the brain.

While vitamin D acts as a neuroactive molecule it participates in neuron differentiation and synapses action. Link between vitamin D deficiency in pregnant women and their child developing ASD exists. Autistic characteristics seem to disappear after administering the vitamin, and conception during low UVB penetration favors ASD children deliveries. In their systematic review concluded that vitamin D deficiency is associated with ASD especially in dark skinned children and the effect of the lack of vitamin D in pregnant mothers is transmitted to the offspring. Few animal model experiments proved the impact of proper vitamin D level during gestation on brain and ventricles sizes, often enlarged in autistic subjects. In general, there is still no conclusive evidence for an association between the mother having vitamin D deficiency and the offspring developing ASD as few studies lacking such evidence exist.

Exposure to various infections (viral and bacterial) in prenatal life increases the risk for developing ASD. Particularly rubella, herpes simplex, cytomegalovirus, measles or viral meningitis in the first pregnancy trimester or bacterial infection during the second trimester are associated with ASD behaviour in their offspring. Maternal infection alters specific cytokine levels as well in maternal body as in placenta and fetal brain. It was proved that cord blood concentration of IL1-B facilitates neurological outcome in infants exposed to neonatal hypoxia. Other study showed no association between mild common infections or febrile episodes and ASD in offspring, but reported increased risk for ASD child delivery after maternal influenza infection or prolonged fever and antibiotics administration. In fact when influenza virus was intranasally infused shortly after fertilization in mice, the offspring’s social behaviour was altered. Intrapерitoneal administration of poly (I:C) mimicking viral infection, at different times of gestional days resulted in various behavioral phenotypes. High anxiety and decreased social interaction was observed when infection factor was administered at gestational day (GD) 12.5 while perseverant behaviour was noted in case of GD17 and additionally unobserved when inflammogen was infected at GD9. It proves that a critical period for the development of certain pro-social behaviors exists and that susceptibility to the development of ASD increases during GD17. Furthermore rodent experiments suggested that maternal immune activation can result in spatially restricted deficit in Purkinje cells.

Additionally, maternal history of any autoimmune disease shows increased risk for ASD. The association was confirmed in epidemiological studies in 40% of studied patients. The significant association was reported for autoimmune thyroiditis or hypothyroidism, rheumatic fever, rheumatoid arthritis, celiac disease, ulcerative colitis, psoriasis and Type 1 diabetes. Also, epigenetic regulation of transcription is believed to be associated with autoimmune diseases, therefore immunogenetical components of ASD may play a crucial role in developing ASD. Variations in MET proto-oncogene tyrosine kinase pathway or serine and threonine kinase C genes can be inherited by ASD children and should be considered as they are involved in innate and adaptive immunity. Other genes that should take attention are those related to NK cells, macrophage inhibitory factor, reelin or mitochondrial respiratory chain disease.

**Altered Immune Function in ASD Patients in Postnatal Period**

**Neuroinflammation**

Ongoing neuroinflammation in post-mortem brain bioplates and in biomarker studies is a proof of dysregulated immunity in ASD. Increased cell packing and small neuronal size in limbic system and paucity of Purkinje and granular cells in cerebellum can partially explain the inflammation, either systemic or local in ASD, however the inflammatory response within CNS has been linked to mast cells and microglia/astrocyte activation.

Microglia cells, present particularly in diencephalon, are mononuclear cells possessing phagocytic activity within CNS. They are responsible for cytokine and reactive oxygen species production within CNS. In addition they regulate synaptogenesis and neurogenesis. Multiple studies reported that microglia cells were prominently activated in ASD brains thereby acted neurodestructively. It was suggested that the activation disrupts brain blood barrier (BBB) in...
Immune related factors in pathogenesis of autism spectrum disorders

ASD via secretion of vascular endothelial growth factor. Moreover, cytokines and chemokines production were highly increased in brain specimens and cerebral spinal fluid (CSF). These were: IFN-γ, IL-1β, IL-6, IL-12p40, TNF-α and chemokine CCL-2. The inflammation was discovered particularly in areas with white matter overgrowth shortly after the collapse of the development. It was shown that neuron-specific reaction is associated with microglial activation in dorsolateral cortex and the neuronal pattern organization may be disrupted in later ASD life. In other post-mortem study it was reported that microglial activation was widespread in the fronto-insular and visual cortices in subjects with ASD. Because of the anatomical distinct of these parts it was discussed whether density of microglia in autistic brains is higher throughout the cerebral cortex.

The probable reason for microglia activation may be linked to perturbations in complement production in ASD individuals. The complement component C1q seems to be a key factor which influences neurodevelopment in humans. The protein C1q is the largest component of the complement system to activate the classical complement pathway, by binding of C1 to initiate the given activator for intense antigen-antibody immune complex. C1q has been found to play a key role in microglia-mediated synaptic pruning in typical developing human brain where it can activate the complement cascade and produce C3/CR3 signaling. This signaling in turn further activates and promotes microglia phagocytosis. Inflammatory insults which result in chronic elevation in serum C1q inhibiting factor leading to relative C1q dysfunction.

Astrocytes play role in repairing brain tissue after injury, lining the BBB, balancing extracellular ion concentration and transporting nutrients to neurons. They are involved in synaptogenesis during development, as well. When permanently activated gliosis occurs brain damage develops. Astrocyte markers concentration, for instance, glial fibrillary acidic protein, are elevated as well in the CSF as in post-mortem ASD brain tissues. Other astrocyte markers concentration, such as aquaporin 4 and connexin 43 have also been found to be increased in autistic brains.

Cytokine and Chemokine Profile

The cytokine profile, as well pro- as anti-inflammatory, in ASD patients is altered. Few studies reported decreased concentration of TNF-β in serum samples from subjects with ASD and its linkage with autism severity was proved. Insufficient amount of TGF-β is critical for inflammation control as the molecule is related to cell migration, apoptosis and regulation within immune system and CNS. Lower concentration of TGF-β were found to be associated with lower adaptive behaviours. Increased TNF-α concentration in ASD brain, cerebrospinal fluid or blood cells is able to block synaptic communication. Plasma level of leptin functionally mimicking IL-6 and IL-12 is also increased and able to pass the BBB. The observation was critically made in children with early onset autism.

Elevated levels of macrophage inhibitory factor (MIF) was additionally observed in individuals with ASD. As the molecule is responsible for maintaining neural and endocrine systems, the highest concentration of MIF was marked in children with the most severe autism. The findings of recent meta-analysis identified significantly altered concentrations of cytokines as IL-1β, IL-6, IL-8, IFN-γ, eotaxin and monocyte chemotactic protein-1 in ASD, strengthening evidence of an abnormal cytokine profile in ASD where inflammatory signals dominate. Cytokines are overexpressed upon toll like receptor 2 (TLR2) and TLR4 but not TLR9 stimulation. The overproduction of cytokines has been associated with altered behaviour and nonverbal communication. Increased plasma concentrations of platelet derived growth factor (PDGF) have been reported in autistic patients with more impaired communicative and behavioral skills as well as restricted stereotypic activities.

On the other hand, it should be noted that peripheral blood mononuclear cells from ASD patients were find to secrete more IL1RN, sTNFRI and sTNFRII, limiting the inflammatory response, in company with higher production of anti-inflammatory IL-10. It was shown that during a fever, when pro-inflammatory proteins concentration is heightened and T lymphocyte subsets activated, hyperactivity and stereotypia or speech impairment are reduced. Taken together, the data indicates that the aforementioned cytokines milieu can influence behaviour and increase risk of developing ASD.

Chemokines serve chemotactic properties within the immune system. Studies confirmed the elevated levels of MCP-1, RANTES, as well as CCL-2 and CCL-5 in autistic patients. Chemokine imbalance was found to be associated with more impaired developmental and adaptive fun-
tion, thereby the exact role of these chemokines in ASD pathogenesis need to be elucidated\(^\text{108}\). The conclusions of the Early Markers for Autism (EMA) study carried out by Zerbo et al\(^{107}\) are promising. The authors suggested that measurement of immune system function, especially chemokines: MCP-1, RANTES, MIP-1\(\alpha\), in the first few days of life may aid in the early identification of abnormal neurodevelopment.

**Immunoglobulin Levels**

The role of antibodies in ASD pathogenesis has been extensively studied. Lower levels of IgM and IgG classes of immunoglobulin were marked in ASD children and linkage with impaired behaviour was proved\(^{118}\). The later studies revealed increased level of neutralizing IgG4 antibodies among IgG subclass\(^{118}\). Additionally, in ASD patients alterations in BBB result in inducing expression of specific autoantibodies of IgM, IgG an IgG classes as a consequence of exposure to neuron-derived antigens. The single specific target have yet to be identified nevertheless single studies indicate antibodies against serotonin receptors, myelin basic protein, heat shock proteins, various brain tissue proteins. The lack of target specificity may be a consequence of cellular damage and the emergence/revealing of sequestered or new epitopes due to antibody generation\(^7\). Such processes promote neuroinflammatory conditions in ADS patients but studies are still continuing to confirm this hypothesis\(^{109}\).

The presence of circulating antibodies directed toward brain or nuclear proteins\(^{108}\) in ASD patients have been mentioned in a few reports. Wills et al\(^{115}\) examined plasma samples of children with ASD for antibodies directed against human cerebellar proteins. Protein analyses revealed that 21\% of subjects with ASD produce antibodies reactive toward a cerebellar 52kDa protein. Intense immunoreactivity was determined morphologically to be the Golgi cell of the cerebellum\(^{25}\). A significantly higher percent seropositivity of anti-nuclear antibodies in ASD patients with/without a family history of autoimmunity in comparison to healthy children was found. Furthermore, ASD individuals have marked serum anti-myelin-associated glycoprotein antibodies\(^{110}\) and elevated serum levels of anti-ganglioside M1 antibodies than healthy children\(^{111}\).

Additionally, the presence of anti-nuclear antibodies has been shown to be positively correlated with disease severity, mental retardation and electroencephalogram abnormalities\(^{112}\).

**Cellular Response**

Several alterations comprising B and T cells, natural killer (NK) cells and monocytes function in company with autoantibodies production have been observed in ASD\(^{113,114}\). There is evidence indicating increased response of Th1 cells instead of Th2\(^{115}\), HLA-DR high level in CD3\(^+\) T cells was stated previously as the allele is associated with late cellular response\(^9,98\). Moreover, CD26 (dipeptidyl peptidase IV) expression was increased on CD8\(^+\) T cells. CD26 being marker associated with effector cell phenotype in human CNS diseases makes the discovery significant\(^9\). In vitro stimulation of co-stimulatory and activation markers resulted in higher levels of CD137 and decreased levels of CD134, CD25 on T cells of children suffering from ASD\(^9\). Increased T lymphocytes activation may be associated with maintaining activated cells due to decreased apoptosis, as seen in Crohn’s disease\(^{116}\). Additionally, adhesion molecules are known to control the passage of T cells across endothelium. In high functioning autistic children, the concentration of such adhesion factors like sPECAM-1, sP-Selectin and sL-Selectin were decreased\(^{117,118}\) and the low selectin level has been found to be associated with altered social skills\(^{118}\).

NK cells serving viral response, tumor cytotoxicity and autoimmunity roles have been shown to be dysregulated in ASD\(^{119,120}\). The expression of few NK cells receptors and effectors were altered and significantly associated with NK cells function. These were perforin, granzyme B, and IFN\(\gamma\) marked under resting conditions in children with ASD. Precise stimulation of NK cells obtained from ASD patients showed decreased cytotoxicity in compare to control cells. It was proposed that low glutathione, IL-2 and IL-15 may be responsible\(^{121}\). In general, the mechanism behind this low NK cell activity need to be clarified.

Monocytes identify pathogens via TLRs and subsequently direct immune response. Dormant, peripheral blood monocyte life is short but upon inflammation they tend to escape apoptosis and via CCL-2 and CCL-7 chemokines are transported to the inflammation area where they differentiate into macrophages\(^{122,123}\). When recruited into CNS they develop into microglial cells and play crucial role in CNS inflammation\(^{114}\). Significant differences in pro-inflammatory cytokine production by monocytes from ASD patients were observed under different TLRs stimulation. While TLR-2 and TLR-4 favoured the condition, TLR-9 stimulation decreased cytokine production. This altered immunity response in ASD subjects can have a wide range impact on neural function in autistic people\(^{110}\).
Gastrointestinal Malfunctions

Immune alterations within gastrointestinal tract have been associated with ASD phenotype as well as its contribution to the neurodevelopment or autism severity. Symptoms of digestive tract diseases were observed in 9-84.1% autistic individuals depending on study type (retrospective vs prospective) and inclusion criteria. Symptoms such as excessive production of intestinal gases, flatulence, abdominal pain, diarrhea, belching, symptoms of gastroesophageal reflux or constipation have been attributed to changes in gut microbiota as well as elevated intestinal permeability and intestinal inflammation. Introducing the parents of ASD patients indicated coexisting severe behavioral symptoms with the ongoing gastrointestinal problems.

Intestinal microbiota is an important factor contributing to the organism homeostasis. Intestinal dysbiosis, being a qualitative-quantitative disorder among particular groups of microorganisms, is considered to be the etiological agent of many types of diseases, inflammatory bowel diseases, obesity and atopic diseases. Gastrointestinal tract microbiome and enteric neurons are directly connected, forming part of the axis of microbe-small intestine-brain. Therefore, regulatory activity of microbiota on the CNS occurs via neuronal, endocrine, metabolic and immune pathways. Shaw et al. attempted to understand the correlation of intestinal microbiota with the occurrence of clinical symptoms of autism in a case study. There were two brothers with ASD in the study who showed the presence of Krebs cycle metabolite analogues and high concentrations of arabinose in the urine. The compounds were not present in the urine of healthy subjects. The described phenomenon is considered as the result of colonization of the gastrointestinal tract by bacteria and/or yeasts, whose metabolites are inhibitors of mitochondrial Krebs cycle. Moreover, potential impact of the microbiota in the initiation/deterioration of ASD symptoms comes from a significant improvement in behaviour of children overtreated with vancomycin. The frequent use of antibiotics, mainly because of ear infections, was thought to interfere strongly intestinal ecosystem, promote the multiplication of pathogenic microorganisms, including toxigenic strains of the Clostridium sp. Autistic neurological symptoms may develop following the direct action of tetanus neurotoxin-tetanospasmin. Accumulation of bacterial toxins in conjunction with excessive intestinal permeability observed in children with ASD, leads to increased blood concentration of toxicants resulting in systemic symptoms. Further studies of the intestinal ecosystem in children with ASD found a significant increase in the number of different species of Clostridium species, in comparison to the control group. These observations were confirmed in Parracho et al. analysis, who described the population of Clostridium histolyticum to be overgrown in ASD patients. Finegold et al., discovered the overgrowth of Clostridium bolteae in the feces of persons with ASD. In addition to the best documented Clostridium spp overgrowth in the gastrointestinal tract of ASD individuals, there are also studies suggesting abnormal commensal bacteria milieu. It was demonstrated that in children with severe ASD Bacteroidetes family predominates, while Firmicutes were more often found in healthy children.

Gastrointestinal disruptions in autistic patients are functional as well as organic disorders. Immune tissue in gastrointestinal tract is the largest and most complex immune system in humans. Epithelial cells provide both innate and adaptive immune responses. Innocuous milieu within intestine makes proinflammatory Th2-dependent, Th1-dependent delayed-type hypersensitivity, IgG antibodies and Th17-dependent granulocytic responses to be suppressed. Histopathological findings such as gastritis, lymphoid nodular hyperplasia, colonic lymphoid nodular hyperplasia, eosinophilic infiltration and others was significantly more common in children with ASD. Additionally, many inflammatory transcripts detected within gastrointestinal tract are common for ASD and Crohn’s disease or ulcerative colitis. Few observed changes (eosinophilic infiltration) can be partially resolved in children with autism as a result of dietary intervention as a result of treating non allergy food hypersensitivity. This is especially evident due to increased permeability of the ASD individuals small intestine mucous membrane which is observed even in patients with nonspecific inflammatory bowel disease, celiac disease or cystic fibrosis. Homeostasis of intestinal barrier provides selective and preferential absorption of nutrients from the intestinal lumen, thereby preventing bloodstream migration of pathogens and toxicants. Selective loss of intestinal barrier function is thought to be associated with IgE-independent food hypersensitivity with delayed mechanism of reaction. Penetration of undigested food particles through the gut barrier activates the immune system, leads to the specific IgG antibodies production, immune complex formation and
Sensitivity Toward Environmental Toxicants

Environmental toxicants can disrupt proper neurodevelopment and immunity. Autistic children susceptibility to halogenated aromatic hydrocarbons (HAH) is specific to ryanodine receptor expression in autistics brain is altered. All in all HAH generally disrupt immunity by diminishing cellular response and causing lymphoid organs atrophied. Among few HAH toxicants polybrominated diphenyl ethers (PBDEs) have been studied. When peripheral mononuclear blood cells from ASD patients were treated with PBDEs followed by bacterial derivative lipopoly saccharide stimulation, cytokines and chemokines production in cell culture was critically increased. Opposite findings were discovered in control cell cultures originated in typically developing children. The toxicant has been, therefore, considered as an immune suppressor in neurotypical people. Many other toxicants such as solvents, phthalates, pesticides or heavy metals have been studied. Mercury and lead may lead to autoantibody production or skewed production of cytokines. Organochlorine pesticides disrupt calcium and sodium channels as well as GABA receptors thereby induce neuro and immunotoxicity. Organophosphates upregulate Th1 and Th2 cytokines which evolve them into malfunction of adaptive response. Pyrethrines were found to suppress IFN-γ and IL-4 production and upregulate production of IL-12 and TNFα.

Conclusions

The immune response in individuals with ASD is altered. These malfunctions may be responsible for ASD development.

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Conflicts of interest

The authors declare no conflicts of interest.

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