Journal of Autoimmunity 44 (2013) 1–7

Contents lists available at SciVerse ScienceDirect

Journal of Autoimmunity

journal homepage: www.elsevier.com/locate/jautimm

Review

Immunological and autoimmune considerations of Autism Spectrum Disorders

Benjamin Gesundheit [a,*], Joshua P. Rosenzweig [a], David Naor [b], Bernard Lerer [c], Ditza A. Zachor [d], Vaclav Procházka [e], Michal Melamed [b], Donald A. Kristt [f], Abraham Steinberg [g], Cory Shulman [h], Paul Hwang [i,j], Gideon Koren [k,l,m], Asnat Walfisch [n,o], Jacob R. Passweg [p,q], John A. Snowden [r,s], Ryad Tamouza [t], Marion Leboyer [u], Dominique Farge-Bancel [v], Paul Ashwood [w,x]

[a] Jerusalem, Israel
[b] Lautenberg Center for General and Tumor Immunology, Hebrew University, Hadassah Medical School, Jerusalem, Israel
[c] Department of Psychiatry, Biological Psychiatry Laboratory, Hadassah – Hebrew University Medical Center, Ein Karem, Jerusalem, Israel
[d] Department of Pediatrics, The Autism Center, Assaf Harofeh Medical Center, Tel Aviv University, Zerifim, Israel
[e] Department of Interventional Neuroradiology and Angiology, Radiodiagnostic Institute, University Hospital Ostrava, Czech Republic
[f] Molecular Pathology Program, Rabin Medical Center, Petach Tikva, Tel Aviv University, Israel
[g] Pediatric Neurology, Shaare Zedek Medical Center, Jerusalem, Israel
[h] School of Social Work, Hebrew University Jerusalem, Israel
[i] Paediatric Neurology Division, Department of Paediatrics, North York General Hospital, Canada
[j] University of Toronto Epilepsy Research Program (UTERP), University of Toronto, Ontario, Canada
[k] Pediatrics, Pharmacology, Pharmacy and Medical Genetics, Motherisk Program, Hospital for Sick Children, University of Toronto, Canada
[l] Pediatrics and Physiology/Pharmacology, Canada
[m] Molecular Toxicology, University of Western Ontario, Canada
[n] High Risk Pregnancy Unit, Hillel Yaffe Medical Center, Hadera, Israel
[o] Technion, Israel Institute of Technology, Haifa, Israel
[p] Department of Hematology, University Hospital, Basel, Switzerland
[q] Severe Aplastic Anemia Working Party of European Bone Marrow Transplantation (EBMT), Switzerland
[r] Department of Haematology, Sheffield Teaching Hospitals NHS Foundation Trust, UK
[s] Department of Oncology, University of Sheffield, Sheffield, UK
[t] Jean Dausset Department and INSERM, UMRS, 940, Saint Louis Hospital, Paris, France
[u] Department of Psychiatry, Paris-Est-Créteil University, GH Mondor, FondaMental Foundation, Psychiatry Genetic Team (INSERM U 955), Hôpital Albert Chenevier, 40 Rue de Mesly, Créteil 94010, France
[v] Unité de Médecine Interne et Pathologie Vasculaire, INSERM U 976, Hopital St Louis, 1 Avenue Cl. Vellefaux, Paris, France
[w] Department of Medical Microbiology and Immunology, University of California Davis, USA
[x] MIND Institute, University of California Davis, USA

ARTICLE INFO

Article history:
Received 15 October 2012
Received in revised form
26 May 2013
Accepted 30 May 2013

Keywords:
Autism Spectrum Disorders
Brain inflammation
Microglia
Autoantibodies
HLA association
Cytokines

ABSTRACT

Autism Spectrum Disorders (ASD) are a group of heterogeneous neurodevelopmental conditions presenting in early childhood with a prevalence ranging from 0.7% to 2.64%. Social interaction and communication skills are impaired and children often present with unusual repetitive behavior. The condition persists for life with major implications for the individual, the family and the entire health care system. While the etiology of ASD remains unknown, various clues suggest a possible association with altered immune responses and ASD. Inflammation in the brain and CNS has been reported by several groups with notable microglia activation and increased cytokine production in postmortem brain specimens of young and old individuals with ASD. Moreover several laboratories have isolated distinctive brain and CNS reactive antibodies from individuals with ASD. Large population based epidemiological studies have established a correlation between ASD and a family history of autoimmune diseases, associations with MHC complex haplotypes, and abnormal levels of various inflammatory cytokines and immunological markers in the blood. In addition, there is evidence that antibodies that are only present in some mothers of children with ASD bind to fetal brain proteins and may be a marker or risk factor for ASD. Studies involving the injection of these ASD specific maternal serum antibodies into pregnant mice

* Corresponding author. Tel.: +972 506558638.
E-mail address: b.gesund@gmail.com (B. Gesundheit).

0896-8411/$ – see front matter
http://dx.doi.org/10.1016/j.jaut.2013.05.005

during gestation, or gestational exposure of Rhesus monkeys to IgG subclass of these antibodies, have consistently elicited behavioral changes in offspring that have relevance to ASD. We will summarize the various types of studies associating ASD with the immune system, critically evaluate the quality of these studies, and attempt to integrate them in a way that clarifies the areas of immune and autoimmune phenomena in ASD research that will be important indicators for future research.

1. Introduction

The underlying causes of Autism Spectrum Disorders (ASD) are still unknown. This limits the current treatment of ASD to intensive behavioral therapy for the core symptoms. There is an urgent need to improve our understanding of the underlying pathology of ASD in order to explore new therapeutic approaches for this severe lifelong condition. We will review studies that investigated various immunological aspects associated with ASD, and attempt to evaluate the quality of these studies, in an effort to direct future research towards the possible underlying mechanisms of ASD.

2. Autism spectrum disorder (ASD)

2.1. General background

In 1943 Leo Kanner [1894–1981] first described autism as a highly variable neuro-developmental disorder. In 1979, Wing and Gould characterized autism clinically by the triad of: a) Impaired social interaction, b) restricted communications skills, and c) unusual repetitive behavior. These categories have over time expanded to include

- *Impaired social interaction*- Infants show reduced attention to social stimuli, smile less, and manifest reduced eye contact and facial/emotional expression, significant impairment in initiating and maintaining peer relationship; poor shared enjoyment and joint attention skills; lack of empathy and poor understanding of social rules
- *Restricted communication skills*- with delayed babbling, diminished responsiveness, no integration of gestures with words, less/no sentence construction, often repeating someone else's words (echolalia), poor conversation skills; immediate and delayed echolalia; poor symbolic and make believe play
- *Unusual repetitive behavior*- including stereotypic repetitive movements, compulsive behavior, ritualistic behavior and self-injury, unusual sensory interests and unusual repetitive patterns of interests

Due to the often broad, heterogeneous clinical presentation of autism, the term Autism Spectrum Disorders (ASD) was coined, which includes Asperger's syndrome, Rett's syndrome, Childhood Disintegrative Disorder, Pervasive Developmental Disorders Not Otherwise Specified (PDD-NOS). In May 2013, the APA (American Psychological Association) plans to release the DSM-5 (Diagnostic and Statistical Manual of Mental Disorders) which will likely merge several previously separate diagnoses. A variety of additional symptoms are associated with ASD including: epilepsy and/or subclinical increase in epileptic waveforms; anxiety disorders; insomnia and nocturnal awakening; mental retardation; sensory abnormalities with poor muscle tone and motor skills; and gastrointestinal dysfunction. Some children with ASD experience developmental regression previously described in the literature as "*autism with regression*", "*autistic regression*", "*setback-type autism*", or "*acquired autistic syndrome*," characterized by a loss of previously-acquired skills, primarily in the areas of language, social interest, adaptive functioning and motor skills. It remains debatable whether or not this represents a distinct subset of autism. Although the numbers of individuals with ASD that have one or more of these additional features are substantial, the exact frequencies that appear in the ASD are not firmly established.

Prevalence estimates for ASD range from 0.7% to 2.64% and represent a dramatic increase since the 1980s. Diagnosis occurs early in childhood but symptoms typically remain stable throughout adulthood. Most individuals have severe disabilities requiring intense care throughout their lifespan including medical care, educational support and many are unable to live independently. The etiology for ASD is likely multi-factorial. Many theories evoking different or inter-related pathways have been suggested:

- A genetic component is likely to exist in some cases, since siblings—in particular twins—are often affected. While various known genetic abnormalities are associated with approximately 10% of individuals with ASD, the critical loci remain unknown [1]. While in the past genetic screening was suggested primarily for children with dysmorphic features, more recently, some geneticists recommend the use of microarray CGH in any child with ASD due to the high rate of copy-number variants (CNVs) found.
- Association of ASD with prenatal risk factors [2] has been suggested including advanced age of both parents, use of psychiatric drugs, bleeding disorders of the mother, teratogenic factors, and familial autoimmune diseases or immune conditions including diabetes, maternal celiac disease, maternal allergies/asthma with onset during pregnancy.
- Recent post-mortem transcriptomic analysis of brain specimens from individuals with ASD and controls revealed the existence of two "modules" of gene co-expression networks; a neuronal module and an immune module. In the neuronal module, known autism susceptibility gene variants are enriched and under expressed while in the immune module immune/inflammatory genes are enriched and up regulated [3].
- Theories blaming vaccine components as a cause for ASD were suggested in the past, but these controversial theories have been largely rejected due to a current lack of scientific evidence.
- Epidemiological studies indicate that ASD might be associated with endocrine dysregulation and in particular steroid function [4].

The pathology of ASD also remains enigmatic with various neurobiological theories suggested [5] and alterations in many brain systems implicated including cortex, limbic system, cerebellum, corpus callosum, basal ganglia and brainstem. However, altered early brain development might be more relevant for ASD than documented pathological findings in adults. Aspects of the early developmental processes that cannot be documented as pathology include: neuronal migration [6], connectivity or plasticity [7], neuronal organization of the white matter [8], reduced synaptic maturation [9], reduced dendritic maturation [5], and abnormal serotonin metabolism/transport [10]. For instance, disrupted synaptic development might be associated with epilepsy in 20% of ASD [11]. However, the linkage between the pathology and the clinical neuro-psychological manifestation is not fully understood [8]. So

far electrophysiological [12] and functional MRI studies [13] that have attempted to document clinical correlations with ASD have provided inconclusive results.

Screening, diagnosis and monitoring of ASD remains a major challenge involving a joint effort by pediatricians, psychologists, psychiatrists and neurologists. Some countries incorporate routine screening tests such as the Modified Checklist for Autism in Toddlers (M-CHAT). Several diagnostic tests based on the DSM-IV-TR and ICD-10 such as ADOS (Autism Diagnostic Observations Schedule) and/or the parents' interview ADI-R (Autism Diagnostic Interview-Revised) are accepted as gold standards. Other tests such as Aberrant Behavioral Checklist (ABC) or Childhood Autism Rating Scale (CARS) are also widely used. Based on clinical symptoms, additional tests such as EEG/QEEG or imaging studies including new MRI techniques might prove helpful in screening and monitoring of ASD.

Frontline psychological treatments are typically individually tailored to the child's needs including early intensive behavioral and educational interventions and support for the family. Behavioral therapies to teach children self-care and social skills such as Applied Behavior Analysis (ABA) enhance overall function in preschool children [14]. However, over 50% of children with ASD in the USA are treated with various medications including anticonvulsants, psychoactive drugs like antidepressants, stimulants and antipsychotics, but with no evidence that the core symptoms of ASD improve, and despite reported severe side effects [15].

Due in part to the elusive etiology of the disease, currently no medications directed at core symptoms of ASD are available and treatment options remain disappointing. This is reflected in the ever expanding list of non-evidence-based treatments offered to parents again, despite serious concerns regarding safety with some of these therapies. A recent comprehensive analysis of the plethora of offered treatments shows that, in the majority of situations, there is very limited/no evidence of effectiveness. This is true as well for a variety of complementary and alternative therapies (CAM) and chelation protocols whilst the commonly offered dietary recommendations including gluten- and casein-free (GFCF) are currently not supported by any evidence-based consensus [16].

Because of the overall lack of effective treatments, there is a need for innovative evidence-based therapies supported by sound scientific evidence. Even minor therapeutic benefits might result in significantly better long-term outcome and improve the daily function and quality-of-life of individuals with ASD.

2.2. ASD and autoimmune disease

While the etiology of ASD remains unknown, various clues suggest a possible association with altered immune responses and ASD (See Fig. 1).

Various studies established an association between ASD and a family history of autoimmune diseases [17–19]. This was first documented in case reports [20] and later confirmed in comprehensive epidemiological studies for approximately 40% of children with autism [21,22]. In particular an association with autoimmune thyroiditis or hypothyroidism [23], rheumatic fever [24], rheumatoid arthritis, celiac disease, ulcerative colitis, psoriasis, family history of type 1 diabetes has been found [22,25]. Some have suggested that these findings support further research into the possibility of an autoimmune component in ASD because, in general, in autoimmune diseases there is a higher prevalence of a family history of autoimmune disease. It is important to note that some of the studies were based on small sample sizes, and relied on self-response questionnaires in order to elicit the family history. This carries with it a potential recall bias. Other studies derived their information from national registries. The extremely large sample size in these studies and the lack of reliance on the questionnaire are certainly advantages of this method. However, national registry studies have their own known limitations based on data selection, inconsistent diagnostic

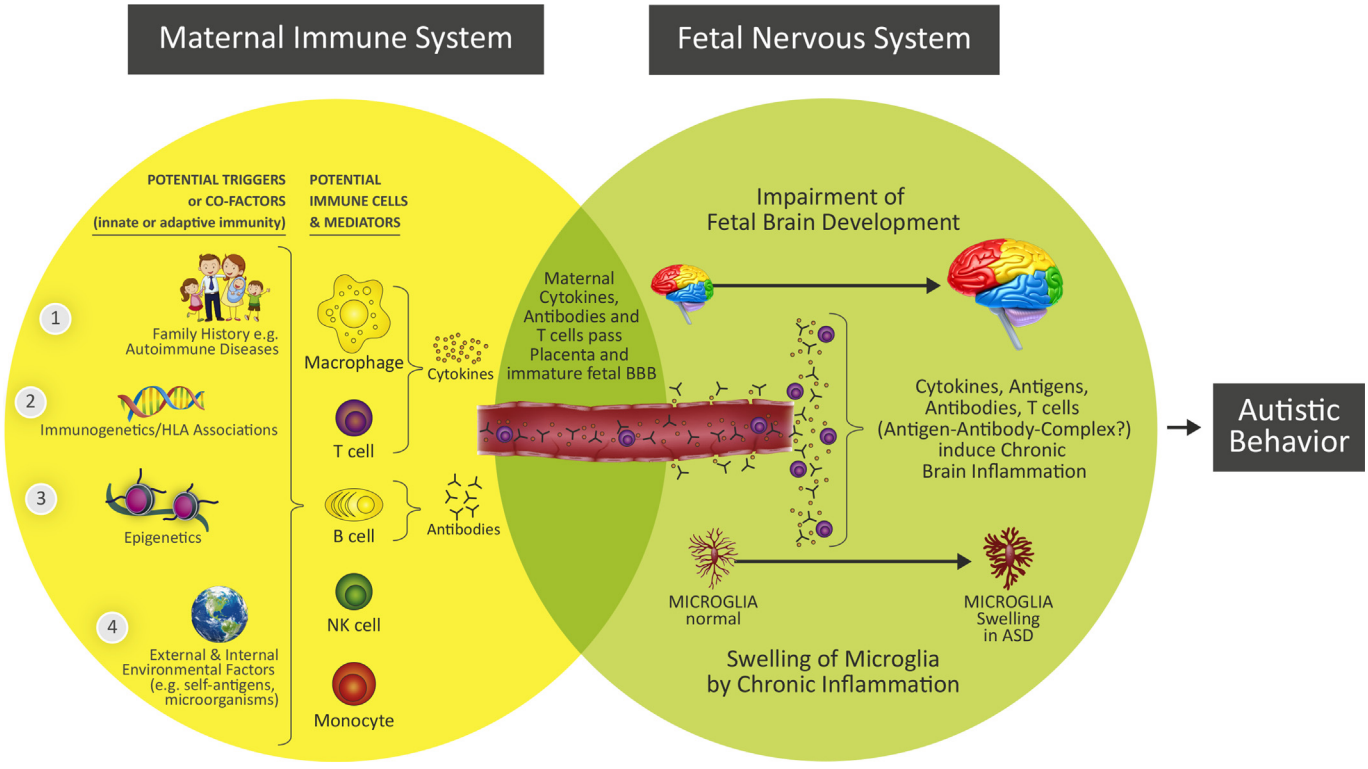

Fig. 1. Proposed immunological basis of some forms of autism.

criteria, quality, and lack of validation. Additionally, since the criteria for ASD and the definition/nomenclature for ASD has vacillated over the years, it is unclear how studies that include data from a long range of time account for this discrepancy.

Immunogenetics provides an opportunity to investigate on a genetic level the relationship between ASD and the immune system. According to Geschwind et al., we now have knowledge of the etiology of ASD for between 10 and 20% of cases based on recent genetic studies [26]. However, no specific gene accounts for the majority of ASD and that even the most common genetic forms account for not more than 1–2% of cases. More specifically, in the area of immunogenetics, the research has mainly focused on HLA associations. In this context, studies have shown an association for classical MHC class I, II and III alleles and ASD. As early as the 1980's Stubbs et al. suggested that parents of children with autism shared HLA antigens significantly more than the controls [27]. More recently, Torres et al. showed an increase of certain HLA-A2 (but not HLA-B) alleles in subjects with autism [28]. Beyond MHC class I associations in the above studies, the MHC class II region has also been implicated in ASD. More specifically, a strong association between HLA-DRB1*04 (the widely admitted major susceptibility allele for rheumatoid arthritis) and ASD has been found in various studies [29,30]. Additionally, other Class II alleles sharing the third hypervariable region (HVR-3) have been found to be linked to ASD. Some children with autism express a higher frequency of both the HLA-A2 and DR11 alleles. A few studies have even suggested that some MHC class II genes might serve a protective function [28]. However, a recent meta-analysis revealed that autism share with rheumatoid arthritis the same protective HLA class II allele namely HLA-DRB1*13 [31]. Important studies by Guerini et al. and Lee et al. have aimed to analyze these associations while taking into account various variables such as ethnicity and genetic background [32,33]. In terms of MHC class III genes, the C4 complement alleles have been linked to ASD [34,35].

In addition, several genes connected to innate and adaptive immune regulation may be involved in ASD [36]. For example, the MET proto-oncogene tyrosine kinase pathway which is involved in immune regulation has been shown to be associated with ASD [37]. Other studies have linked the serine and threonine kinase C gene PRKCB1 to ASD [38]. This gene has been shown to be involved in B cell activation as well as neuronal function. Other susceptibility genes that alter immune function including those related to NK cells, macrophage inhibitory factor (MIF), PTEN tumor suppressor gene, Reelin, and mitochondrial respiratory chain disease have been associated with ASD.

Recent studies have demonstrated that disrupting the epigenetic regulation of transcription plays crucial roles in the development of autoimmune diseases [39–41]. Beyond studies investigating the potential immunogenetics component of ASD, there is new interest in evaluating potential epigenetic mechanisms associated with ASD [42–45]. In light of the fact that Rett syndrome, Fragile X, and other genetic syndromes comorbid with ASD, have been shown to be associated with epigenetic modifications, the theory that epigenetic mechanisms might potentially be associated with the etiology of ASD deserves more attention. More specifically, methyl CpG binding protein 2 (MECP2) gene mutations seem to be worthy of further investigation. This is particularly the case, since the reversible nature of epigenetic regulation, and the potential interaction with environmental factors, generates potentially fertile ground for research into therapeutic interventions.

An exciting new direction in the research of immune markers in ASD is the investigation of microglia in autism. Microglia, the macrophages of the central nervous system which serve as the first active immune defense in the CNS, were identified by Del Rio-Hortega as playing an important role in various pathological conditions back in the 1930's. Our understanding of their function within neuro-immunology has continued to develop since that time. Their role ranges from antigen presenting cells, to promoting repair, to phagocytosis, to producing cytokines as well as many others. There is also much research focusing on their role within both autoimmune and neurological diseases.

Recently, researchers have begun investigating the possible role of microglia within patients with ASD. In a landmark study in 2005, Vargas et al. demonstrated a marked increase in neuroglial responses, characterized by activation of microglia and astroglia, in the brains of individuals with ASD [46,47]. The neuroglial activation was particularly prominent in the granular cell layer and white matter of the cerebellum. In addition, they found the presence of a marked increase of MCP-1 (monocyte chemoattractant protein-1) in CSF supporting the hypothesis that proinflammatory pathways are activated in the brain of individuals with ASD and that its presence may be associated with mechanisms of macrophage/microglia activation observed in the brain tissue studies [46]. These results must be tempered by the conflicting research done by Kemper that did not show evidence of astrogliosis or microglial reactions in infantile autism. However, possible support for the role of microglia in autism has recently been provided by Morgan and Courchesne. In 2010, they demonstrated that microglia appeared markedly activated in 5 of 13 cases with autism, including 2 of 3 under the age of 6 years old, and marginally activated in an additional 4 of 13 cases [48]. More recently, they showed that at least some microglial activation in the dorsolateral prefrontal cortex in autism is associated with a neuron-specific reaction, and that neuronal organization may degrade later in life in the disorder [49].

In addition, signs of lymphocytic infiltration in the mucosa might point to autoimmune processes. A few studies have investigated the presence of a lymphocytic infiltration in the gut of patients with ASD [37–39]. However, there is no evidence to suggest that excess lymphocytic infiltration is present in the brains of adults or older children with ASD. This does not rule out the possibility that lymphocytes could infiltrate the brain early in neurodevelopment such as *in-utero* or during early postnatal development. The statistical association of ASD with various MHC (Major Histocompatibility Complex) haplotypes is another area of research that points to an association between ASD and the immune system. A number of studies have been conducted that demonstrate a clear statistical association [50]. The research in this direction is ongoing and promising.

At this stage, there is no clear consensus in the literature about whether maternal antibodies and child autoantibodies are linked. In fact, it seems that they are likely two separate phenomena and should be considered as separate and not necessarily interrelated. Many studies have isolated brain and CNS reactive antibodies from individuals with ASD [19]. However, so far no studies have conclusively identified the antigens to which these antibodies bind. Some evidence suggests that the putative antigen(s) appears to be of molecular weight of 52kD [51] and to be present on GABAergic interneurons in the brain [36,52]. However, a number of other targets such as GFAP and MBP have also been suggested. Although these results remain inconsistent and even contradictory, with no apparent specific targets conclusively identified yet, these findings do indicate that auto-immune mechanisms might play an important role in the pathogenesis of at least a subgroup of ASD [53]. It is unclear if the increase in antibodies to cerebellum proteins and the disputed finding of increased antimyelin basic protein antibodies have direct pathologic significance, or if they are merely a response to previous injury. To the best of our knowledge, nobody has reproduced ASD or ASD-like symptoms by direct transfer of

autoantibodies isolated from children or adults with ASD into animal models.

Beyond the studies investigating autoantibodies in children with ASD, there are studies that analyze the presence of maternal antibodies in mothers of children with ASD. These maternal antibodies are distinct from the autoantibodies of children with ASD, as mentioned above. There is yet no direct evidence that transplacental transmission of antibody from an affected mother to a fetus has resulted in ASD. However, there seems to be significant evidence that an antibody not recognized as pathogenic in the mother can produce ASD in the infant [54–58] (See Fig. 2A, B, C). In 2003, Dalton et al. reported injecting serum antibodies from a mother of children with ASD into pregnant mice during gestation and found altered behavior in the offspring compared with offspring of mice injected with sera from mothers of healthy children (See Fig. 2A, D, E). Similarly, in 2008, Martin et al. demonstrated that Rhesus monkeys gestationally exposed to IgG class antibodies from mothers of children with ASD consistently displayed atypical stereotyped behaviors compared to controls injected with IgG from mothers of typically developing children. In mouse models, Singer et al. demonstrated that exposure to IgG from mothers of children with ASD during gestation resulted in offspring with alterations of sociability. The direct pathogenicity of these antibodies or their targets have not yet been identified.

Various inflammatory cytokines and immunological markers reflecting immune dysfunction have been documented in ASD [59]. Large studies of antibodies in ASD suggest clinical correlation with disease's severity [60]. A variety of studies demonstrate statistically significant alterations in various immune mediators, including serum antibodies, brain antibodies, serum cytokines, chemokines, NK cells, and adhesion molecules in children with ASD. Some of these studies were conducted without proper controls, some relied on a small number of patients, and some have yet to be replicated or have been contradicted. Nevertheless, viewed together as a unit, these results seem to suggest an association between alterations in immune mediators and ASD that demands further larger studies with proper controls. More specifically, the decrease in IgG and IgM, levels in children with ASD might indicate immune dysfunction. Similarly, the increase in proinflammatory cytokines such as IL-6, IL-12 and IFN-γ and the decrease in anti-inflammatory cytokines such as IL-10 and TGF-β1 demonstrates a possible hyperimmune state in ASD. Though studies have suggested that adhesion molecules play a key role in immune responses in the CNS and that they are important mediators of inflammation, the precise meaning of the finding of decreased PECAM-1 in children with ASD remains unclear. Chemokines serve an important role as chemotactic factors in the immune system. The finding of increased MCP-1, RANTES, and eotaxin in children with ASD and their association with increased impairments in behavior in these children suggests that an altered immune response might be associated with behavioral changes in ASD. Further research is necessary to elucidate the precise role that these chemokines might play in the pathogenesis of ASD. Furthermore, research over the last decades has documented immune abnormalities in children with ASD. Autoimmune associated phenomena such as hyperactivation of monocyte, NK cell and T cell responses [61–63], increased pro-inflammatory cytokine and chemokine production, decreased frequencies of regulatory T cells or IL-10 and TGFβ1 production [60,62] have been observed in children with ASD. While no clear link between ASD and specific autoimmune diseases are yet to be described, these findings indicate that abnormal regulation of the immune response or immune dysfunction may be associated with ASD.

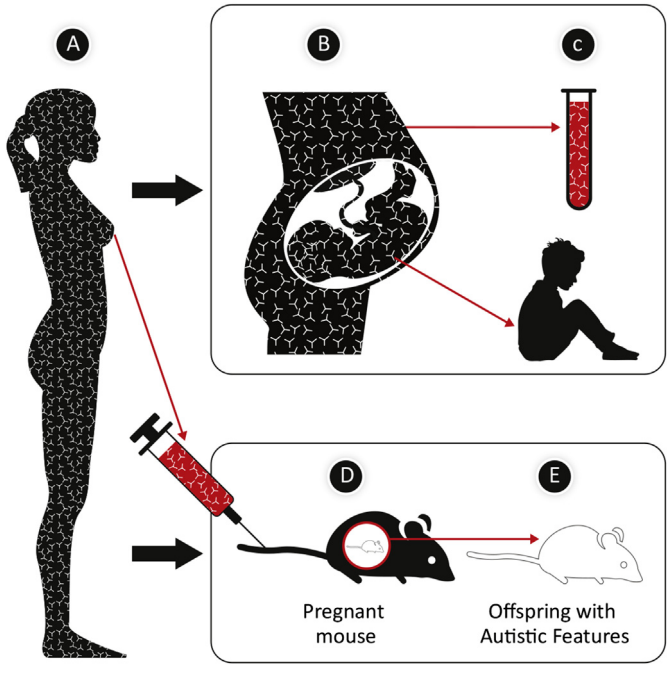

Fig. 2. Schema of proposed immunologic issues and development of some forms of autism.

Response to immune suppression is an important area of research that might help clarify the nature of the involvement of the immune system in ASD. Immune based therapy for ASD has been suggested in the past, however, large placebo controlled clinical trials have not been performed [18]. Studies investigating the benefits of steroids, IVIG, and Vitamin D for ASD remain controversial and contradictory. The conflicting results in treating ASD with steroids are consistent with the mixed results in treating other autoimmune diseases. For example, in the case of scleroderma, one study combining high-dose methotrexate and oral corticosteroids demonstrated efficacy in controlling localized scleroderma without significant adverse reaction [64]. Similarly, a study combining high-dose immunosuppressive therapy and autologous progenitor cell transplantation for systemic sclerosis showed prolonged (>3 years) improvements of skin thickening and functional ability, together with a stabilization of pulmonary function in two-thirds of treated patients with severe systemic sclerosis [65]. However, unlike other autoimmune diseases, broadly blocking the immune response (e.g., with steroids) does not appear to be generally useful in scleroderma [66]. One theory for the lack of effectiveness of steroids in scleroderma is that the immune system has strong anti-fibrotic components, and that broad-spectrum inhibitors may exacerbate the fibrosis associated with the disease. Similarly, in primary biliary cirrhosis, one study demonstrated that immune suppression through the use of prednisolone was associated with a better overall hepatic outcome and little evidence of increased bone loss [67]. Other studies, however, have demonstrated that, in fact, oral corticosteroids are minimally effective in treating primary biliary cirrhosis [68]. Clearly the heterogeneity of auto-immune disorders [69] makes it difficult to generalize about the effectiveness of immune suppressive therapy. Therefore, it is not surprising to find conflicting studies in ASD, which as a spectrum is by definition heterogeneous, independent of its characterization as an autoimmune disease. However, enough promising results exist to form a basis to perform more rigorous studies with a subgroup of patients with ASD both to evaluate the efficacy of treatment and to elucidate the complex relationship between ASD and the immune system.

While research suggests that a subgroup of ASD may have an autoimmune component, we are aware of the complications associated with this theory. For example, while many of the immune marker studies have been reproduced, some studies have yet to be confirmed and others have been contradicted. Even assuming that in fact the markers are reliable, one can challenge whether there is any correlation between antibody level and anticipated pathological effect. Indeed, Rumsey has already outlined the obstacles in identifying specific pathology in autism via functional neuroimaging [70]. Furthermore, the slow progress in confirming an HLA association despite familial history of autoimmune diseases leaves reason for doubt. The poor results for steroid treatment also pose concerns about the theory of autism as an autoimmune disease. Combined, these concerns are not reasons to dismiss the theory, but rather they justify a critical approach to the issue which takes into account all of the factors involved.

One possible hypothesis that would connect the autoimmune components elaborated upon above with the clinical findings in autism would be an early-life immune insult leading to changes in the vulnerable embryonic and infantile brain. Immunologic mechanisms involving antibodies against brain epitopes may induce auto-immunity, altered immune response in CNS, or neuroinflammation.

In conclusion, various types of immunological evidence (brain antibodies, serum cytokines, family history, and immunogenetics) point to a relationship between ASD and the immune system. Since some of these studies lack robust controls and many focus on only one type of immunological evidence in isolation, our international group suggests that future research focus on intensifying studies analyzing immunological aspects of ASD with proper controls in a more integrative fashion. One question for further research involves the precise characterization of the nature of the relationship between ASD and the immune system. None of these studies sufficiently explain whether the immune system underlies the pathology of ASD in a causative way, whether immune interferences create vulnerability to other pathogens responsible for ASD, or whether a third, yet unknown factor is responsible for both the pathology of ASD and for the aberrant immune response in ASD. Further research should consider the potential relevance of assessing the autoimmune aspects associated with ASD according to systematic guidelines for autoimmune diseases described by Shoenfeld et al. and Rose and Bona [71].

Acknowledgments

We would like to thank Dr. Noel R. Rose (Director, Johns Hopkins Center for Autoimmune Disease Research) for graciously reviewing the manuscript. Thanks also to Professors Ronald Goldstein, Edwin Cook for their input and to Sarah Tal for her assistance in designing the figures.

References

[1] Abrahams BS, Geschwind DH. Advances in autism genetics: on the threshold of a new neurobiology. Nat Rev Genet 2008;9:341–55.
[2] Gardener H, Spiegelman D, Buka SL. Prenatal risk factors for autism: comprehensive meta-analysis. Br J Psychiatry 2009;195:7–14.
[3] Voineagu I, Wang X, Johnston P, Lowe JK, Tian Y, Horvath S, et al. Transcriptomic analysis of autistic brain reveals convergent molecular pathology. Nature 2011;474:380–4.
[4] Frye CA, Llaneza DC. Corticosteroid and neurosteroid dysregulation in an animal model of autism, BTBR mice. Physiol Behav 2010;100:264–7.
[5] Levy SE, Mandell DS, Schultz RT. Autism. Lancet 2009;374:1627–38.
[6] Schmitz C, Rezaie P. The neuropathology of autism: where do we stand? Neuropathol Appl Neurobiol 2008;34:4–11.
[7] Mostofsky SH, Powell SK, Simmonds DJ, Goldberg MC, Caffo B, Pekar JJ. Decreased connectivity and cerebellar activity in autism during motor task performance. Brain 2009;132:2413–25.
[8] Minshew NJ, Williams DL. The new neurobiology of autism: cortex, connectivity, and neuronal organization. Arch Neurol 2007;64:945–50.
[9] Betancur C, Sakurai T, Buxbaum JD. The emerging role of synaptic cell-adhesion pathways in the pathogenesis of autism spectrum disorders. Trends Neurosci 2009;32:402–12.
[10] Lam KS, Aman MG, Arnold LE. Neurochemical correlates of autistic disorder: a review of the literature. Res Dev Disabil 2006;27:254–89.
[11] Kelleher RJ, Bear MF. The autistic neuron: troubled translation? Cell 2008;135:401–6.
[12] Roberts TP, Schmidt GL, Egeth M, Blaskey L, Rey MM, Edgar JC, et al. Electrophysiological signatures: magnetoencephalographic studies of the neural correlates of language impairment in autism spectrum disorders. Int J Psychophysiol 2008;68:149–60.
[13] Just MA, Cherkassky VL, Keller TA, Kana RK, Minshew NJ. Functional and anatomical cortical underconnectivity in autism: evidence from an FMRI study of an executive function task and corpus callosum morphometry. Cereb Cortex 2007;17:951–61.
[14] Eikeseth S. Outcome of comprehensive psycho-educational interventions for young children with autism. Res Dev Disabil 2009;30:158–78.
[15] Rossignol DA. Novel and emerging treatments for autism spectrum disorders: a systematic review. Ann Clin Psychiatry 2009;21:213–36.
[16] Buie T, Campbell DB, Fuchs GJ, Furuta GT, Levy J, Vandewater J, et al. Evaluation, diagnosis, and treatment of gastrointestinal disorders in individuals with ASDs: a consensus report. Pediatrics 2010;125(Suppl. 1):S1–18.
[17] Comi AM, Zimmerman AW, Frye VH, Law PA, Peeden JN. Familial clustering of autoimmune disorders and evaluation of medical risk factors in autism. J Child Neurol 1999;14:388–94.
[18] Careaga M, Van de Water J, Ashwood P. Immune dysfunction in autism: a pathway to treatment. Neurotherapeutics 2010;7:283–92.
[19] Onore C, Careaga M, Ashwood P. The role of immune dysfunction in the pathophysiology of autism. Brain Behav Immun 2012;26:383–92.
[20] Money J, Bobrow NA, Clarke FC. Autism and autoimmune disease: a family study. J Autism Child Schizophr 1971;1:146–60.
[21] Sweeten TL, Bowyer SL, Posey DJ, Halberstadt GM, McDougle CJ. Increased prevalence of familial autoimmunity in probands with pervasive developmental disorders. Pediatrics 2003;112:e420.
[22] Atladóttir HO, Pedersen MG, Thorsen P, Mortensen PB, Deleuran B, Eaton WW, et al. Association of family history of autoimmune diseases and autism spectrum disorders. Pediatrics 2009;124:687–94.
[23] Molloy CA, Morrow AL, Meinzen-Derr J, Dawson G, Bernier R, Dunn M, et al. Familial autoimmune thyroid disease as a risk factor for regression in children with autism spectrum disorder: a CPEA study. J Autism Dev Disord 2006;36:317–24.
[24] Keil A, Daniels JL, Forssen U, Hultman C, Cnattingius S, Söderberg KC, et al. Parental autoimmune diseases associated with autism spectrum disorders in offspring. Epidemiology 2010;21:805–8.
[25] Croen LA, Grether JK, Yoshida CK, Odouli R, Van de Water J. Maternal autoimmune diseases, asthma and allergies, and childhood autism spectrum disorders: a case-control study. Arch Pediatr Adolesc Med 2005;159:151–7.
[26] Geschwind DH. Genetics of autism spectrum disorders. Trends Cogn Sci 2011;15:409–16.
[27] Stubbs EG, Magenis RE. HLA and autism. J Autism Dev Disord 1980;10:15–9.
[28] Torres AR, Maciulis A, Stubbs EG, Cutler A, Odell D. The transmission disequilibrium test suggests that HLA-DR4 and DR13 are linked to autism spectrum disorder. Hum Immunol 2002;63:311–6.
[29] Warren RP, Singh VK, Cole P, Odell JD, Pingree CB, Warren WL, et al. Possible association of the extended MHC haplotype B44-SC30-DR4 with autism. Immunogenetics 1992;36:203–7.
[30] Johnson WG, Buyske S, Mars AE, Sreenath M, Stenroos ES, Williams TA, et al. HLA-DR4 as a risk allele for autism acting in mothers of probands possibly during pregnancy. Arch Pediatr Adolesc Med 2009;163:542–6.
[31] Crespi BJ, Thiselton DL. Comparative immunogenetics of autism and schizophrenia. Genes Brain Behav 2011;10:689–701.
[32] Guerini FR, Bolognesi E, Manca S, Sotgiu S, Zanzottera M, Agliardi C, et al. Family-based transmission analysis of HLA genetic markers in Sardinian children with autistic spectrum disorders. Hum Immunol 2009;70:184–90.
[33] Lee LC, Zachary AA, Leffell MS, Newschaffer CJ, Matteson KJ, Tyler JD, et al. HLA-DR4 in families with autism. Pediatr Neurol 2006;35:303–7.
[34] Warren RP, Singh VK, Cole P, Odell JD, Pingree CB, Warren WL, et al. Increased frequency of the null allele at the complement C4b locus in autism. Clin Exp Immunol 1991;83:438–40.
[35] Corbett BA, Kantor AB, Schulman H, Walker WL, Lit L, Ashwood P, et al. A proteomic study of serum from children with autism showing differential expression of apolipoproteins and complement proteins. Mol Psychiatry 2007;12:292–306.
[36] Enstrom AM, Van de Water JA, Ashwood P. Autoimmunity in autism. Curr Opin Investig Drugs 2009;10:463–73.
[37] Campbell DB, Warren D, Sutcliffe JS, Lee EB, Levitt P. Association of MET with social and communication phenotypes in individuals with autism spectrum disorder. Am J Med Genet B Neuropsychiatr Genet 2010;153B:438–46.
[38] Lintas C, Sacco R, Garbett K, Mirnics K, Militerni R, Bravaccio C, et al. Involvement of the PRKCB1 gene in autistic disorder: significant genetic association and reduced neocortical gene expression. Mol Psychiatry 2009;14:705–18.

[39] Brooks WH, Le Dantec C, Pers JO, Youinou P, Renaudineau Y. Epigenetics and autoimmunity. J Autoimmun 2010;34:J207–19.
[40] Lu Q, Renaudineau Y, Cha S, Ilei G, Brooks WH, Selmi C, et al. Epigenetics in autoimmune disorders: highlights of the 10th Sjögren's syndrome symposium. Autoimmun Rev 2010;9:627–30.
[41] Chaste P, Leboyer M. Autism risk factors: genes, environment, and gene-environment interactions. Dialogues Clin Neurosci 2012;14:281–92.
[42] Schanen NC. Epigenetics of autism spectrum disorders. Hum Mol Genet 2006;15(Spec. No. 2):R138–50.
[43] Grafodatskaya D, Chung B, Szatmari P, Weksberg R. Autism spectrum disorders and epigenetics. J Am Acad Child Adolesc Psychiatry 2010;49:794–809.
[44] Persico AM, Bourgeron T. Searching for ways out of the autism maze: genetic, epigenetic and environmental clues. Trends Neurosci 2006;29:349–58.
[45] Mehler MF, Purpura DP. Autism, fever, epigenetics and the locus coeruleus. Brain Res Rev 2009;59:388–92.
[46] Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. Ann Neurol 2005;57:67–81.
[47] Tetreault NA, Hakeem AY, Jiang S, Williams BA, Allman E, Wold BJ, et al. Microglia in the cerebral cortex in autism. J Autism Dev Disord 2012;42:2569–84.
[48] Morgan JT, Chana G, Pardo CA, Achim C, Semendeferi K, Buckwalter J, et al. Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. Biol Psychiatry 2010;68:368–76.
[49] Morgan JT, Chana G, Abramson I, Semendeferi K, Courchesne E, Everall IP. Abnormal microglial-neuronal spatial organization in the dorsolateral prefrontal cortex in autism. Brain Res 2012;1456:72–81.
[50] Torres AR, Sweeten TL, Cutler A, Bedke BJ, Fillmore M, Stubbs EG, et al. The association and linkage of the HLA-A2 class I allele with autism. Hum Immunol 2006;67:346–51.
[51] Cabanlit M, Wills S, Goines P, Ashwood P, Van de Water J. Brain-specific autoantibodies in the plasma of subjects with autistic spectrum disorder. Ann N Y Acad Sci 2007;1107:92–103.
[52] Wills S, Cabanlit M, Bennett J, Ashwood P, Amaral DG, Van de Water J. Detection of autoantibodies to neural cells of the cerebellum in the plasma of subjects with autism spectrum disorders. Brain Behav Immun 2009;23:64–74.
[53] Stigler K, Sweeten T, Posey D, McDougle C. Autism and immune factors: a comprehensive review. Res Autism Spectr Disord 2009;3:840–60.
[54] Malkova NV, Yu CZ, Hsiao EY, Moore MJ, Patterson PH. Maternal immune activation yields offspring displaying mouse versions of the three core symptoms of autism. Brain Behav Immun 2012;26:607–16.
[55] Goines PE, Croen LA, Braunschweig D, Yoshida CK, Grether J, Hansen R, et al. Increased midgestational IFN-γ, IL-4 and IL-5 in women bearing a child with autism: a case-control study. Mol Autism 2011;2:13.
[56] Braunschweig D, Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Croen LA, et al. Autism: maternally derived antibodies specific for fetal brain proteins. Neurotoxicology 2008;29:226–31.
[57] Braunschweig D, Duncanson P, Boyce R, Hansen R, Ashwood P, Pessah IN, et al. Behavioral correlates of maternal antibody status among children with autism. J Autism Dev Disord 2011;42.
[58] Martin LA, Ashwood P, Braunschweig D, Cabanlit M, Van de Water J, Amaral DG. Stereotypies and hyperactivity in rhesus monkeys exposed to IgG from mothers of children with autism. Brain Behav Immun 2008;22:806–16.
[59] Ratajczak HV. Theoretical aspects of autism: biomarkers–a review. J Immunotoxicol 2011;8:80–94.
[60] Ashwood P, Wills S, Van de Water J. The immune response in autism: a new frontier for autism research. J Leukoc Biol 2006;80:1–15.
[61] Enstrom AM, Onore CE, Van de Water JA, Ashwood P. Differential monocyte responses to TLR ligands in children with autism spectrum disorders. Brain Behav Immun 2010;24:64–71.
[62] Ashwood P, Enstrom A, Krakowiak P, Hertz-Picciotto I, Hansen RL, Croen LA, et al. Decreased transforming growth factor beta1 in autism: a potential link between immune dysregulation and impairment in clinical behavioral outcomes. J Neuroimmunol 2008;204:149–53.
[63] Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah I, Van de Water J. Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. Brain Behav Immun 2011;25:40–5.
[64] Torok KS, Arkachaisri T. Methotrexate and corticosteroids in the treatment of localized scleroderma: a standardized prospective longitudinal single-center study. J Rheumatol 2012;39:286–94.
[65] van Laar JM, McSweeney PA. High-dose immunosuppressive therapy and autologous progenitor cell transplantation for systemic sclerosis. Best Pract Res Clin Haematol 2004;17:233–45.
[66] Leask A. Emerging targets for the treatment of scleroderma. Expert Opin Emerg Drugs 2012;17:173–9.
[67] Mitchison HC, Palmer JM, Bassendine MF, Watson AJ, Record CO, James OF. A controlled trial of prednisolone treatment in primary biliary cirrhosis. Three-year results. J Hepatol 1992;15:336–44.
[68] Angulo P, Jorgensen RA, Keach JC, Dickson ER, Smith C, Lindor KD. Oral budesonide in the treatment of patients with primary biliary cirrhosis with a suboptimal response to ursodeoxycholic acid. Hepatology 2000;31:318–23.
[69] Charles C, Clements P, Furst DE. Systemic sclerosis: hypothesis-driven treatment strategies. Lancet 2006;367:1683–91.
[70] Rumsey JM, Ernst M. Functional neuroimaging of autistic disorders. Ment Retard Dev Disabil Res Rev 2000;6:171–9.
[71] Shoenfeld Y, Selmi C, Zimlichman E, Gershwin ME. The autoimmunologist: geoepidemiology, a new center of gravity, and prime time for autoimmunity. J Autoimmun 2008;31:325–30.