



2010 Research Grant Program Winning Abstract

Innate Immunity and Autism

By **Paul Ashwood**

Autism spectrum disorders (ASD) are neurodevelopmental disorders characterized by stereotyped repetitive behavior, impairments in social interaction and deficits in communication. According to current estimates from the CDC as many as 1 in 98 children have ASD. The etiologies of autism are largely unknown but there is a growing body of evidence that suggests immune responses play a role in the pathophysiology of autism.

Numerous immune system abnormalities have been described in individuals with autism. Chief among these is the increased activation of innate immune responses in autistic brain specimens. Importantly, both young and adult (ages 5-44 years) autistic brains exhibit signs of active, ongoing inflammation. There is marked activation of microglia and astrocytes, up-regulation of HLA-DR expression and striking increases in many cytokines in the brain and CSF of autistic subjects. Gene expression analysis of autistic brain tissue has revealed differential expression of several genes involved in cytokine signaling, TOLL like signaling and immune regulatory pathways. In addition, in monocytes, which are thought to be precursors of brain macrophages and microglia, there is evidence of atypical function in autism. Increased numbers of monocytes have been reported in autism, as well as atypical responses to TLR stimulation. Monocytes from children with autism showed increased cytokine production in response to TLR2 stimulation and TLR4 stimulation, and decreased response to TLR9 stimulation. Moreover, increased levels of IL-1beta in response to TLR stimulation positively correlated with impairments in social behavior, suggesting a link between immune activation and behavioral features characteristic of autism.

Adverse activity of brain macrophages and microglia has been implicated in the pathology of several neurological diseases including Parkinson's, Multiple Sclerosis, and Alzheimer's. Macrophages and microglia are capable of polarizing into two major subtypes, categorized as M1 or M2. The "classical" or M1 subtype typically releases large quantities of pro-inflammatory cytokines and promote cell-mediated immunity and is characterized by production of high levels of interleukin (IL)-12, and low levels of IL-10. "Alternatively activated" or M2 macrophages function to resolve inflammation and to clear cellular debris following an inflammatory event and produce very low levels of IL-12 and high amounts of anti-inflammatory molecules IL-10 and TGFbeta. Increased M1 polarization is consistent with increased IL-12 observed in plasma and brain specimens in autism, and may play a role in the pathophysiology of autism.

As autism is a disorder that manifests in early childhood, it is difficult to find suitable research tools and accessible tissues for experimentation. For example, post mortem human tissue can never provide the substrate for dynamic functional studies and finding suitable control material is problematic. Immune cells, in contrast, provide a readily accessible model system which has many advantages for autism research including easy acquisition, high availability and fine matching with controls. Macrophages can be



obtained in-vitro by maturing monocytes into monocyte-derived macrophages (mo-MDMs). Due to the conserved function and cellular processes between macrophages and microglia, these mo-MDMs can serve as surrogates for examinations of microglia phenotype and function. For this study, monocytes will be collected from autistic participants and controls and developed into mo-MDMs which will be analyzed for M1/M2 polarization, TLR expression and dynamic cellular function.

The specific aims of this proposal are (1) to determine the activation and polarization of mo-MDM from children with autism compared with typically developing controls, (2) to determine expression patterns of TLR2, TLR4, and TLR9 in mo-MDMs from children with autism and controls, and (3) to determine the dynamic cellular function of mo-MDM from children with autism compared with controls in response to TLR stimulation. Monocytes will be collected from patients and typically developing controls and cultured in culture media (10% FBS, 1% pen/strep, RPMI 1640) + 50 ng/ml M-CSF for 7 days, in order to develop mo-MDMs. Cultured macrophages will be conditioned in cell media alone, or stimulated with agonists for TLR2 (lipoteichoic acid), TLR4 (lipopolysaccharide), and TLR9 (CpG) for 24 hours with BD GolgiPlug™ prior to intracellular cytokine analysis using standard protocols for flow cytometry. Using standard techniques, the cultured cells will be fixed and permeabilized with BD Cytofix/Cytoperm™ solution and labeled with BD Pharmingen™ fluorescently conjugated anti-CD14, and CD11b antibodies to verify macrophage phenotype, anti-CD86, CD80, CD40, and HLA-DR to measure activation and co-stimulatory ability, and anti-IL-12 (M1) and anti-IL-10 (M2) to determine M1/M2 polarization, and anti-TLR2, anti-TLR4, and anti-TLR9 antibodies. All samples will be analyzed on a BD™ LSR II flow cytometer. Gene expression of TLR2, TLR4 and TLR9 will also be assessed by RT-PCR. In parallel experiments, mo-MDMs will be stimulated with or without TLR agonists for 24 hours. Supernatants will be harvested and cytokine levels of TNF α , IL-1 β , IL-6, IL-12, IL-10, CXCL10, CCL22, and TGF β assessed using BD™ Cytometric Bead Array Flex Sets, according to standard protocols and analyzed on a BD LSR II flow cytometer. Cytokines revealed to be significantly different will be confirmed by BD OptEIA™ ELISA kits.

This study will examine a component of the immune system that has not previously been examined in autism and will help further elucidate the role innate immunity plays in the pathophysiology of autism.

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