Do the Stem Cells Really Work with Autism Spectrum Disorders Associated with Neuro-Immune Interaction?

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Abstract

Autism spectrum disorders (ASDs), namely neurodevelopmental disorders encompassing impairments in communication, social interactions and restricted stereotypical behaviors, induces a relatively high morbidity and mortality ratio (1/166) in modern children's life. One of the serious factors accounting for ASDs is the failure of the appropriate neuro-immune interaction. Although a relationship between altered immune responses and ASDs was firstly recognized nearly 40 years ago, only recently has new evidence started to shed light on the complex multifaceted communication between neuro-immune dysfunction and behavior in ASDs. Extensive alterations in immune function have now been described in both children and adults with ASDs, including ongoing inflammation in brain specimens, elevated pro-inflammatory cytokine profiles in the Cerebro-Spinal Fluid (CSF) and blood, increased presence of brain-specific auto-antibodies and altered immune cell function. Accumulated data both from clinical and lab research proposed the essential role of neuro-immune interaction during the pathogenesis of ASDs. Stem cells, which account for normal turnover and injury repair, might do great favors on ASDs due to their ability to give rise to new functional cells as a cell replacement source, paracrine secretion as trophic and cytokine contributor, immune modulator to balance the pro-inflammation and anti-inflammation as well as the inhibitor of chronic inflammation in ASDs brain, etc. Here in this review, we focus on the current development of stem cell administration in ASDs especially on mesenchymal stem cells (MSCs), which proved to be the most plastic and efficient to interfere with ASDs neuro-immune interaction, moreover summarize the probable mechanism and efficient therapeutic methods to treat ASDs withMSCs.

Keywords: Stem cells; Mesenchymal stem cells (MSCs); Autism spectrum disorders (Asds); Neuro-immune interaction

Introduction

Autism spectrum disorders (ASDs) are a series of pervasive development disorders including autistic disorder, Ret’s disorder, childhood disintegrative disorder, Asperger’s syndrome or pervasive developmental disorder not otherwise specified (PDD-NOS). ASDs incidence is reaching epidemic proportions, afflicting approximately 1 in 166 children [1]. It is currently characterized in several areas of development: reciprocal social interaction skills, communication skills, or the presence of stereotyped behavior, interests and activities (APA, 2000) [2]. ASDs are considered complex, heterogeneous diseases caused by an interaction between genetic vulnerability and environmental factors. In an effort to better target the underlying roots of ASDs for diagnosis and treatment, efforts to identify reliable biomarkers in genetics, neuroimaging, gene expression, and measures of the body’s metabolism are growing [3]. Besides the immense efforts on the genetic and transcriptome analysis, increasing evidence points to a central role for immune system and many maternal immune system-related risk factors-including autoimmunity, infection and fetal reactive antibodies-which are closely associated with ASDs [2]. Moreover, there is close correlation between ongoing immune dysregulation and ASDs either in individuals or animal models [4]. Recently, several molecular signalling pathways-including those downstream of cytokines, the receptor MET, major histocompatibility complex class I molecules, microglia and complement factors have been identified as the links mediating immune activation to ASDs phenotypes [5-7]. Together, these findings indicate that the immune system is a point of convergence for multiple ASDs-related genetic and environmental risk factors [8-10]. Simone Gupta’s group revealed dysregulation of innate immune response genes and neuronal activity-dependent genes between region-matched autism and control brains by transcriptome analysis [11]. These accumulated data from epidemiology, clinician observers and neurological scientists led to a point that brain immune dysfunction mediate the genetic factors to the environmental ones and the ultimate ASDs. In this way, one of the most probable efficient ASDs treatments is to perform immune modulation. Modern science witnessed the revolution of stem cell
research which has brought new insights into the intervention of traditional intractable diseases such as ASDs, Amyotrophic Lateral Sclerosis (ALS), Rett Syndrome, Spinal Muscular Atrophy (SMA) etc. due to stem cell's intrinsic properties of regeneration against degeneration, immune regulation resisting the immune dysfunction, neurotrophic ability to reverse the insufficient neurotrophic capacity of these diseases. Stem cells have undoubtedly become one of the most promising and effective treatment methods for most of the Central Nerve System (CNS) diseases.

What kind of stem cells rank as the best appropriate for treating ASDs?

Stem cells are group of cells that can keep self-renewal and at the same time give rise to terminal functional adult cells. Among them, mesenchymal stem cells (MSCs) exist in most of the mesenchymal tissues such as bone marrow, adipose tissue, umbilical cord, etc. at relatively high ratio and are easy to be isolated and expanded in vitro. MSCs are firstly isolated by Friedenstein and coworkers, identified as a nonphagocytic cell population with fibroblast-like appearance, able to originate discrete fibroblastic colonies in vitro [12,13]. In 1991, they were defined as mesenchymal stem cells (MSCs) by Caplan and regarded as new therapeutic tools for tissue repair, due to their capacity of differentiation and commitment to unique tissue types (e.g., cartilage and bone) [14]. Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT) determined that multipotent mesenchymal stromal cells (with the acronym MSCs) were the more appropriate term to be used. In addition, this committee proposes that these cells must be defined by three minimal criteria. First, they must be plastic-adherent when maintained under standard culture conditions. Second, they must present CD105, CD73, and CD90 expression (≥95%) and lack expression of CD45, CD34, CD14, or CD11b, CD79alpha or CD19 and HLA-DR (≤2% positive). Third, they must be able to differentiate into osteoblasts, adipocytes, and chondroblasts when cultured under standard in vitro differentiating conditions [15]. Given the disturbing rise in incidence rates for ASDs, and the fact that no pharmacological therapy for ASDs has been approved by the Food and Drug Administration (FDA), there is an urgent need for new therapeutic options. Research in the therapeutic effects of MSCs for other immunological and neurological conditions has shown promising results in preclinical and even clinical studies [16]. MSCs have demonstrated the ability to suppress the immune system and to promote neurogenesis with a anticipated safety profile. These characteristics may be attributed to the fact that MSCs are intrinsically derived from perivascular cells, pericytes, liberated from their basement membrane tethers surrounding blood vessels upon injury or inflammation. Many experiments have accumulated related data on the treatment of ASDs using MSCs with high plasticity. Actually, MSCs are in late phases of clinical trials for treatment of two immune dysregulation conditions of graft versus host disease (GVHD) and Crohn's Disease. Cord blood CD34+ cells are known to be potent angiogenic stimulators, having demonstrated positive effects in not only peripheral ischemia, but also in models of cerebral ischemia. Additionally, anecdotal clinical cases have reported responses in autistic children receiving cord blood CD34+ cells. Therefore, Ichim TE et al proposed that combined use of MSC and cord blood CD34+cells may be useful in the treatment of autism [1]. Ly Y T et al provides us a clinical evidence by a non-randomized, open-label, single center phase I/II trial investigating the safety and efficacy of combined transplantation of human cord blood mononuclear cells (CBMNCs) and umbilical cord-derived mesenchymal stem cells (UCMSCs) in treating children with autism [17]. They concluded that transplantation of CBMNCs demonstrated efficacy compared to the control group; however, the combination of CBMNCs and UCMSCs showed larger therapeutic effects than the using CBMNC alone. There were no safety issues noted during infusion and the whole monitoring period. Simberlund J et al suggested that MSC transplantation for the treatment of autism spectrum disorders is a novel approach that deserves further investigation, however substantial methodological and theoretical challenges and pitfalls remain before this can be considered a viable therapeutic option [18]. If MSCs can be fluently translated into clinical usage, the mechanism by which MSC modulate immune system should be firstly explored and clarified. Siniscalco D et al proposed the following MSC mechanism: stimulation of repair in the damaged tissue, e.g., inflammatory bowel disease; synthesizing and releasing anti-inflammatory cytokines and survival-promoting growth factors; integrating into existing neural and synaptic network, and restoring plasticity [19,20].

How does failure of neuro-immune dysfunction induce ASDs?

McDougle et al reviewed the evidence and the research results of immune mediated subtype of ASDs [21]. A role for immunological involvement in ASDs has long been hypothesized. Although Kanner (1943) did not specifically address this in his initial description of the syndrome, a detailed review of the original 11 patient cases reveals some potentially important and pertinent observations and comments. In 10 of the 11 case descriptions, clinical information was provided that could represent, in part, immune dysregulation. This is the earliest indication of correlation between immune and ASDs. The most important factor which mediates immune dysfunction to ASDs is microglia in the brain. Microglia are the tissue macrophages of the CNS and provide immune surveillance [22]. When neuronal injury occurs, microglia change their morphology and turns into macrophage-like cells and mediate inflammation [23,24]. However, the multiple roles of microglia in maintaining brain health have been under appreciated until current research focusing on microglia appeared. Microglia have been wisely called 'the constant gardeners' of the brain for their roles in synaptic pruning [25]. Once considered as passive custodians until activated by foreign signals, a immense body of literature has emerged over the past years pointing to the critical roles of microglia in removing weak, damaged or dysfunctional synaptic connections to maintain healthy brain function. Since faulty synaptic pruning has been associated with ASDs, it was logical for investigators to begin to study the role of microglia in the neuronal pathobiology of ASDs [26-28]. Paul Patterson's laboratory first demonstrated that a maternal immune response induces autism-like behaviors in mouse offspring using maternal respiratory infection with influenza virus [29]. For safe and easy to use, preclinical models have utilized antigen mimics that effectively induce an innate immune response without infection. A well-established maternal immune activation (MIA) procedure involves administration of the viral mimic Poly I:C to pregnant mice during a critical period of fetal brain development. Poly I:C is a synthetic double-stranded RNA with high affinity for the pattern recognition receptor Toll-like Receptor 3 (TLR3), which induces an acute-phase immune response and closely resembles the systemic symptoms seen with acute viral infections [30]. MIA induces long-lasting and region-specific changes in brain cytokines in offspring, including alterations during peak periods of synaptogenesis and plasticity. Poly I:C enhances the production and release of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α
and type I interferons IFN-alpha and IFN-beta [31-33]. Accordingly, a single maternal injection of the cytokine IL-6 causes some of the deficits observed in Poly I:C MIA offspring, while co-administration of an anti-IL-6 antibody with Poly I:C prevents these deficits [34], suggesting that IL-6 is critical in mediating some of the behavioral changes observed in Poly I:C MIA offspring. An extensive study of 23 cytokines in the blood and three brain regions (frontal cortex, cingulate cortex, and hippocampus) of Poly I:C MIA offspring demonstrated significant alterations in various pro-inflammatory, anti-inflammatory, and regulatory cytokines, as well as chemokines [35]. Taken together, these findings support the hypothesis that disruptions of cytokine signaling influence the behavioral and physiological abnormalities observed in MIA offspring. Therefore, effective intervention of immune dysfunctional ASDs should return to the immune modulation or anti-inflammation drugs or live cells, for example stem cells or in vitro adaptive immune cells.

The general mechanism through which MSCs interfere with ASDs

Site-activated MSCs produce a curtain of immuno-modulation behind which slow and specific tissue regeneration takes place. In addition, MSCs sense the tissue microenvironment and adjusts the curtain and regenerative activity accordingly. This includes the production of antibiotic proteins like LL37 that both kills intruding bacteria on contact, but calls forth macrophage and other members of the hematopoietic system to further mediate the injury site. Thus, MSCs appear to be local managers of the tissues' innate regenerative potential. In this way, MSC should denote 'Medicinal Signaling Cells' since they serve as 'drug stores' for sites of injury or inflammation [36]. Moreover MSCs have the ability to communicate with damaged tissues, where they can trigger immunosuppression or immune enhancement depending on the milieu, and engraft at sites of inflammation or injury [37]. At the same time, MSC possess unique immunological properties including expression of major histocompatibility complex (MHC) class I molecules but not MHC class II molecules [38-41]. Therefore, they normally do not act as antigen presenting cells [42,43], a feature that becomes important in their clinical use and they demonstrate a so-called 'stealth' ability to go undetected by a host immune system [44]. MSC have demonstrated complex immunomodulatory effects [45-48] on both humoral and cell mediated immune responses [49-53]. In the cell-mediated immune response [52,53] MSC inhibit T cell proliferation, decrease pro-inflammatory cytokine production like tumor necrosis factor-alpha (TNF-α), interferon gamma (IFN-γ) and decrease cell-mediated cytotoxicity [54-60]. MSC have also been found to inhibit natural killer (NK) cell proliferation, NK cell cytokine production and NK cell-mediated cytotoxicity through various mechanisms [60] still under investigation. In the humoral response, MSC inhibit B cell proliferation, maturation, migration, and immunoglobulin and antibody production [61,62]. Beyond the effect of MSC on T cells and B cells, MSC also exert an inhibitory effect on dendritic cell maturation, activation, and antigen presentation [63-65]. Furthermore, MSC have been found to block recruitment of neutrophils, likely protect neutrophils from apoptosis, and block production of TNF-a from activated macrophages [66,67]. It is not clear that the protective effect against neutrophil apoptosis is beneficial, as neutrophils are supposed to die off quickly. MSC can also suppress the delayed type hypersensitivity response in C57/BL6 (H2b) mice [68]. When MSC enter injured tissues, inflammatory triggers such as cytokines stimulate the release of many growth factors by MSC [37] including: epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-b), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), insulin growth factor-1 (IGF-1), angiopoietin-1 (Ang-1), keratinocyte growth factor (KGF) and stromal cell-derived factor-1 (SDF-1) [69-71]. These factors produced by MSCs can maintain endothelial integrity and regulate endothelial cell proliferation [37]. Inconsistently to the above reports about the MSC anti-inflammation function, Lee S H, et al demonstrated that MSCs combined with enzyme chondroitinase ABC (chABC) introduction into the spinal cord of chronic spinal cord injury dog models did not resulted in anti-inflammatory effects because of improved expression of pro-inflammatory cytokine factors such as COX2 and TNF-a [72]. This result may attribute to the degradation of chondroitin sulfate proteoglycans (CSPGs) by chABC, which may incur a second longer inflammation following the administration of MSCs and chABC beyond the inflammation modulation ability of MSCs.

The molecular mechanism in which MSCS affect ASD neuro-immune interaction for rescuing ASDs?

The growing body of literature suggests that the immune system might be dysregulated in individuals affected by autism spectrum disorder (ASDs) or their unaffected family members [73,74]. Studies of biological markers of immune function in individuals with ASDs have found neuro-inflammation in brain tissues [75-77], immunoglobin imbalances, including increased levels of plasma IgG4 [78], reduced levels of IgG and IgM [79,80] or of total IgG [81]; imbalances in cytokine/chemokine levels [8,82-84], abnormal ratios of CD4+ to CD8+ T-cells or increased blood levels of nitric oxide metabolites [74]. Most importantly, various studies demonstrated a specific effect of MSC on microglia [85], which play a crucial role in ASDs. In experimental ALS, the number of microglia cells was significantly decreased in the spinal cord after administration of MSC [86]. Similar results were reported in a rat focal ischemia model of transient middle cerebral artery occlusion [87] and neonatal hypoxic-ischemic brain injury, where MSC reduced expansion of microglia and favoring the formation of new neurons. Consistently, our lab used human adipose derived stem cells (hADSCs), one type of mesenchymal stem cell isolated from adipose tissue, to treat C57/BL6 mouse middle of cerebral artery occlusion (MCAO) and Spinal Cord Injury (SCI) models, decreased ionized calcium-binding adapter molecule 1 (Iba1) positive and glial fibrillary acidic protein (GFAP) was observed indicating suppression the reactivation of microglia cells and astrocytes induced by inflammation [88-90]. In experimental Parkinson’s disease, hMSC treatment significantly decreased lipopolysaccharide (LPS)-induced microglial activation [91,92]. One controversy still exists whether the immunosuppressive effect of MSC is a direct effect or requires activation via some specific cytokines or pathways [42, 43, 93, 94]. While further investigation is needed, the majority of the evidence points to an inhibitory role of MSC on immune function. Various theories try to elucidate the possible mechanisms of action of MSC on the immune systemMSC mediate the suppression of T cell proliferation via an indoleamine 2,3-dioxygenase (IDO) immunosuppressive pathway [57,95]MSC bring in two negative feedback loops in the very early phase of inflammation by secretion of prostaglandin (PGE2) and TNF-stimulated gene 6 protein (TSG-6) [53,60,96-98]. PGE2 is known to inhibit T cell proliferation, to affect apoptosis of T cells in either direction depending on the cell maturation and activation state [98], and influence the production of cytokines by T cells [98]. PGE2 is also known to induce and suppress B
cells depending on the maturity of the B cells. In addition, PGE2 can modulate the function of antigen presenting cells such as dendritic cells and macrophages [99]. In fact, PGE2 released by MSC can reprogram macrophages to produce more IL-10, inhibit dendritic cell maturation, and shift the balance between TH1 and TH2 [37,53,65,100]. TSG-6 is expressed at sites of inflammation and has been shown to reduce inflammatory damage through inhibiting neutrophil trans-endothelial migration induced by CXCL8- [101-104]. It is known that at the presence of PGE2, the effects of IDO in MSC-mediated immunoregulation of T-cell proliferation and NK cell activation can also be enhanced [60,94]. What should be pointed out is that, the above mechanisms might be species-specific [105]. The underlying mechanism of the immune modulation of MSC intervention with many diseases especially CNS ones has been broadly and deeply explored. The concise flow chart to explain the mechanism is drawn as below in Figure 1.

Administration timing, dosage and delivery route of MSC affect the treatment effects of ASDs

Previous research on MSC intervention of ASDs seems efficient, attracts enormous pre-clinical and clinical trials and deserves deeper exploration. However, the pivotal factors that affect the outcomes are the window timing for cell delivery, the cell dosage, the method of cell delivery and the objective effect detection. MSC treating ASDs is an extremely complicated course in which MSCs interact with various endogenous cells such as T cells, B cells, macrophages, microglia cells, astrocytes, neurons, oligodendrocytes, etc. This process is automatic and time consuming. Thus, the time to detect the effects becomes ambiguous since there is absence of good parameters and experiences. In addition, different MSC delivery methods may incur totally different treatment effects. Basically, clinical trials adopt their cell delivery as intravenous injection. This method theoretically cannot recruit sufficient MSCs onto the damaged or affected region, leading to unsatisfied clinical outcomes. In situ injection seems harder to handle than intravenous delivery, however, it can make sure enough amounts of cells possibly localized in the affected region, which thus results in better clinical efficacy.

Figure 1: The network crosstalk by which MSC interfere with the host nerve immune system: (1) MSCs can suppress the over reactivity of glia cells such as microglia and astrocytes through cytokine secretion; (2) MSCs can directly manipulate the cytokine secretion profile of immune cells such as B cells, T cells, Neutrophil, Dendritic cells, leukocytes, etc.. At the same time, the cytokine secreted by the immune cells can modulate the function of MSCs; (3) MSCs can secrete some trophic factors to support the endogenous cells such as neurons, oligodendrocytes and endothelial cells recovery from injury shock. → means promote the downstream events, ┌ means suppress the downstream events, ↑ means secretion upregulated, ↔ means bidirectional modulation.

The possible side-effects and ethical problems of MSC intervention with ASDs

Though previous enormous lab and clinical evidence has pointed to an important conclusion that MSC could be an alternative stem cells therapy source with bright future application especially in their positive immune modulation, there are still many uncertainties including the specific underlying mechanism, the side-effects, the delivery methods and window timing of MSC intervention, etc. One of the mostly concerned side-effect and biological safety problems is their...
Conclusions and Future Research Directions

Taken together, dysfunction of neuro-immune interaction make up large percentage of ASDs. Active interference with chronic inflammation and immune modulation may be a good choice for ASDs treatment. MSCs can act as a perfect cell source candidate due to their allogeneic transplantation possibility, easy isolation and expansion, abundance in various mesenchymal tissues, multi-potential differentiation capacity, low immunogenecity and superior immune modulation. The mechanism of MSC rescuing ASDs has been excellently unravelled as mainly through close crosstalk with the host immune system such as suppression of reactive glia cells, manipulate the cytokine secretion profile by negative feedback loops, and secrete trophic factors to protect the endogenous cells from injured damages. The most important job to do in the future research is to evaluate the clinical safety, efficiency, correlation between cell dosage administered and efficacy output as well as the deeper mechanism of MSCs for ASDs treatment and translate MSCs into treating various clinical immune related diseases or degenerative diseases.

References


