Future perspectives on cell therapy for autism spectrum disorder

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Abstract: Autism spectrum disorder (ASD) is a complex neurodevelopmental condition characterized by impairments in social communication, abnormal to absent verbal communication, the presence of repetitive stereotypic verbal and non-verbal behaviors and restricted interests, with onset early in life. We showed cognitive and behavioral characteristics of ASD by impairment of communication, cognition, perception, motor skills, executive function, theory of mind and emotion control. Recently, pathogenesis of immune pathology in the brains of individuals with ASD has been focused. New therapeutic approaches in the viewpoints of immune modulation and microglial function are logical for novel treatments for individuals with ASD. Cell therapies such as umbilical cord blood cells and mesenchymal stromal cells for ASD will be a challenging and encouraging field in the future clinical study with progress of biotechnological science.

Abbreviations

ASD: Autism spectrum disorder
MSCs: mesenchymal stromal cells
UCBs: umbilical cord blood cells
EVs: extracellular vesicles
HGF: hepatocyte growth factor
GDNF: glial cell line–derived neurotrophic factor
VEGF: vascular endothelial growth factor
HIE: hypoxic-ischemic-encephalopathy
CP: cerebral palsy
IL-6: interleukin 6
TNF-α: tumor necrosis factor-alpha
BDNF: brain derived neurotrophic factor
LPS: lipopolysaccharide
GTPase: guanosine triphosphatase

Introduction

Autism spectrum disorder (ASD) is a complex neurodevelopmental condition characterized by impairment in social communication, verbal communication, the presence of repetitive stereotypic verbal and non-verbal behavior and restricted interests, with an onset early in life. Prevalence of ASD has been increasing in the last two decades. In Japan, the prevalence of individuals with intellectual disabilities needing special educational program in school, including those with ASD, increased more than threefold from 21,000 in 2002 to 74,000 in 2013, similar to findings in the other countries (Ministry of Education, Culture, Sports, Science and Technology–Japan, 2013; Blumberg et al., 2013; Guthrie et al., 2019).

Cognitive and Behavioral Characteristics of ASD Due to Various Factors

The main cognitive and behavioral characteristics of ASD are impairment of communication, cognition, perception, motor skills, executive function, theory of mind and emotion control (Gabrielsen et al., 2018; Robertson and Baron-Cohen, 2017; Mottron et al., 2006; Ketcheson et al., 2017; Demetriou et al., 2018; Jones et al., 2018; Haney et al., 2018; Conner et al., 2019; Baron-Cohen et al., 1985) (Fig. 1) and various behavioral therapies based on neurophysiological viewpoints have been considered the foremost strategies for management of individuals on ASD (Sharma et al., 2018b).

Yet, there is currently no apparent effective therapy for ASD (Tachibana et al., 2017).

Genetic factors (Sullivan and Geschwind, 2019; Ruzzo et al., 2019; Parikshak et al., 2018), chronic inflammation, impairment of the microglia, oxidative stress, hormones/neurotransmitters abnormality (Gao and Penzes, 2015; Volk et al., 2015) and environmental factors (Oudin et al., 2019; Rossignol et al., 2014) are likely contributing to the development of ASD, however the precise pathophysiology is unknown.

More recently, pathogenesis of immune pathology in the brain of individuals with ASD has been a focus of research.

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Voineagu et al. (2011) reported over expression of immune-related gene networks (Voineagu et al., 2011), Braunischweig et al. (2013) reported the presence of maternal antibodies in fetal brain tissue (Braunschweig et al., 2013), Vargas et al. (2005) described atypical levels of pro-inflammatory cytokines (IL-6, TNF-a) in cerebrospinal fluid (CSF) of patients, and further reports suggested that excessive microglial activation leads to aberrant neural connections (Morgan et al., 2010; Suzuki et al., 2013).

From the recent basic research, abnormal activation of microglia by activation of TLR4 signaling pathway following maternal Lipopolysaccharide exposure, which in turn was involved in excessive synaptic pruning to decrease synaptic plasticity in the offspring may be one of the reasons for the autism-like behavior in the offspring mice (Xiao et al., 2021). On the other hand, early postnatal allergic airway inflammation has been reported to induce dystrophic microglia that exhibit defective synaptic pruning upon short- and long-term allergen exposure resulting in excitatory postsynaptic surplus and ASD-like behavior (Saitoh et al., 2021). These basic research suggest abnormal microglial activation which is responsible for synaptic pruning partially contributes to ASD pathology. These resulted in a novel therapeutic approach for an immunomodulation or repair of microglial function. Preclinical and clinical studies have shown that umbilical cord blood contains hematopoietic stem cells, endothelial progenitor cells, and mesenchymal stromal cells (MSCs) that, through paracrine signaling, alter brain connectivity and modulate inflammation (Meier et al., 2006; Sun et al., 2017). Infusion of autologous cord blood cells (UCBs) have been shown to be safe in individuals with cerebral palsy (CP), ASD, and other acquired brain injuries. Dawson et al. reported safe and feasible autologous UCBs infusions in young children with ASD and several promising outcomes were published (Dawson et al., 2017; Dawson and Fletcher-Watson, 2021; Dawson et al., 2020; Li et al., 2015). Recently, MSCs for individuals with ASD had favorable outcomes (Lv et al., 2013; Riordan et al., 2019; Sun et al., 2020) (Table 1).

Recent clinical trials in ASD were done by BM-MNC (n = 3), UCB (n = 2), UC-MSC (n = 2) and UCB + UC-MSC (n = 1). Frequency of administration are 1 times (n = 4), 2 times (n = 1), 3 times (n = 2) or 4 times (n = 1). Number of cells of administration are various (each dose 1 × 10^6–5 × 10^7 cells/kg). UCB and UC-MSC are performed mainly using IV, while BM-MNC is performed using IT. IV injection is easier compared to IT, and UC-MSC and UCB are distributed to the central nervous system without being trapped in the lung and blood-brain-barrier. UC-MSCs therapy for ASD is performed considering neurotrophic effects of UC-MSCs in addition to immunomodulation against chronic inflammation. Few serious adverse events were observed after transplantation. Autologous BM-MNC transplantation is required in order to avoid transplant rejection, but the procedure to take BM-MNC might be harmful and administration of BM-MNC intrathecal injection in ASD could be harmful. On the other hand, most reports suggest that UC-MSCs has a therapeutic potential with relative safety and allogeneic MSCs can be ordered as a most suitable critical timing.

**UCBs for individuals with ASD**

UCBs deliver a protective effect from impairment of microglia, chronic inflammation and oxidative stress, as well as enhance neurological regeneration. Human CD34 positive cells have been shown to secrete various growth factors such as brain derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), vascular endothelial growth factor (VEGF), and numerous angiogenic factors, including hepatocyte growth factor (HGF) and insulin-like growth factor-1 (Yoshihara et al., 2008; Majka et al., 2001). Considering that human CD34 cells have an effect on BDNF production and knowing that this neurotrophic factor is widely described as altered in ASD, this mechanism could be a potential therapeutic effect of UCBs” (Santos-Terra et al., 2021; Kern et al., 2013; Saghazadeh and Rezaei, 2017). Marchezan et al. (2018) reported that immunological and microglial changes are related to behavioral outcomes and that immunological and microglial changes in the TEA, as well as a behavioral improvement after managing these changes (Marchezan et al., 2018).

Considering the angiogenic and vascular reparative capabilities of endothelial progenitor cells, there is an altered expression of genes associated with blood brain barrier integrity coupled with increased neuroinflammation and possibly impaired gut barrier integrity in the ASD brain (Fiorentino et al., 2016). In 2004, Taguchi et al. (2004) reported that endothelial progenitor cells have angiogenic and vascular reparative capabilities that make them ideal for neurovascular repair (Taguchi et al., 2004). With a rich vascular environment, along with the generation of other nurturing neuronal mediators from CD34 positive cells, such as VEGF, epidermal growth factor 2, and insulin-like growth factor 1, endothelial progenitor cells enhance subsequent neuronal regeneration (Nakatomi et al., 2002). Some reports suggest that UCBs could suppress chronic inflammation in addition to paracrine and angiogenesis (Shahaduzzaman et al., 2013). Impairment of microglia has been shown to have profound effect on neural development, possibly resulting in ASD (Takano, 2015). Therefore, modulation of the phenotype of the microglia may be a novel therapeutic strategy for the treatment of neurological disorders accompanied by inflammation. Li et al. (2016)
reported that UCBs administration at 12 h after hypoxic-ischemic event reduces white matter injury by affecting activated microglia (Li et al., 2016).

In terms of glucose metabolism in ASD, Mitelman et al. (2018) reported that glucose metabolic rates were decreased in the parietal lobe, frontal premotor and eye-fields areas, and amygdala. Anil Kumar et al. (2017) reported that 4 out of the 10 patients with autism had abnormal PET scan findings, while none of the patients in the control group had abnormal PET scan. Findings of their study support the view of hypometabolism of glucose in subjects with ASD (Anil Kumar et al., 2017). Sharma et al. (2017) reported that [18F] 2-fluoro-2-deoxy-D-glucose PET scan was performed on 45 patients with ASD to study age-related developmental changes in the brain metabolism. Results showed that, in contrary to control data, the median standardized uptake values in patients with ASD decrease linearly with increase in age. As compared to controls, autism children below 5 years showed greater metabolism and older children showed lower metabolism. In ASD group, comparison of absolute standardized uptake values within different regions of the brain revealed relatively lower metabolism in amygdala, hippocampus, parahippocampal gyrus, caudate nucleus, cerebellum, mesial temporal lobe, thalamus, superior and middle temporal pole, and higher metabolic uptake in calcarine fissure and Heschl’s gyrus. These results help in understanding the baseline metabolism and developmental changes of brain metabolism among different age groups in ASD (Sharma et al., 2017). Zhao et al. (2020) reported that characterized changes on glucose metabolism.

### TABLE 1

Recent clinical review of cell therapy for Autism Spectrum Disorder

| Source             | Number Age (range) | Route of administration | Number of cells | Results                                                                 | Severe adverse events |
|--------------------|--------------------|-------------------------|-----------------|-------------------------------------------------------------------------|-----------------------|
| Sharma et al. (2012) | BM-MNC 1 8         | IT                      | 1 × 10⁶/kg      | Improvement                                                             | No                    |
| Nguyen Thanh et al. (2021) | BM-MNC 30 3–7    | IT                      | 1st: 4.2 × 10⁷/kg 2nd: 4.1 × 10⁷/kg | Significantly Improvement in the Childhood Autism Rating Scale (CARS), and the median Vineland Adaptive Behavior Scales | No                    |
| Lü et al. (2013)    | UCB/UC-MSC 37 3–12 | IV/IT + IV | UCB: 2 × 10⁶/kg UC-MSC: 1 × 10⁶/kg | Improvement in the Childhood Autism Rating Scale (CARS), Clinical Global Impression (CGI) scale and Aberrant Behavior Checklist (ABC) | No                    |
| Li et al. (2015)    | UCB 14 3–12        | IV/IT                   | 2–6 × 10⁶ cells | Improvement in the Childhood autism rating scale (CARS) and increase of NGF levels in the CSF | No                    |
| Dawson et al. (2017) | UCB 25 2.26–5.97  | IV                      | 1–5 × 10⁵ cells | Significant improvements in children’s behavior                           | No                    |
| Riordan et al. (2019) | UC-MSC 20 6–16    | IV                      | 3.6 × 10⁷ cells × 4 | The CARS and ATEC scores of eight subjects decreased                       | No                    |
| Sun et al. (2020)   | UC-MSC 12 4–9      | IV                      | 2 × 106/kg × 3 | 6/12 participants demonstrated improvement in at least two ASD-specific measures | 5 participants developed new class I anti-human leukocyte antigen (HLA) antibodies |
| Sharma et al. (2020) | BM-MNC 254 Under 5–over 15 | IT immediately after isolation | 1 time | Improvement of eye contact, attention and concentration, hyperactivity, sitting tolerance, social interaction, stereotypical behavior, aggressiveness, communication, speech, command following and self-stimulatory behavior | No                    |

Note: UCB = umbilical cord blood; IT = intrathecal; IV = intravenous.
metabolism, and also ASD-like behaviors in 1st and 2nd generations from 12- and 18-month-old male mice, respectively. Whole Genome Bisulfite Sequencing of sperm from advanced paternal age mice identified differentially methylated regions within the whole genome, and differentially methylated regions within promoter regions, suggesting that specific genes and relevant pathways might be associated with ASD and aberrant glucose metabolism in the offspring from advanced paternal age males. These results strongly suggest that epigenetic reprogramming induced by aging in male sperm may lead to high risks of aberrant glucose metabolism and the development of ASD behaviors in intergenerational and transgenerational offspring (Zhao et al., 2020). UCBs and peripheral blood mononuclear cells infusion therapy for patients with CP improved brain glucose metabolism by PET study (Min et al., 2013; Rah et al., 2017). Autologous bone marrow mononuclear cells therapies for patients with ASD also showed improvement of glucose metabolism and various cognitive and behavioral symptoms such as eye contact, attention and concentration, hyperactivity, sitting tolerance, social interaction, stereotypical behavior, aggressiveness, communication, speech, command following and self-stimulatory behavior as well as improvement of glucose metabolism and motor function in patients with CP (Sharma et al., 2015; Sharma et al., 2020). In 2020, Kikuchi-Taura et al. (2020) reported that angiogenesis is activated by bone marrow mononuclear cells via gap junction–mediated cell–cell interaction and that cell–cell interaction via gap junction is the prominent pathway for activation of angiogenesis at endothelial cells and improvement of glucose uptake. Transplanted BM-MNCs transferred small molecules to endothelial cells via gap junction followed by activated Hif-1a and suppressed autophagy at endothelial cells (Kikuchi-Taura et al., 2020). The mechanism of UCs to improve glucose metabolism might lead to therapeutic potential for individuals with ASD.

**MSCs for individuals with ASD**
Sanagi et al. (2019) reported that microglia in patients and animals with ASD symptoms could frequently be in the apoptotic phase with high turnover rates of microglia found in some pathological conditions (Sanagi et al., 2019). MSCs have been reported to secrete heterogeneous lipid bilayer vesicles called extracellular vesicles (EVs), which act as mediators for inter-cell communication. These exosomes/EVs secreted from MSCs are known to improve neuronal function in neurologically injured models (Fuloria et al., 2021). Perets et al. (2020) reported long term beneficial effect of neurotrophic factors-secreting MSCs transplantation in the autism mouse model (Perets et al., 2020). Hadar et al. (2016) reported that transplantation of MSC resulted in a reduction of stereotypical behaviors, a decrease in cognitive rigidity and an improvement in social behavior. Tissue analysis revealed elevated BDNF protein levels in the hippocampus accompanied by increased hippocampal neurogenesis in the MSC-transplanted mice compared with sham treated mice. This result might indicate a possible mechanism underpinning the behavioral improvement (Segal-Gavish et al., 2016). Perets et al. (2017) reported that exosomes derived from MSC would have a direct beneficial effect on the behavioral autistic-like phenotype of the genetically modified Shank3B KO mouse model of ASD. They indicated that intranasal treatment with exosomes derived from MSC improves the core ASD-like deficits of this mouse model of ASD and therefore has the potential to treat ASD patients carrying the Shank3 mutation (Perets et al., 2017). Our group also demonstrated the amelioration of neuronal injury followed by functional improvement in MSC-administered mice models, which resulted from the secretion of trophic factors such as BDNF and HGF rather than neuronal differentiation and eternal cell replacement by MSCs (Mukai et al., 2017; 2018). Human CD34 positive cells have been shown to secrete various growth factors such as BDNF, GDNF, VEGF, and numerous angiogenic factors, including HGF and insulin-like growth factor-1.

We also demonstrated that secretomes from MSCs can change the phenotype of activated microglia. Umbilical cord derived MSCs immunomodulated microglia and changed the phenotype of LPS-activated microglia restoring actin dynamics and phagocytosis by increasing active Rho GTPase, in which microglia changed their amoeboid to a more ramified pattern (Mukai et al., 2021). This suppression and immunomodulation of activated microglia by MSCs may lead to therapeutic potential for individuals with ASD in which excessive microglial activation lead to aberrant neural connections. On the other hand, human UC-MSC therapies for individuals with CP showed improvement of motor function and much increase of glucose metabolism by PET-CT scan (Gu et al., 2020). The mechanism of MSCs to improve glucose metabolism might lead to therapeutic potential for individuals with ASD.

**Future perspectives**
Immune modulation and microglial function repair may serve as novel therapies for individuals with ASD and other neurological diseases. Multiple mechanisms of paracrine, angiogenesis, immunomodulation, glucose metabolism and repair of microglial function by cell therapies might lead to clinical therapeutic potential for individuals with ASD. These cell therapies for patients with ASD have been often directed at children who cannot consent clearly. We should consider cell therapy clinical trials in patients with ASD, once the ethical and diagnostic barriers can be resolved.

**New strategy of ASD treatment**
The main cognitive and behavioral characteristics of ASD are impairment of communication, cognition, perception, motor skills, executive function, theory of mind and emotion control. And various behavioral therapies based on neurophysiological viewpoints have been considered the foremost strategies for management of individuals on the Autism spectrum. Genetic factors, chronic inflammation, impairment of the microglia, oxidative stress, hormones/neurotransmitters abnormality and environmental factors are likely contributing to the development of ASD. These strategies such as behavioral therapies, medications to hormones/neurotransmitters abnormality and health care to prevent environmental factors show no apparent effectiveness for ASD. Cell therapies in the viewpoints of immune
modulation and microglial function could be new strategies of ASD treatments.

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