Cell-based therapy proposals for the treatment of autism: Sertoli cells included in the ‘tool box’?

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Abstract
Introduction Autism is a neurodevelopmental condition characterised by severe abnormalities in communication, social awareness and skills, and the presence of restrictive as well as stereotyped patterns of behaviours. Its aetiology is not exactly known to date. Without any definitive cure and just a few effective biomedical interventions, cell-based therapy approaches seems to be gaining interest by researchers in the topic. In this critical review, we summarised the current proposals and speculated whether testis-derived Sertoli cells could be used as an additional tool for cell therapy in such a context, considering their immune-privileged characteristics, safety and efficacy already reported when transplanted into the mammalian brain.

Conclusion We believe that transplants of Sertoli cells, alone or in combination with other cell types, have potential to be a very useful tool (in addition to other cell sources), not only for acute and chronic neurodegenerative conditions, but also for neuropsychiatric disorders.

Discussion The ‘tool box’: cell-based therapy proposals for the context of ASD In this article, it would be extremely difficult, if not virtually impossible, to describe in detail the aberrant synaptic functioning and brain connectivity in ASD at the genetic, molecular, cellular, regional and system levels due to space limitations; but the literature is already rich in studies that well illustrate the neuropathological events that characterise ASD. Nevertheless, there are a number of characteristic features we would like to briefly highlight: (I) There are accumulating data suggesting that a common characteristic in autism cases may be oxidative stress; and such an event would represent the mechanism through which environmental factors may exert their deleterious effects (either prenatally, perinatally or postnatally), further exacerbated by the interaction of genetically susceptible alleles, and lead to the developmental abnormalities observed in the disorder. Furthermore, evidence of reactive oxygen species-mediated damage to mitochondrial DNA in children with autism has also been reported.

superior frontal cortex and cerebellum) that have previously been implicated in the pathogenesis of the disorder, suggesting widespread GABAergic dysfunction in the brains of these subjects. In contrast, significantly higher glutamate levels have been observed in the hippocampus, frontal regions and serum of autistic patients when compared to controls. Glutamate is the primary excitatory neurotransmitter produced in the central nervous system, and overactivity of glutamate and its receptors lead to excitotoxicity that can account for the neuronal dysfunction observed in these patients. Other studies have described neuropathological changes in brain tissues of autistic patients (especially, in the cerebellum) in the form of extensive neuroglial responses characterised by both microglial and astroglial activation. Moreover, the results suggest that ASD subjects carrying more microglial activation correlates with higher impairment in their cognitive skills. Activated microglia and astrocytes in postmortem brain tissue represent evidence for neuroinflammation. As a matter of fact, a number of studies have shown that the levels of glial fibrillary acidic protein are increased in autism. For instance, Purkinje cell loss in ASD was sometimes found to be accompanied by gliosis and increased expression of glial fibrillary acidic protein. Cao et al. reported astrocytosis in the frontal cortex with decrease in Wnt and β-catenin proteins in autistic subjects. In that study, branching processes, total branching length and cell body sizes of astrocytes from autistic patients were significantly reduced. Additionally, the neuroinflammatory process is characterised by the increase of transforming growth factor-β1 (TGF-β1), interleukin-6 (IL6) and IL10 in the brain of patients together with higher levels of inflammatory cytokines, such as tumour necrosis factor-α, interferon-γ (IFNy), IL1, IL6, IL8 and IL12 in blood mononuclear cells, serum, plasma and cerebrospinal fluid of autistic subjects, as well as higher plasma heat shock protein-70, TGF-β2, caspase 7 and IFNγ when compared to age- and gender-matched controls. Dendritic spines are the major sites of information processing in the brain, and as for other cognitive disorders, aberrant spine morphology seems to be a characteristic feature in autism. Abnormalities in dendritic spines have additionally been observed in brain specimens from epilepsy patients and animal models of epilepsy. Interestingly enough, a significant overlap between epilepsy and ASD has been detected but it is not clear how often ASD may impact epilepsy and the effect of epilepsy on social cognition. Autism has been considered as a paediatric autoimmune neuropsychiatric disorder since some studies have found serum antineuronal antibodies (e.g. serum levels of antiganglioside M1) in a subgroup of autistic children, which significantly correlated with the severity of the disorder. A number of studies have reported cerebral hypoperfusion in autistic patients, correlated with certain symptomatology such as repetitive behaviours, desire for sameness, impairments in processing facial expressions and emotions as well as decreased language development.

To the best of our knowledge, one can basically find four elegant proposals in the form of ‘review article’ and ‘letter to the editor’ that suggest the potential use of cellular transplantation for the treatment of ASD (Table 1). Ghanizadeh proposed two different (but complementary) alternatives for such an approach, based on the use of a GABAergic cell line or c-Kit+ cells with the aim of compensating the existing imbalance in the ratio of GABA to glutamate in autistic brains. Unfortunately, no preclinical trials on animal models of autism have been performed to date, in order to check the plausibility of these promising hypotheses. The other two proposals presented MSCs as the most adequate candidate cell source for cell therapy in these patients due to their peculiar characteristics. MSCs are multipotent adult stem cells, able to differentiate into cells not only of mesodermal origin (e.g. adipocytes, chondrocytes and osteocytes) but also of representative lineages of the three embryonic layers. These stem cells can be isolated from several and perhaps most postnatal organs and tissues such as umbilical cord, adipose

### Table 1: Current proposals for the use of cell-based therapy in the context of autism

<table>
<thead>
<tr>
<th>Cell source</th>
<th>Aim</th>
<th>Year</th>
<th>PMID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cord blood expanded CD34+ cells together with MSCs</td>
<td>To compensate immune abnormalities and neural hypoperfusion</td>
<td>2007</td>
<td>17597540</td>
</tr>
<tr>
<td>GABAergic cell line</td>
<td>To compensate the GABA inhibitory neurotransmitter deficiency</td>
<td>2010</td>
<td>20934920</td>
</tr>
<tr>
<td>c-Kit+ cells</td>
<td>To prevent excitotoxicity by targeting the hyperglutamatergic state with the increase of GLT-1 transporter levels</td>
<td>2011</td>
<td>21225454</td>
</tr>
<tr>
<td>MSCs</td>
<td>To inhibit immune alterations To rescue the loss of Purkinje cells, cortical and synaptic plasticity To restore brain-damaged functions</td>
<td>2012</td>
<td>22496609</td>
</tr>
</tbody>
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tissue, placenta, amnion, dental pulp, cord blood and especially, bone marrow that seems to be the most often utilised source. MSCs have become very popular among other cell sources belonging to what we could call ‘the tool box’ of cell-based therapy, probably due to their ability to modulate the immune response and thus, to exert immunosuppressive effects and their well-described potential to differentiate into neurons, hepatocytes, myocytes, chondrocytes, osteocytes and adipocytes. In contrast to embryonic stem cells, which are obtained from the inner cell mass of the blastocyst, are associated with tumourigenesis, and their use involves ethical and legal considerations; the use of MSCs seems to be less problematic with regard to these issues. Perhaps, the main reason why MSCs were suggested as a candidate source for cell therapy in these subjects was their strong immunosuppressive activity and the capability to inhibit the release of proinflammatory cytokines that makes them a powerful tool for autologous and heterologous transplantations. Ichim et al. additionally proposed the combined use of MSCs together with CD34+ cells with a potential to produce angiogenic factors and to give rise to endothelial cells themselves, for the treatment of hypoperfusion defect that has been associated with autism. MSCs are not prone to tumour formation and have a decade record of biosafety data in vivo making these cells an exciting therapeutic option for the future. Nevertheless, as for Ghanizadeh’s proposals, it still remains to be elucidated the real potential of these cells when transplanted in different in vivo models of autism, and the tissue source from where these MSCs should be isolated for such a purpose, since it has been shown that many genes were differentially expressed in MSCs from different ontogenetic sources or from different culture conditions and they even possess different proliferative and differentiation potential.

The potential therapeutic use of SCs in ASD: why not?

It is generally accepted that the use of stem cells could potentially provide benefits when transplanted into a patient by integrating, differentiating and restoring functional and behavioural deficits in the central nervous system. On the other hand, major concerns still reside in avoiding tumourigenesis and graft rejection due to cellular transplantation. Since the use of pharmacological immunosuppression has been associated with toxicity issues in both human and animal models, the search and election of safe immunoprivileged cell sources, like MSCs, appears to be mandatory for ensuring long-term viability of future clinical trials in humans.

Testis is an example of immune privileged site, and such a characteristic is critical for preventing a detrimental immune response against the autoimmunogenic germ cells. It is also critical for the tolerance of neo-antigens from developing germ cells that appear after the constitution of self-tolerance, but imposes the paradoxical challenge of providing sufficient and enough protection against pathogens and tumourigenic cells. Somatic SCs are considered the main structural component of the seminiferous tubule, they give rise to the blood–testis barrier and their functions are essential for the generation of spermatozoa since these cells produce growth factors that stimulate self-renewal (GDNF and FGF2) and differentiation (activin A, BMP4 and SCF) of spermatogonial stem cells. They also secrete TGF-β1, which is a potent immunosuppressive factor that suppresses the secretion of IFN-γ and tumour necrosis factor-α by immune cells. These characteristics could actually confer local immunoprotection when transplanted alone or in combination with other cells. For instance, preclinical trials with positive results have been performed in animal models of Parkinson’s disease, Huntington’s disease and amyotrophic lateral sclerosis. Furthermore, different research groups are developing novel protocols to genetically engineer SCs for providing continuous delivery of therapeutic proteins of interest. Then, considering the autoimmune characteristics of autism with evidence of neuroinflammation, oxidative stress and both increased microglial and astroglial activation, could SCs represent an interesting candidate cell source to be tested for transplantations in the brain of animal models of autism? Already, one can find different successful studies in the literature that inspire optimism. For instance: (I) It has been demonstrated that transplanted testis-derived SCs into adult tracts survive, produce localised immunoprotective and suppress microglial response when co-transplanted with bovine adrenal chromaffin cells (xenograft), and without the administration of systemic immunosuppressive drugs. (II) In addition, it has been shown that intravenous infusion with SCs in islet transplantation induced systemic immune tolerance, reduced the peripheral blood lymphocyte and cytokine levels and effectively prolonged the survival of islet grafts. (III) Besides, the survival and immunoprotective capability of SCs isolated from neonatal pigs have also been tested after transplantation in humans, in a type 1 diabetes mellitus patient with an islet xenotransplant. This study showed evidence that co-xenotransplantation of these cells into a subcutaneous autologous collagen pouch could be considered a safe alternative for the control of type 1 diabetes.

Conclusion

We believe that transplants of SCs, alone or in combination with other cell types, have the potential to be a very useful tool (in addition to other cell sources), not only for acute and...
chronic neurodegenerative conditions, but also for neuropsychiatric disorders. In general, a major challenge for developing cell therapies is the use of appropriate animal models for testing both safety and efficacy of the approach and to collect the most complete preclinical data. In the context of autism, we face the same obstacle because the available in vivo models are of several, quite different, kinds and sometimes parallels with autism are uncertain. Optimal approaches to select animal models for either neurodegenerative or neuropsychiatric conditions require a deep understanding of the nature of the disease/disorder. We need a better understanding of the pathophysiologic mechanisms underlying autism and its symptoms. In other words, we have a putative tool, but where do we test it?

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Critical review

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