



Review Article

The emergence of amnion epithelial stem cells for the treatment of Multiple Sclerosis

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Multiple Sclerosis (MS) is an autoimmune inflammatory disease of the central nervous system (CNS). Whilst a number of immune modulatory agents are available to treat the disease, their efficacy is relatively low, but more problematically, is the fact that they are associated with significant side effects. Most compellingly is that whilst these drugs slow the disease progression, they are not curative. Therefore there is an urgent need for the development of new therapies that have fewer side effects and treat a larger proportion of patients. One such potential therapy is the use of stem cells. A number of different stem cell types have been shown to be efficacious in murine models of MS, thus paving the way for their potential application to the clinic. A novel stem cell source that is gaining attention is amnion epithelial cells (AECs). These cells have been shown to engraft, suppress immune responses, migrate to inflamed sites within the CNS and differentiate towards neural lineages. Given their immune suppressive and neuroregenerative potential, AECs are therefore attractive vehicles for the therapy of MS and other inflammatory and neurodegenerative disorders that effect the CNS.

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1. Introduction

Multiple Sclerosis (MS) affects 2.5 million people worldwide and is characterized as an inflammatory disease of the central nervous system (CNS)¹⁾. The cardinal pathological features of the disease are demarcated plaques, which are composed of infiltrating immune cells, localised myelin destruction, loss of oligodendrocytes and axonal degeneration²⁻⁴⁾. The disease most commonly occurs in young adults aged 20-40 years old and affects women more commonly than men at a ratio of approximately 2:1. Symptoms of MS are often variable, but the most prominent features include weakness, loss of function of one or more limbs, blurred vision, ataxia, bladder dysfunction, fatigue, memory loss and paralysis¹⁾. MS is a multi-factorial disease of unknown cause⁴⁾. However, it is now apparent that genetic background and exposure to certain environmental cues increases the chance of developing MS^{2,4,5)}. Data from mouse models of MS, such as experimental autoimmune encephalomyelitis (EAE) also suggest that the disease is initiated by an unrelenting autoimmune attack directed against CNS autoantigens, including myelin proteins such as myelin basic protein (MBP), proteolipid protein (PLP), myelin oligodendrocyte glycoprotein (MOG), oligodendrocyte-specific protein (OSP) and possibly $\alpha\beta$ crystallin³⁻⁷⁾.

1.1 Multiple Sclerosis- clinical categories

MS can be divided into 4 clinical categories, which include, relapsing-remitting (RR), secondary progressive (SP), primary progressive (PP) and progressive relapsing (PR). RR-MS is the most common form of MS occurring in ~80% of patients and is characterized by sudden attacks of neurological dysfunction, followed by periods of remission, sometimes with full recovery²⁾. Approximately 50% of RR-MS patients go on to develop SP-MS after their initial course of RR-MS. The SP-MS phase is characterized by a steady increase in the level of disability, with little or no recovery periods⁸⁾. A decrease in inflammatory and demyelinating processes with an increase in neurodegeneration has been suggested as a possible cause for the transition from RR-MS to SP-MS. However the opinions on this issue remains divided. PP-MS is the most severe form of MS and affects approximately 10% of all MS patients and is characterized by continual neurological deterioration from the onset of disease with no obvious remitting

periods⁹⁾. Finally, PR-MS occurs in nearly 5% of cases, exhibits disease progression from onset, but involves clear acute relapses, with or without recovery from these relapses⁹⁾.

1.2 Current MS therapies

Whilst both genetic and environmental factors contribute to the development of MS^{2,4,5)}, initiators of the disease have yet to be clearly defined. While much effort is being invested in trying to understand and treat this disease, there is at present no cure for MS. Nonetheless, most of the current drugs that are used in the clinic, such as Betaferon, Copaxone, Ty-sabri and Fingolimod (FTY720) act in an immunomodulatory or immunosuppressive manner¹⁰⁻¹²⁾. While these treatments are beneficial in approximately 30% of RR-MS patients, they have little impact on disease progression and have limited efficacy in patients with progressive disease. For this reason there is a clear need for improved therapies that are aimed at providing neuroprotection and preventing the progression of disease to chronic disability. To that end, future treatment strategies will need to involve a combination of both immunomodulatory and neuroprotective regimes. Current clinical practice at best, only targets the inflammatory components of the disease.

1.3 Stem cells

Broadly speaking, two classes of stem cells have been defined; embryonic and adult. Embryonic stem cells (ESCs) are able to generate cells from all three germ layers and can theoretically be maintained in culture indefinitely, providing a limitless source of precursor cells for the regeneration of damaged tissue. Adult stem cells can differentiate, maintain and repair the tissue in which they were isolated from. For example, adult bone marrow mesenchymal stromal cells (MSCs) can differentiate into mesodermal lineages producing osteocytes and adipocytes¹³⁾. Because such MSCs are also highly immunosuppressive¹⁴⁾ they are, in principle, ideal for targeting the inflammatory process in MS as well as potentially promoting and/or enhancing spontaneous remyelination⁵⁾. As such, small phase 1-2 clinical trials are now underway in the UK, and shortly the US, to assess the safety and efficacy of MSCs in treating MS¹⁵⁾. However, both sources of stem cells have their own inherent usage limitations, such as spontaneous differentiation or ethical issue as manifest by ESCs, whilst a dearth in



the number of adult stem cells restricts their use for autologous transplantation in adults.

A novel alternate source of stem cells that is gaining interest among the stem cell research community are amnion epithelial cells (AECs). These cells are obtained from discarded term placenta and have been reported to possess properties similar to both ESCs and MSCs. Given that there are approximately 300,000 births a year in Australia alone, amnions provide an abundant source of regenerative cellular material and do not possess ethical constraints associated with ESCs. Akin to ESCs, AECs are pluripotent and have the ability to be expanded in culture. Importantly and in contrast to ESCs, and MSCs isolated from other tissues, AECs do not form teratomas *in vivo*. Significantly, AECs display strong immunomodulatory–immunosuppressive properties and thus offer significant practical advantages for potential clinical applications¹⁶.

1.4 The use of stem cells in EAE

In EAE, the commonly used animal model to study MS, both human and murine-derived MSCs, as well as neural precursor cells (NPCs), ameliorate the clinical signs of disease as well as reducing its pathology^{5,10,13,14,16–19}. While the mechanism by which MSCs produce their effect is not clearly established, it is worth mentioning that when injected intraventricularly into EAE mice, such cells are attracted to areas of CNS inflammation¹⁹. When analysed *in situ*, these cells can upregulate neural markers such as galactocerebroside (GalC), oligodendrocyte marker O4, glial fibrillary acidic protein (GFAP) and beta-tubulin III, suggesting transdifferentiation towards neural lineages and thus implying that MSCs may facilitate in the regeneration process. However, it should be noted that whilst cell replacement represents a potential mechanism by which MSCs act, it is not excluded that this process also involved cell fusion events, rather than cell replacement. Indeed extensive cell fusion of hematopoietic stem cells, including MSCs, with purkinje neurons, has been shown to occur under pathological situations, such as chronic inflammation^{20,21}. Beside their proposed neuronal regeneration properties, MSCs injected intravenously exhibited systemic immunomodulatory effects including the ability to decrease T cell responsiveness against myelin antigens, decrease CNS inflammation with improved axonal integrity and reduce EAE severity^{19,22}. The mechanism by which MSCs produced their immunomodulatory effect

within the CNS remains to be fully determined but this could occur via upregulation of $\alpha 4$ integrins, allowing MSCs to adhere to the inflamed brain endothelium²². Injection of MSCs is also reported to enhance the recruitment of endogenous oligodendrocyte precursor cells to the demyelinated area and thereby help the process of regeneration²³. Whether or not this includes direct cellular contact or the release of trophic factors remains to be elucidated.

In this context, it is noteworthy that like MSCs, NPCs are also capable of influencing the development of EAE. As such, NPCs injected intraventricularly shortly after EAE induction, resulted in NPCs migrating to the white matter, where they are claimed to aid in decreasing inflammation and injury, and reduce EAE clinical severity^{24,25}. Interestingly, NPCs injected intravenously into mice with EAE, migrate to inflamed and demyelinated areas within the CNS²⁶. These cells appear to differentiate into mature brain cells as visualized by the expression of the neuronal marker NeuN and the oligodendrocyte precursor marker, PDGF α . It is suggested that this leads to active remyelination and the regulation of reactive astrogliosis, a process known to hamper axonal remyelination²⁶.

Collectively these results suggest that MSCs and NPCs could offer a means of modulating inflammatory CNS autoreactive T cells, as well as promoting tissue repair within the target tissue. Given the functional similarities that NPCs and MSCs have with AECs, these cells should be considered as a possible alternative for the treatment of inflammatory and neurodegenerative conditions such as MS.

2. Development, Structure and Function of the Amnion

Developmentally, the amnion arises from the ectoderm²⁷, forms the innermost layer of the fetal membranes and is bathed by amniotic fluid²⁸. The amniotic membrane consists of an inner layer of epithelial cells that is in direct contact with the amniotic fluid, referred to as the amniotic epithelium. These epithelial cells can secrete glycoproteins, collagens and laminins²⁹ that constitute the underlying basement membrane. Directly beneath the epithelial layer is the amniotic mesoderm, which includes a compact stromal layer and fibroblast layer³⁰ (Figure 1). Two cell types of different embryological origin are present in the amniotic membrane: AECs derived from embryonic ectoderm and amnion mesenchymal cells derived from embryonic mesoderm^{31,32}. A ba-

sis function of the amniotic membrane is to provide the developing embryo protection from external assault. This offers a supportive environment, in which the embryo can grow without distortion by pressures

from surrounding organs¹⁶⁾. Another function of the amnion is to provide the fetus protection against infections and toxins³³⁾.

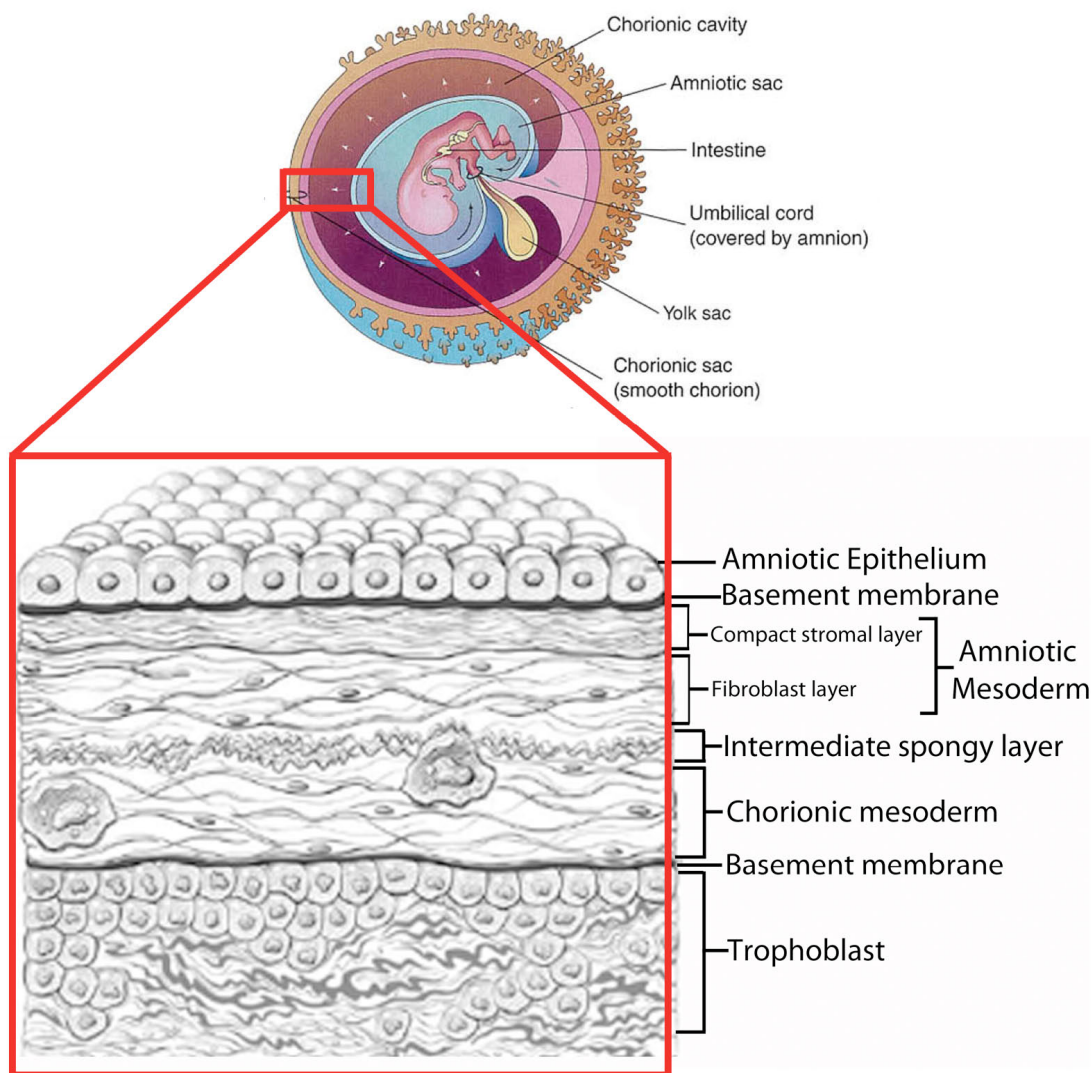


Figure 1. Structure of the amnion.

The amniotic membrane is the innermost membrane that surrounds the fetus, this is shown in the top figure in blue. This membrane provides structural support from external insult and is made up of two distinct layers, the amniotic epithelium and the amniotic mesoderm, separated by a basement membrane. The amniotic mesoderm is made up of two layers referred to as the compact stromal layer and fibroblast layer (shown in the bottom figure). Directly underlying the amniotic membrane is the chorion (chorionic mesoderm) and the trophoblast layer. Two stem-like cells are located in the amniotic membrane; amniotic epithelial cells are found in the amniotic epithelium and amniotic mesenchymal cells are located in the amniotic mesoderm. Adapted from Ilancheran⁹⁸⁾. With permission from Elsevier.

An often-held misconception surrounding AECs is that they are thought to be derived from extra-embryonic tissue. Differentiation of AECs from the epiblast occurs at day 8, a point where cells are pluripotent and will develop into all the tissues of the

body. This time point is also well before gastrulation, when cell fate is determined. Upon differentiation from the epiblast, AECs themselves are formed from amnioblasts, which are directly adjacent to the developing cytotrophoblasts³⁴⁾, (Figure 2).

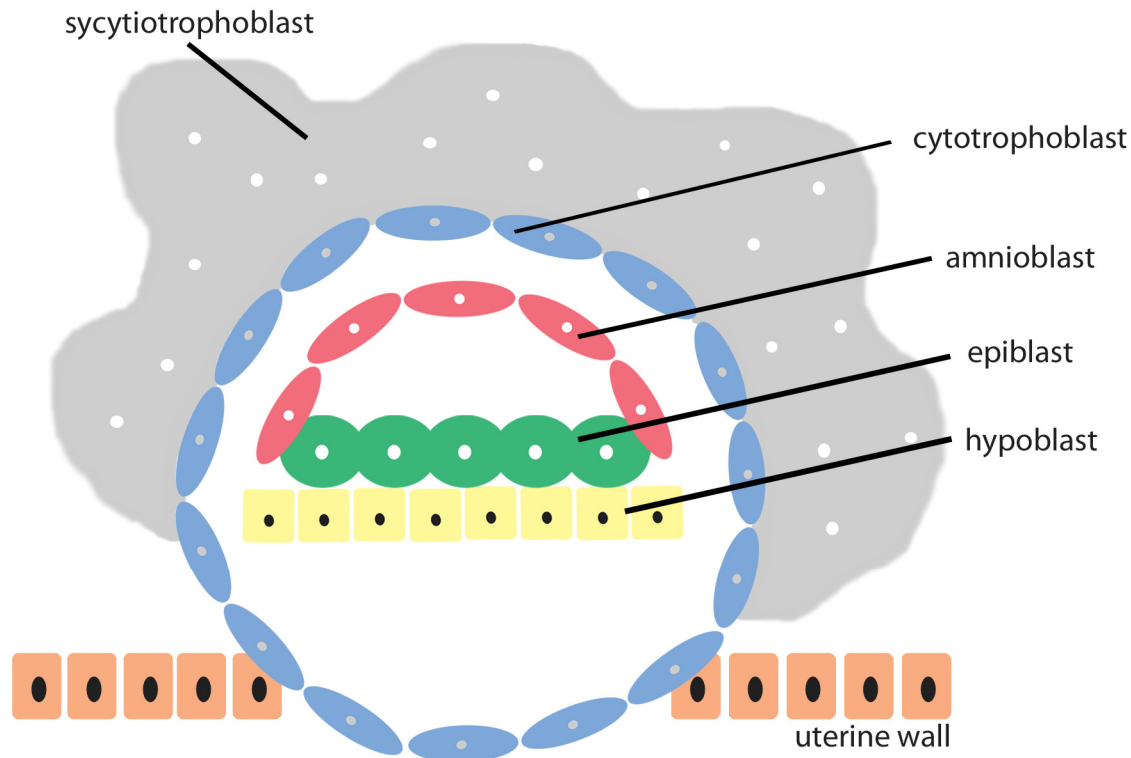


Figure 2. Schematic representation of the development of the amnion.

At day 8 during embryo formation, the inner cell mass differentiates into the epiblast and hypoblast. Directly adjacent to the cytotrophoblasts, the amnioblasts differentiate from the epiblast. The amnioblasts will later form the amniotic epithelial layer. Adapted from Ilancheran⁹⁸. With permission from Elsevier.

3. Multipotentiality of AECs

AECs are readily obtained from the amniotic membrane by mechanical and enzyme dissociation, with each amnion yielding approximately 100 million viable cells³⁵. Once harvested, these cells can easily be cultured under either serum or serum-free conditions³⁶. A well-characterised feature of all stem cells is their ability to differentiate into various cell types within the body. Due to AECs originating from the epiblast and separating early in embryonic development, they possess a high level of pluripotency. As such, several embryonic stem cell markers have been identified in AECs including OCT-4, nanog, SSEA-3, SSEA-4, TRA 1-60 and c-kit^{16,37-39}, (Table

1). They also express markers common to MSCs, including CD90, CD44, CD105 and CD166^{13,40,41}, thus suggesting AECs may share similar functional properties with MSCs. Studies have shown that AECs can differentiate into all three germ layers including endoderm, ectoderm and mesoderm³⁹. Notably, they can generate clinically relevant cell types such as cardiomyocytes, myocytes, osteocytes, adipocytes, pancreatic cells, hepatocytes, as well as neural and astrocytic cells^{16,38,42}. A considerable advantage that AECs have over MSCs is that they are unable to form teratomas after being transplanted into SCID mice but are nonetheless highly clonogenic and can be maintained in culture over numerous passages^{38,39}.

**Table 1. Phenotypic characteristics of amnion epithelial cells**

Embryonic Markers	MSC markers	Neuronal Markers	Immune Markers
SSEA-1, SSEA-3, SSEA-4, TRA 1-60, TRA 1-81, c-kit, STRO-1, OCT-4, GATA-4, nanog, SOX-2, PAX-6, GCTM-2	CD90, CD44, CD73, CD166, CD105, CD29	Nestin, musashi, RC-1, vimentin, A2B5, MAP-2, GFAP, CNPase, MBP, β 3-tubulin	MHC class 1, HLA-G

4. Immunological properties of AECs

Some controversy exists regarding the immune privileged status of AECs. A few studies have found that whole amniotic epithelium does not express human leukocyte antigens (HLA)-A, B or C on the cell surface, suggesting that acute rejection would not occur after cell transplantation^{38,43,44}. However other reports have demonstrated widespread expression of HLA class I⁴⁵⁻⁴⁷. Despite the discrepancies over HLA class I expression, it is clear that AECs do not express HLA class II antigens, nor the co-stimulatory factors CD80 and CD86⁴⁸, (Table 1). Moreover, the lack of expression of HLA class II after IFN- γ treatment⁴⁸ suggests that AECs may be cloaked from an activated immune system, given that activation of the host CD4⁺ T-helper cells should, in theory, be limited. Significantly, AECs express HLA-G⁴⁹, a non-classical HLA class I antigen. The presence of soluble HLA-G in embryos is associated with increased pregnancy rates and is used as a non-invasive marker for embryo selection in assisted reproduction⁵⁰. One possible role of HLA-G is to provide protection for the fetus, from rejection by the maternal immune system⁵¹. Therefore, it has been hypothesised that in an allo-transplant setting, cells expressing HLA-G may be able to evade immune surveillance. Although, the mechanism behind this phenomenon is not fully understood, HLA-G has been shown to induce apoptosis of activated CD8⁺ cells and inhibit CD4⁺ cell proliferation^{48,52} (Figure 3 (i)). Cell surface expression of HLA-G is maintained on AECs, but gradually decreases in primary cultures⁵³. Interestingly, in the presence of IFN γ , HLA-G cell surface expression

is restored^{48,53}. In the context of using these cells for clinical application, these results suggest that within an inflammatory environment, AECs could evade an activated immune system, thus resulting in their increased persistence within the host environment. Among the alternative mechanisms by which AECs can evade immune detection is by secreting immunomodulatory factors. Indeed, supernatant from a hAEC culture has the ability to inhibit cells of the innate and adaptive immune system, as shown by the reduction of both T and B cell proliferation, via apoptosis²⁸. Alpha-fetoprotein (AFP), for example, is a immunomodulatory protein produced by AECs and MSCs⁵⁴ which has been shown to reduce lymphocyte reactivity and suppress the extent of neuroinflammation in mice with EAE by modulating immune cell apoptosis^{55,56}. Additional *in vitro* studies have demonstrated that AECs exhibit a dose-dependant inhibition of peripheral blood mononuclear cell (PBMC) immune responses in mixed lymphocyte reactions as well as T cell cultures stimulated with the cell mitogen, phytohemagglutinin (PHA)⁵⁷. One possible mechanism involved in this suppression could be due to AECs acting on responder CD4⁺ T cells, by reducing their proliferation and consequently decreasing the amount of Th1 and Th2 cytokines⁵⁸. In addition, AECs secrete factors that inhibit migration of neutrophils and macrophages *in vitro*. One such factor is macrophage inhibitory factor (MIF), a potent inhibitor of macrophage activity and NK-cell mediated cytotoxicity²⁸. Other important factors that may contribute further to AECs ability to suppress immune responses include Fas ligand, TRAIL, and transforming growth factor-beta (TGF- β)²⁸ (Figure 3 (ii)).

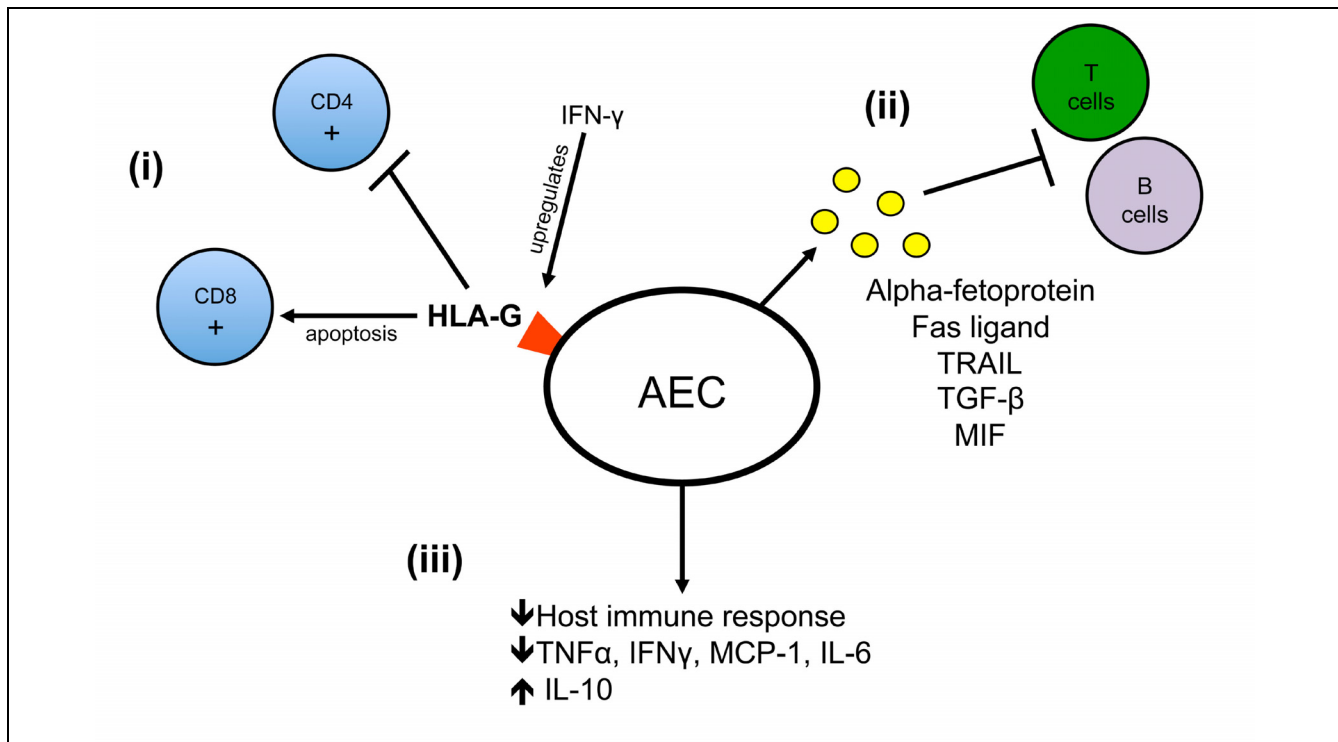


Figure 3. Immunosuppressive/immunomodulatory properties of AECs.

(i) AECs express HLA-G, which is known to suppress the proliferation of CD4⁺ T-cells and induce apoptosis of CD8⁺ T-cells. HLA-G is also upregulated by IFN γ . (ii) AECs secrete many factors that are able to suppress T and B cells, including AFP, Fas ligand, TRAIL, TGF- β and MIF. (iii) AECs have been shown to modulate host immune systems *in vivo*, by decreasing the production of TNF α , IFN γ , MCP-1 and IL-6 and increasing the production of the anti-inflammatory cytokine IL-10.

In view of the aforementioned properties, AECs have been tested in a number of experimental systems to assess their immunomodulatory properties⁴⁷⁾. Upon transplantation to the ocular surface, AECs can create a local environment that modulates the surrounding inflammatory responses⁴³⁾. This effect is likely to involve the suppression of MHC class II antigen-presenting cells entry in the inflamed cornea, since the amnion grafts were accepted with little cell infiltration of CD4⁺ and CD8⁺ T cells. In an inflammatory lung injury model, where damage is induced by administration of bleomycin, a potent stimulator of lung fibrosis, AECs modulated the host inflammatory response, reduced lung fibrosis and prevented loss of lung function^{44,59)}. This effect is concomitant with a reduction in pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF α), IFN γ , monocyte chemotactic protein-1 (MCP-1) and interleukin 6 (IL-6), resulting in a decrease in inflammatory cell infiltration⁵⁹⁾ and an increase in the anti-inflammatory cytokine, interleukin 10 (IL-10)⁴⁴⁾ (Figure 3 (iii)). Interestingly, in immune-competent mice following bleomycin treatment, AECs were not found

to engraft, yet were still able to mediate their immunosuppressive effect⁵⁹⁾.

In view of the paucity of data pertaining to the effect of AECs on the immune response in autoimmune-mediated demyelination, we have begun to investigate the ability of AECs to modulate both the TCR-dependant and-independent proliferation of T cells, as well as their effect on EAE. *In vitro* studies have shown that AECs can significantly reduce the proliferation of MOG specific T cells (Figure 4B) from transgenic 2D2 mice⁶⁰⁾. AECs can also significantly decrease the secretion of pro-inflammatory cytokines IFN γ (Figure 4C) and TNF α (Figure 4D), from MOG specific T cells. Preliminary studies from our laboratory show that intraperitoneal injection of 1 million AECs on day 8, can significantly reduce clinical symptoms (Figure 4A) and decrease CNS inflammation, demyelination and axonal degeneration, in stained sections of the spinal cord and brain (data not shown). Taken together, these results suggest a possible immunomodulatory mechanism by which AECs can suppress the development of EAE.

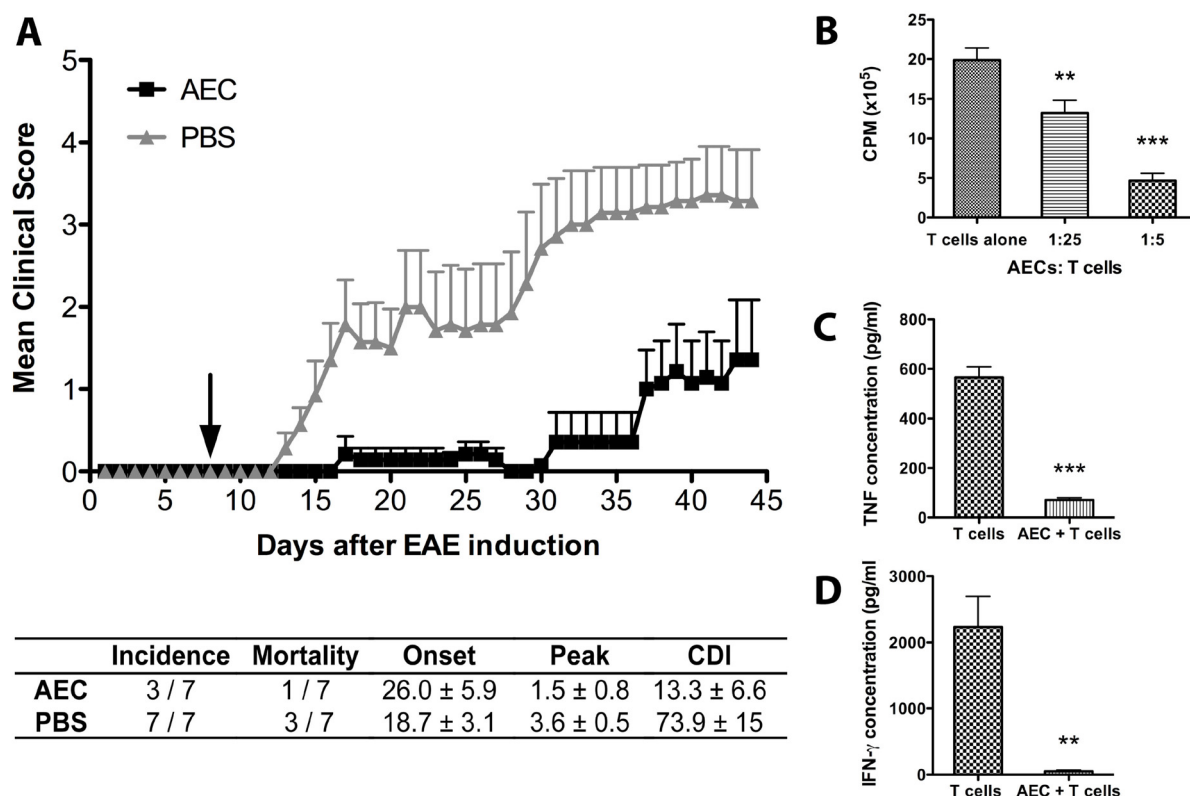


Figure 4. IP injection of AECs suppresses the development of RR-EAE.

(A) EAE was induced in female NOD/It mice with 65 μ g rMOG emulsified in CFA and 350ng pertusis toxin was injected ip on day 0 and 2. Mice received either 1 million AECs or PBS alone in the peritoneum, on day 8 (indicated by the arrow). Mice were monitored daily for their signs of disease. Mice were scored according to the following scheme: 0- no disease, 1- limp tail, 2- weakness in hind limbs, 3- paralysis of hind limbs, 4- paralysis of hind limbs and fore limbs and 5- moribund. Injection of AECs at day 8 delays significantly reduces severity of EAE. (n=7, results shown as mean \pm sem, *P<0.05). (B) MOG specific T cells, from transgenic 2D2 mice, were stimulated with 20 μ g MOG35-55 either alone or in the presence of AECs at two different ratios 1:5 and 1:25. At both ratios AECs are able to significantly suppress T cell proliferation. (n=6, performed in triplicate, results shown as mean \pm sem, **P<0.01, ***P<0.0001). AECs were co-cultured with MOG specific T cells at 1:5 ratio and culture supernatant was collected at 72 hours and analysed for the presence of IFN- γ (C) and TNF- α (D). AECs significantly decreased the secretion of both proinflammatory cytokines *in vitro*. (n=3, results shown as mean \pm sem, **P<0.01, ***P<0.0001)

5. Trafficking to the CNS

A critical aspect for the clinical utility of any stem cell population is their ability to home to target lesioned areas. Whilst very little is known regarding the mechanisms of AEC migration in a transplant setting, several studies have clearly defined molecules which direct the migration of adult stem cells within injured sites. For example, MSCs have been shown to secrete a host of different chemokines, cytokines, growth factors and express various chemokine receptors under resting conditions⁶¹. However in the

presence of proinflammatory cytokines, they can upregulate the expression of chemokine receptors, such as CXCR4⁶¹, which has been found to play a role in the homing of cells to areas of ischemic brain injury⁶². Notably, CXCR4 has also been implicated to play a role in lymphocyte and monocyte trafficking into the CNS in EAE⁶³. More recently, it has been suggested that CXCR7 is important for the trafficking of interneurons within the CNS. This chemokine receptor is also involved in lymphocyte trafficking across the blood brain barrier⁶⁴. Interestingly, both, CXCR4 and CXCR7, have been shown to be



expressed on AECs (McDonald et al, unpublished). Given their functional similarities with MSCs, it is plausible that these receptors have similar attributes to AECs in the context of their ability to traffic to the CNS.

6. Ability of AECs to engraft and differentiate *in vivo*

The efficacy of AECs in various disease models, highlights their ability to engraft within host tissue and differentiate into specific lineages (Table 2). In a liver disease model, induced by administration of carbon tetrachloride (CCl₄), transplantation of AECs led to hepatic engraftment, which resulted in a reduction in hepatocyte apoptosis and a decrease in inflammation and fibrosis³⁵). Moreover intravenous administration of AECs in a lung injury model in severe combined immunodeficiency (SCID) mice, led to their short-term engraftment and production of surfactant proteins A, B, C and D⁴⁴). Along the same line, direct transplantation of AECs into the spleen of diabetic SCID mice, led to the integration of AECs in the host splenic parachyma. Notably, one month post transplantation, AECs were found in in-

sulin-producing anatomical sites such as the liver and pancreas and were shown to produce human-specific insulin⁶⁵). AECs have also been investigated in stroke and spinal cord injury (SCI) models. Results from these studies have shown that transplanted AECs are able to potentially migrate to the CNS and possibly differentiate towards neural lineages as indicated by the expression of markers such as nestin and MAP2. Some investigations have reported that transplanted AECs can survive in the host for up to 60 days, suggesting that these cells can escape initial immunological rejection within the CNS⁶⁶). However, further long-term studies are required to validate these early findings. Finally, intraperitoneal injection of AECs into neonatal swine and rats, resulted in microchimerism in many organs including the lung, bone marrow, spleen, kidney, liver and most importantly the brain. In the context of treating CNS-related disorders with AECs, these data support the notion that such cells are able to home to the CNS, cross the blood brain barrier where they can engraft, reduce inflammation and regenerate damaged tissues⁶⁷).

Table 2. Summary of transplantation studies investigating the therapeutic efficacy of AECs in various disease models

Disease	Outcome	References
Diabetes	AECs differentiated into β -cells and normalised blood glucose levels	65)
Liver Disease	AECs engrafted within the liver and reduced hepatocyte apoptosis and hepatic inflammation.	35)
Occular surface disease	AECs successfully treated persistent trophic ulcers of the cornea and improved vision.	90)
Parkinson's disease	AECs decreased rotational asymmetry and grafts expressed nestin and vimentin	72)
Respiratory disease	AECs modulated host inflammatory response, reduced fibrosis and improved lung function	44,59)
Spinal cord injury	AECs promoted regeneration and sprouting of host axons and prevented glial scar formation	66,77,96)
Stroke	AECs migrated to ischemic area, reduced brain oedema and improved motor deficits	73,74)



7. Potential of AECs to regenerate cells from neural lineages and aid in host repair

7.1 Neuronal differentiation

While the inflammatory processes are important in the pathology of MS, the disease is also characterised by significant neurodegeneration associated with the death of oligodendrocytes and axonal damage and loss. Accordingly, any successful MS treatment not only needs to incorporate strategies to dampen inflammation but also regenerate and/or replace the damaged tissue. AECs offer a potential solution to this problem, in that they can differentiate into various neuronal cell types. Notably, AECs express a plethora of markers present on both immature and mature neural and glial cells such as microtubule-associated protein 2 (MAP-2), GFAP and the neural stem cell markers nestin and musashi^{34,68-70} (Table 1). Given the expression of a number of these neural markers on freshly isolated AECs, it is likely that these cells are already primed to differentiate towards neural lineages *in vitro*⁶⁹. Moreover, differentiated AECs have been shown to acquire functional activity with the synthesis and release of acetylcholine, catecholamines, dopamine, neurotrophic factors and noggin^{16,71}. Based on these characteristics, the therapeutic effects of AECs have been investigated in various experimental neurodegenerative diseases. Using a Parkinson's model, Yang and colleagues injected AECs into the lateral ventricle of rats and found that AECs ameliorated rotational asymmetry and after 5 weeks, transplanted cells were still detectable within the CNS and expressed nestin and vimentin⁷². In a haemorrhagic stroke model, in which brain injury and degeneration occurs, AECs transplanted into the brain were found to survive for at least 4 weeks, reduce brain oedema and improve motor deficits⁷³. In a rat middle cerebral artery occlusion model, transplanted AECs genetically modified to express the glial derived neurotrophic factor, were found to migrate to ischemic areas and maintain the expression of the neuronal markers MAP2, nestin and GFAP. Moreover, in such treated mice, the behavioural dysfunction was ameliorated and the infarct volume associated with the disease was reduced⁷⁴. Collectively, these experiments indicate that AECs can readily undergo neural differentiation *in vivo*, and possess the capability of homing to sites of injury.

7.2 Promotion of host repair

Along with their ability to replace damaged cells, AECs offer beneficial support for the growth and differentiation of host tissue stem cells. This characteristic has been shown to be independent of cell-cell contact, as conditioned medium from AEC cultures promote significant improvements in the survival of NSCs cultured *in vitro*⁷⁵. Identification of trophic factors secreted by AECs, include brain derived neurotrophic factor, neurotrophin-3, nerve growth factor^{34,75} and novel epidermal growth-like factors⁷⁶. Further confirmation of the efficacy of AECs to promote neural cell survival and development has been demonstrated in a number of transplantation settings. In a rodent SCI model, administration of AECs directly into the transection cavity led to the assisted penetration of host axons and the complete abolishment of glial scar formation⁶⁶. It would be interesting to know if AECs in this model of injury, have allowed the axotomized cells to survive and have facilitated the attraction of collateral neurite sprouting. As a further example, when AECs over-expressing bFGF were co-transplanted with NSCs, significant locomotor improvement was observed when compared with just NSCs alone⁷⁷. In summary these results suggest that AECs have the ability to promote the survival and differentiation of endogenous NSCs.

8. AECs in the clinic

While AECs promise to be an adjunct for the treatment of inflammatory and neurodegenerative diseases, an important question pertaining to their usage is whether or not they are safe, once transplanted into a patient. In this context it is worth noting that amniotic membrane and its epithelial layer (from which these AECs originate from) has been used for over 3 decades to treat several clinical conditions⁷⁸, (Table 3). As early as 1981, amniotic tissue was used to treat dural defects in infants and young children with severe and moderately severe craniocerebral trauma without post-operative complications, such as graft rejection⁷⁹. In more recent times, amniotic membrane has also been used for ocular surface reconstruction in conditions such as keratopathy⁸⁰, phenylketonuria⁸¹, conjunctival Bowen's disease⁸², as well as for other conditions that affect corneal epithelium⁸³⁻⁸⁸. Amniotic membranes are also highly successful in preventing corneal neovascularisation⁸⁹, and in treating persisting trophic ulcers of the cornea⁹⁰. Amnion has also been



widely used for the treatment of burn lesions, with results showing that grafts are easily tolerated and

successfully assist in the healing of damaged tissue^{91,92}.

Table 3. Summary of publications for the use of amnion in the clinic

Year	Publication title	Ref.
1981	<i>Use of amnion to repair a dural defect</i>	79)
2000	<i>Reconstruction of damaged corneas by transplantation of autologous limbal epithelial cells</i>	83)
2001	<i>Amniotic membrane graft in ocular surface disease. Prospective study with 31 cases</i>	90)
2006	<i>Ultrastructural analysis of in vivo expanded corneal epithelium on amniotic membrane</i>	84)
	<i>Amniotic membrane transplantation in palliative treatment of bullous keratopathy</i>	80)
	<i>Treatment of large conjunctival nevus by resection and reconstruction using amniotic membrane</i>	85)
	<i>Amniotic membrane transplantation for treatment of symblepharon in a patient with recessive dystrophic epidermolysis bullosa</i>	86)
2007	<i>Use of amniotic membrane graft and corneal transplantation in a patient with bilateral keratomalacia induced by uncontrolled phenylketonuria</i>	81)
2010	<i>Clinical study of the effect of amnion membrane transplantation on diminishing corneal neovascularization induced by alkali burn</i>	89)
	<i>Dried irradiated human amniotic membrane as a biological dressing for facial burns--a 7-year case series</i>	92)
	<i>Functional and anatomic results of amnion vaginoplasty in young women with Mayer-Rokitansky-Kuster-Hauser syndrome</i>	97)
	<i>Use of amniotic membrane transplantation in isolated conjunctival Bowen disease: a case report</i>	82)
	<i>Amniotic membrane transplantation in the treatment of persistent epithelial defect on the corneal graft</i>	87)
2011	<i>Comparisons of the effects of biological membrane (amnion) and silver sulfadiazine in the management of burn wounds in children</i>	91)
	<i>In vivo analysis of stromal integration of multilayer amniotic membrane transplantation in corneal ulcers</i>	88)

Given the current safety profile of amnion, it is likely that AECs could be investigated, at least in Phase I clinical trials. Nonetheless issues associated with their immunogenicity and other factors will need to be carefully assessed. For example, the propagation of these cells will require the absence of xenogeneic feeder or serum support. Close monitoring of AECs in culture will also be required, as it has been documented that certain stem cell types can upregulate MHC class I during differentiation⁹³, thus making them potential targets for immune clear-

ance. This aspect is particularly pertinent, given that AEC graft survival can significantly be reduced in pre-immunised animals⁹⁴. For example, serial transplantation of amniotic membrane into the eye (which is immune privileged) has been shown to generate an inflammatory response after the second and third transplantation⁹⁵. These results suggest that the continuous immunosuppressive and regenerative effects of AECs in a transplant setting will require several immunological barriers to be overcome.



9. Conclusion

Results from pre-clinical animal models, suggests that AECs have the capacity to strongly suppress immune responses, potentially induce peripheral tolerance and reverse ongoing inflammatory damage. There is also evidence that AECs may be able to differentiate into neural cells *in vivo* and possibly assist in tissue regeneration. Encouragingly, amniotic tissue has already been shown to be effective and safe in the clinic. As such, the transition of AECs from pre-clinical stages to the clinical setting may be hastened. However, further research is required to decipher the underlying mechanisms that contribute to their anti-inflammatory and regenerative potential. Nonetheless, AECs have many properties that make them exploitable for regenerative medicine and more specifically the treatment of neurodegenerative disorders.

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References

- 1) McQualter JL, Bernard CCA: Multiple sclerosis: a battle between destruction and repair. *Journal of Neurochemistry*. 2007; 100: 295-306.
- 2) Hauser SL, Oksenberg JR: The Neurobiology of Multiple Sclerosis: Genes, Inflammation, and Neurodegeneration. *Neuron*. 2006; 52: 61-76.
- 3) Bernard CCA, Johns TG, Slavin A, Ichikawa M, Ewing C, et al.: Myelin oligodendrocyte glycoprotein: a novel candidate autoantigen in multiple sclerosis. *Journal of Molecular Medicine*. 1997; 75: 77-88.
- 4) Ewing C, Bernard CCA: Insights into the aetiology and pathogenesis of multiple sclerosis. *Immunol Cell Biol*. 1998; 76: 47-54.
- 5) Payne N, Siatskas C, Barnard A, Bernard CC: The Prospect of Stem Cells as Multi- Faceted Purveyors of Immune Modulation, Repair and Regeneration in Multiple Sclerosis. *Curr Stem Cell Res Ther*. 2010.
- 6) Quintana FJ, Farez MF, Viglietta V, Iglesias AH, Merbl Y, et al.: Antigen microarrays identify unique serum autoantibody signatures in clinical and pathologic subtypes of multiple sclerosis. *Proc Natl Acad Sci U S A*. 2008; 105: 18889-18894.
- 7) Menon KN, Steer DL, Short M, Petratos S, Smith I, et al.: A novel unbiased proteomic approach to detect the reactivity of cerebrospinal fluid in neurological diseases. *Mol Cell Proteomics*. 2011.
- 8) Rovaris M, Confavreux C, Furlan R, Kappos L, Comi G, et al.: Secondary progressive multiple sclerosis: current knowledge and future challenges. *The Lancet Neurology*. 2006; 5: 343-354.
- 9) Zuvich RL, McCauley JL, Pericak-Vance MA, Haines JL: Genetics and pathogenesis of multiple sclerosis. *Semin Immunol*. 2009; 21: 328-333.
- 10) Payne N, Siatskas C, Bernard CC: The promise of stem cell and regenerative therapies for multiple sclerosis. *J Autoimmun*. 2008; 31: 288-294.
- 11) Keegan BM, Noseworthy JH: Multiple Sclerosis. *Annual Review of Medicine*. 2002; 53: 285-302.
- 12) Jones JL, Coles AJ: New treatment strategies in multiple sclerosis. *Exp Neurol*. 2010; 225: 34-39.
- 13) Bochev I, Elmadjian G, Kyurkchiev D, Tzvetanov L, Altankova I, et al.: Mesenchymal stem cells from human bone marrow or adipose tissue differently modulate mitogen-stimulated B-cell immunoglobulin production *in vitro*. *Cell Biology International*. 2008; 32: 384-393.
- 14) Aggarwal S, Pittenger MF: Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood*. 2005; 105: 1815-1822.
- 15) Singer NG, Caplan AI: Mesenchymal stem cells: mechanisms of inflammation. *Annu Rev Pathol*. 2011; 6: 457-478.
- 16) Toda A, Okabe M, Yoshida T, Nikaido T: The Potential of Amniotic Membrane/Amnion-Derived Cells for Regeneration of Various Tissues. *Journal of Pharmacological Sciences*. 2007; 105: 215-228.
- 17) Zappia E, Casazza S, Pedemonte E, Benvenuto F, Bonanni I, et al.: Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. *Blood*. 2005; 106: 1755-1761.
- 18) Uccelli A, Zappia E, Benvenuto F, Frassoni F,



- Mancardi G: Stem cells in inflammatory demyelinating disorders: a dual role for immunosuppression and neuroprotection. *Expert Opinion on Biological Therapy*. 2006; 6: 17-22.
- 19) Kassis I, Grigoriadis N, Gowda-Kurkalli B, Mizrahi-Kol R, Ben-Hur T, et al.: Neuroprotection and immunomodulation with mesenchymal stem cells in chronic experimental autoimmune encephalomyelitis. *Arch Neurol*. 2008; 65: 753-761.
 - 20) Johansson CB, Youssef S, Koleckar K, et al.: Extensive fusion of haematopoietic cells with Purkinje neurons in response to chronic inflammation. *Nature Cell Biology*. 2008; 10: 575-583.
 - 21) Kemp K, Gordon D, Wraith DC, Mallam E, Hartfield E, et al.: Fusion between human mesenchymal stem cells and rodent cerebellar Purkinje cells. *Neuropathol Appl Neurobiol*. 2011; 37: 166-178.
 - 22) Gordon D, Pavlovskaya G, Uney JB, Wraith DC, Scolding NJ: Human mesenchymal stem cells infiltrate the spinal cord, reduce demyelination, and localize to white matter lesions in experimental autoimmune encephalomyelitis. *J Neuropathol Exp Neurol*. 2010; 69: 1087-1095.
 - 23) Constantin G, Marconi S, Rossi B, Angiari S, Calderan L, et al.: Adipose-derived mesenchymal stem cells ameliorate chronic experimental autoimmune encephalomyelitis. *Stem Cells*. 2009; 27: 2624-2635.
 - 24) Einstein O, Grigoriadis N, Mizrahi-Kol R, Reinhartz E, Polyzoidou E, et al.: Transplanted neural precursor cells reduce brain inflammation to attenuate chronic experimental autoimmune encephalomyelitis. *Exp Neurol*. 2006; 198: 275-284.
 - 25) Aharonowicz M, Einstein O, Feinstein N, Lassmann H, Reubini B, et al.: Neuroprotective effect of transplanted human embryonic stem cell-derived neural precursors in an animal model of multiple sclerosis. *PLoS One*. 2008; 3: e3145.
 - 26) Pluchino S, Quattrini A, Brambilla E, Gritti A, Salani G, et al.: Injection of adult neurospheres induces recovery in a chronic model of multiple sclerosis. *Nature*. 2003; 422: 688-694.
 - 27) Kendal-Wright CE: Stretching, Mechanotransduction, and Proinflammatory Cytokines in the Fetal Membranes. *Reproductive Sciences*. 2007; 14: 35-41.
 - 28) Li H, Niederkorn JY, Neelam S, Mayhew E, Word RA, et al.: Immunosuppressive Factors Secreted by Human Amniotic Epithelial Cells. *Invest. Ophthalmol. Vis. Sci*. 2005; 46: 900-907.
 - 29) Takashima S, Yasuo M, Sanzen N, Sekiguchi K, Okabe M, et al.: Characterization of laminin isoforms in human amnion. *Tissue Cell*. 2008; 40: 75-81.
 - 30) Baergen RN: *Manual of Benirschke and Kaufmann's: Pathology of the Human Placenta*, Springer Science+Business Media, Inc., New York; 2005.
 - 31) Whittle WL, Gibb W, Challis JR: The characterization of human amnion epithelial and mesenchymal cells: the cellular expression, activity and glucocorticoid regulation of prostaglandin output. *Placenta*. 2000; 21: 394-401.
 - 32) Sakuragawa N, Kakinuma K, Kikuchi A, Okano H, Uchida S, et al.: Human amnion mesenchyme cells express phenotypes of neuroglial progenitor cells. *J Neurosci Res*. 2004; 78: 208-214.
 - 33) Dobrev MP, Pereira PN, Deprest J, Zwijsen A: On the origin of amniotic stem cells: of mice and men. *Int J Dev Biol*. 2010; 54: 761-777.
 - 34) Sakuragawa N, Thangavel R, Mizuguchi M, Hirasawa M, Kamo I: Expression of markers for both neuronal and glial cells in human amniotic epithelial cells. *Neurosci Lett*. 1996; 209: 9-12.
 - 35) Manuelpillai U, Tchongue J, Lourensz D, Vaghjiani V, Samuel CS, et al.: Transplantation of human amnion epithelial cells reduces hepatic fibrosis in immunocompetent CCl₄-treated mice. *Cell Transplant*. 2010; 19: 1157-1168.
 - 36) Murphy S, Rosli S, Acharya R, Mathias L, Lim R, et al.: Amnion epithelial cell isolation and characterization for clinical use. *Curr Protoc Stem Cell Biol*. 2010; Chapter 1: Unit 1E 6.
 - 37) Miki T, Mitamura K, Ross MA, Stolz DB, Strom SC: Identification of stem cell marker-positive cells by immunofluorescence in term human amnion. *J Reprod Immunol*. 2007; 75: 91-96.
 - 38) Ilancheran S, Michalska A, Peh G, Wallace EM, Pera M, et al.: Stem cells derived from human fetal membranes display multilineage differentiation potential. *Biol Reprod*. 2007; 77: 577-588.



- 39) Diaz-Prado S, Muinos-Lopez E, Hermida-Gomez T, Rendal-Vazquez ME, Fuentes-Boquete I, et al.: Multilineage differentiation potential of cells isolated from the human amniotic membrane. *J Cell Biochem.* 2010; 111: 846-857.
- 40) Wang M, Yang Y, Yang D, Luo F, Liang W, et al.: The immunomodulatory activity of human umbilical cord blood-derived mesenchymal stem cells in vitro. *Immunology.* 2009; 126: 220-232.
- 41) Baptista LS, do Amaral RJ, Carias RB, Aniceto M, Claudio-da-Silva C, et al.: An alternative method for the isolation of mesenchymal stromal cells derived from lipoaspirate samples. *Cytotherapy.* 2009; 11: 706-715.
- 42) Miki T, Lehmann T, Cai H, Stolz DB, Strom SC: Stem cell characteristics of amniotic epithelial cells. *Stem Cells.* 2005; 23: 1549-1559.
- 43) Hori J, Wang M, Kamiya K, Takahashi H, Sakuragawa N: Immunological characteristics of amniotic epithelium. *Cornea.* 2006; 25: S53-58.
- 44) Moodley Y, Ilancheran S, Samuel C, Vaghjiani V, Atienza D, et al.: Human amnion epithelial cell transplantation abrogates lung fibrosis and augments repair. *Am J Respir Crit Care Med.* 2010; 182: 643-651.
- 45) Hsi BL, Samson M, Grivaux C, Fenichel P, Hunt JS, et al.: Topographical expression of class I major histocompatibility complex antigens on human amniotic epithelium. *J Reprod Immunol.* 1988; 13: 183-191.
- 46) Hunt JS, Andrews GK, Fishback JL, Feess M, Wood GW: Amnion membrane epithelial cells express class I HLA and contain class I HLA mRNA. *J Immunol.* 1988; 140: 2790-2795.
- 47) Kubo M, Sonoda Y, Muramatsu R, Usui M: Immunogenicity of human amniotic membrane in experimental xenotransplantation. *Invest Ophthalmol Vis Sci.* 2001; 42: 1539-1546.
- 48) Banas RA, Trumpower C, Bentlejewski C, Marshall V, Sing G, et al.: Immunogenicity and immunomodulatory effects of amnion-derived multipotent progenitor cells. *Human Immunology.* 2008; 69: 321-328.
- 49) Houlihan JM, Biro PA, Harper HM, Jenkinson HJ, Holmes CH: The human amnion is a site of MHC class Ib expression: evidence for the expression of HLA-E and HLA-G. *J Immunol.* 1995; 154: 5665-5674.
- 50) Rebmann V, Switala M, Eue I, Grosse-Wilde H: Soluble HLA-G is an independent factor for the prediction of pregnancy outcome after ART: a German multi-centre study. *Human Reproduction.* 2010; 25: 1691-1698.
- 51) Kovats S, Main EK, Librach C, Stubblebine M, Fisher SJ, et al.: A class I antigen, HLA-G, expressed in human trophoblasts. *Science.* 1990; 248: 220-223.
- 52) Parolini O, Alviano F, Bagnara GP, Bilic G, Buhring HJ, et al.: Concise review: isolation and characterization of cells from human term placenta: outcome of the first international Workshop on Placenta Derived Stem Cells. *Stem Cells.* 2008; 26: 300-311.
- 53) Lefebvre S, Adrian F, Moreau P, Gourand L, Dausset J, et al.: Modulation of HLAG expression in human thymic and amniotic epithelial cells. *Hum Immunol.* 2000; 61: 1095-1101.
- 54) Moon JH, Lee JR, Jee BC, Suh CS, Kim SH, et al.: Successful vitrification of human amnion-derived mesenchymal stem cells. *Hum Reprod.* 2008; 23: 1760-1770.
- 55) Irony-Tur-Sinai M, Grigoriadis N, Loubopoulos A, Pinto-Maaravi F, Abramsky O, et al.: Amelioration of autoimmune neuroinflammation by recombinant human alphafetoprotein. *Exp Neurol.* 2006; 198: 136-144.
- 56) Irony-Tur-Sinai M, Grigoriadis N, Tsiantoulas D, Touloumi O, Abramsky O, et al.: Immunomodulation of EAE by alpha-fetoprotein involves elevation of immune cell apoptosis markers and the transcription factor FoxP3. *J Neurol Sci.* 2009; 279: 80-87.
- 57) Wolbank S, Peterbauer A, Fahrner M, Hennerbichler S, van Griensven M, et al.: Dose-dependent immunomodulatory effect of human stem cells from amniotic membrane: a comparison with human mesenchymal stem cells from adipose tissue. *Tissue Eng.* 2007; 13: 1173-1183.
- 58) Ueta M, Kweon MN, Sano Y, Sotozono C, Yamada J, et al.: Immunosuppressive properties of human amniotic membrane for mixed lymphocyte reaction. *Clinical & Experimental Immunology.* 2002; 129: 464-470.



- 59) Murphy S, Lim R, Dickinson H, Acharya R, Rosli S, et al.: Human amnion epithelial cells prevent bleomycin-induced lung injury and preserve lung function. *Cell Transplant*. 2010.
- 60) Bettelli E, Pagany M, Weiner HL, Linington C, Sobel RA, et al.: Myelin oligodendrocyte glycoprotein-specific T cell receptor transgenic mice develop spontaneous autoimmune optic neuritis. *J Exp Med*. 2003; 197: 1073-1081.
- 61) Croitoru-Lamoury J, Lamoury FM, Zaunders JJ, Veas LA, Brew BJ: Human mesenchymal stem cells constitutively express chemokines and chemokine receptors that can be upregulated by cytokines, IFN-beta, and Copaxone. *J Interferon Cytokine Res*. 2007; 27: 53-64.
- 62) Wang Y, Deng Y, Zhou GQ: SDF-1alpha/CXCR4-mediated migration of systemically transplanted bone marrow stromal cells towards ischemic brain lesion in a rat model. *Brain Res*. 2008; 1195: 104-112.
- 63) Kohler RE, Comerford I, Townley S, Haylock-Jacobs S, Clark-Lewis I, et al.: Antagonism of the chemokine receptors CXCR3 and CXCR4 reduces the pathology of experimental autoimmune encephalomyelitis. *Brain Pathol*. 2008; 18: 504-516.
- 64) Cruz-Orengo L, Holman DW, Dorsey D, Zhou L, Zhang P, et al.: CXCR7 influences leukocyte entry into the CNS parenchyma by controlling abluminal CXCL12 abundance during autoimmunity. *J Exp Med*. 2011; 208: 327-339.
- 65) Wei JP, Zhang TS, Kawa S, Aizawa T, Ota M, et al.: Human amnion-isolated cells normalize blood glucose in streptozotocin-induced diabetic mice. *Cell Transplant*. 2003; 12: 545-552.
- 66) Sankar V, Muthusamy R: Role of human amniotic epithelial cell transplantation in spinal cord injury repair research. *Neuroscience*. 2003; 118: 11-17.
- 67) Bailo M, Soncini M, Vertua E, Signoroni PB, Sanzone S, et al.: Engraftment potential of human amnion and chorion cells derived from term placenta. *Transplantation*. 2004; 78: 1439-1448.
- 68) Uchida S, Suzuki Y, Araie M, Kashiwagi K, Otori Y, et al.: Factors secreted by human amniotic epithelial cells promote the survival of rat retinal ganglion cells. *Neurosci Lett*. 2003; 341: 1-4.
- 69) Shinya M, Komuro H, Saihara R, Urita Y, Kaneko M, et al.: Neural differentiation potential of rat amniotic epithelial cells. *Fetal Pediatr Pathol*. 2010; 29: 133-143.
- 70) Niknejad H, Peirovi H, Ahmadiani A, Ghanavi J, Jorjani M: Differentiation factors that influence neuronal markers expression in vitro from human amniotic epithelial cells. *Eur Cell Mater*. 2010; 19: 22-29.
- 71) Uchida S, Inanaga Y, Kobayashi M, Hurukawa S, Araie M, et al.: Neurotrophic function of conditioned medium from human amniotic epithelial cells. *J Neurosci Res*. 2000; 62: 585-590.
- 72) Yang XX, Xue SR, Dong WL, Kong Y: Therapeutic effect of human amniotic epithelial cell transplantation into the lateral ventricle of hemiparkinsonian rats. *Chin Med J (Engl)*. 2009; 122: 2449-2454.
- 73) Dong W, Chen H, Yang X, Guo L, Hui G: Treatment of intracerebral haemorrhage in rats with intraventricular transplantation of human amniotic epithelial cells. *Cell Biol Int*. 2010; 34: 573-577.
- 74) Liu: Human amniotic epithelial cells ameliorate behavioral dysfunction and reduce infarct size in the rat middle cerebral artery occlusion model. *Shock*. 2008; 29: 603- 611.
- 75) Meng XT, Chen D, Dong ZY, Liu JM: Enhanced neural differentiation of neural stem cells and neurite growth by amniotic epithelial cell co-culture. *Cell Biol Int*. 2007; 31: 691-698.
- 76) Venkatachalam S, Palaniappan T, Jayapal PK, Neelemegan S, Rajan SS, et al.: Novel neurotrophic factor secreted by amniotic epithelial cells. *Biocell*. 2009; 33: 81-89.
- 77) Meng X-t, Li C, Dong Z-y, Liu J-m, Li W, et al.: Co-transplantation of bFGF-expressing amniotic epithelial cells and neural stem cells promotes functional recovery in spinal cord-injured rats. *Cell Biology International*. 2008; 32: 1546- 1558.
- 78) Takashima SS: Human amniotic epithelial cells possess hepatocyte-like characteristics and functions. *Cell structure and function*. 2004; 29: 73.
- 79) Kudriashov AF, Artarian AA, Putsillo MV: Use of amnion to repair a dural defect. *Zh Vopr Neurokhir Im N N Burdenko*. 1981: 37-40.
- 80) Zemba M, Andrei S, Cucu B, Bratulescu M, Stinghe A, et al.: Amniotic membrane transplantation in palliative treatment of bullous keratopathy. *Oftalmolo-*



- gia. 2006; 50: 51-53.
- 81) Habot-Wilner Z, Spierer A, Barequet IS, Greenbaum A: Use of amniotic membrane graft and corneal transplantation in a patient with bilateral keratomalacia induced by uncontrolled phenylketonuria. *Cornea*. 2007; 26: 629-631.
 - 82) Motolese I, Mazzera L, Frezzotti P, Motolese PA, Motolese E: Use of amniotic membrane transplantation in isolated conjunctival Bowen disease: a case report. *Eur J Ophthalmol*. 2010; 20: 604-607.
 - 83) Tsai RJ, Li LM, Chen JK: Reconstruction of damaged corneas by transplantation of autologous limbal epithelial cells. *N Engl J Med*. 2000; 343: 86-93.
 - 84) Ha HS, Song KY, Kim JC: Ultrastructural analysis of in vivo expanded corneal epithelium on amniotic membrane. *J Korean Med Sci*. 2006; 21: 544-549.
 - 85) Tomita M, Goto H, Muramatsu R, Usui M: Treatment of large conjunctival nevus by resection and reconstruction using amniotic membrane. *Graefes Arch Clin Exp Ophthalmol*. 2006; 244: 761-764.
 - 86) Altan-Yaycioglu R, Akova YA, Oto S: Amniotic membrane transplantation for treatment of symblepharon in a patient with recessive dystrophic epidermolysis bullosa. *Cornea*. 2006; 25: 971-973.
 - 87) Dekaris I, Mravicic I, Barisic A, Draca N, Pauk M: Amniotic membrane transplantation in the treatment of persistent epithelial defect on the corneal graft. *Coll Antropol*. 2010; 34 Suppl 2: 15-19.
 - 88) Nubile M, Dua HS, Lanzini M, Ciancaglini M, Caillenno R, et al.: In Vivo Analysis of Stromal Integration of Multilayer Amniotic Membrane Transplantation in Corneal Ulcers. *Am J Ophthalmol*. 2011.
 - 89) Yin L, Pi Y: Clinical study of the effect of amnion membrane transplantation on diminishing corneal neovascularization induced by alkali burn. *Yan Ke Xue Bao*. 2010; 25: 70-71.
 - 90) Muraine M, Descargues G, Franck O, Villeroy F, Toubreau D, et al.: Amniotic membrane graft in ocular surface disease. Prospective study with 31 cases. *J Fr Ophtalmol*. 2001; 24: 798-812.
 - 91) Mostaque AK, Abdur Rahman KB: Comparisons of the Effects of Biological Membrane (Amnion) and Silver Sulfadiazine in the Management of Burn Wounds in Children. *J Burn Care Res*. 2011.
 - 92) Bujang-Safawi E, Halim AS, Khoo TL, Dorai AA: Dried irradiated human amniotic membrane as a biological dressing for facial burns--a 7-year case series. *Burns*. 2010; 36: 876-882.
 - 93) Kadereit S, Trounson A: In vitro immunogenicity of undifferentiated pluripotent stem cells (PSC) and derived lineages. *Semin Immunopathol*. 2011.
 - 94) Wang M, Yoshida A, Kawashima H, Ishizaki M, Takahashi H, et al.: Immunogenicity and antigenicity of allogeneic amniotic epithelial transplants grafted to the cornea, conjunctiva, and anterior chamber. *Invest Ophthalmol Vis Sci*. 2006; 47: 1522-1532.
 - 95) Gabler B, Lohmann CP: Hypopyon after repeated transplantation of human amniotic membrane onto the corneal surface. *Ophthalmology*. 2000; 107: 1344- 1346.
 - 96) Wu ZY, Hui GZ, Lu Y, Wu X, Guo LH: Transplantation of human amniotic epithelial cells improves hindlimb function in rats with spinal cord injury. *Chin Med J (Engl)*. 2006; 119: 2101-2107.
 - 97) Fotopoulou C, Sehouli J, Gehrmann N, Schoenborn I, Lichtenegger W: Functional and anatomic results of amnion vaginoplasty in young women with Mayer-Rokitansky-Kuster-Hauser syndrome. *Fertil Steril*. 2010; 94: 317-323.
 - 98) Ilancheran S, Moodley Y, Manuelpillai U: Human Fetal Membranes: A Source of Stem Cells for Tissue Regeneration and Repair? *Placenta*. 2009; 30: 2-10.