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REVIEW

Amniotic membrane-derived stem cells: immunomodulatory properties and potential clinical application

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Carmen L Insausti[1]
Miguel Blanquer[1]
Ana M García-Hernández[1]
Gregorio Castellanos[2]
José M Moraleda[1]

[1]Unidad de Trasplante Hematopoyético y Terapia Celular, [2]Servicio de Cirugía, Hospital Clínico Universitario Virgen de la Arrixaca, IMIB, Campus Mare Nostrum, Universidad de Murcia, El Palmar, Murcia, Spain

Abstract: Epithelial and mesenchymal cells isolated from the amniotic membrane (AM) possess stem cell characteristics, differentiation potential toward lineages of different germ layers, and immunomodulatory properties. While their expansion and differentiation potential have been well studied and characterized, knowledge about their immunomodulatory properties and the mechanisms involved is still incomplete. These mechanisms have been evaluated on various target cells of the innate and the adaptive system and in animal models of different inflammatory diseases. Some results have evidenced that the immunomodulatory effect of AM-derived cells is dependent on cell-cell contact, but many of them have demonstrated that these properties are mediated through the secretion of suppressive molecules. In this review, we present an update on the described immunomodulatory properties of the derived amniotic cells and some of the proposed involved mechanisms. Furthermore, we describe some assays in animal models of different inflammatory diseases which reveal the potential use of these cells to treat such diseases.

Keywords: epithelial cells, mesenchymal cells, cell therapy, immunomodulation

Introduction

The amniotic membrane (AM) is an avascular tissue that forms the innermost layer of the fetal membranes. It is composed of five layers: an epithelial cells monolayer, an acellular basement membrane layer, a compact layer, a mesenchymal cells layer, and a spongy layer placed in close proximity to the chorion.[1]

Using a variety of established protocols, two types of cells have been isolated from the AM and their properties have been studied.[2–7] Isolated cells have been identified as human amniotic epithelial cells (HAECs) and human amniotic mesenchymal stromal cells (HAMSCs).[2] It has been shown that both types of cells possess stem cell characteristics, differentiation potential toward lineages of different germ layers,[5–12] and immunomodulatory properties.[13–19]

While the expansion and differentiation potential of the AM-derived cells has been well studied and characterized for different groups, the available knowledge about their immunomodulatory behavior is relatively scarce and disperse. However, in the last few years, increasing experimental findings have pointed toward the immunomodulatory properties of these cells, which it is hoped could dramatically expand their therapeutic potential clinical applications.

In this paper, we present an update on the described immunomodulatory properties of the derived amniotic cells, and an overview of the current theories regarding the potential use of these cells to treat inflammatory diseases.

Correspondence: Carmen L Insausti
Unidad de Trasplante Hematopoyético y Terapia Celular, Hospital Universitario Virgen de la Arrixaca, Ctra Madrid-Cartagena, s/n, 30120 El Palmar, Murcia, Spain
Tel +34 968 369036
Fax +34 968 369438
Email caymed@gmail.com

Definition of amniotic membrane-derived cells

Freshly isolated HAECs are medium-sized cells, circular in shape, with a central or eccentric nucleus, one or two nucleoli, and abundant cytoplasm, usually vacuolated.[3–6] They express cell surface markers associated with embryonic stem cells such as SSEA-3 and SSEA-4 (stage-specific embryonic antigen 3 and 4), and TRA 1-60 and TRA 1-81 (tumor rejection antigen 1-60 and 1-81). They also express molecules, such as E-Cadherin, CD9, CD29, CD104, CD49e, CD49f, CD49d, and CD44, among other molecules involved in cell-cell interactions and cell adhesion[5,6,13,15,20] (Table 1). HAECs express transcription factors specific for pluripotential stem cells: Oct-4, Sox-2, Nanog, and Rex-1.[2,5,6,8–11] In culture, these cells proliferate, showing numerous mitotic events, and form a confluent single layer with typical cobblestone epithelial morphology.[2,3,5,6,8–11] Cultured HAECs undergo epithelial to mesenchymal transition through the autocrine production of transforming growth factor beta (TGF-β).[21] Under appropriate culture conditions, these cells can be induced to differentiate in cells of the three germinal layers (ectoderm, mesoderm, and endoderm).[2,5,6,8–11]

HAMSCs are defined as a population of cells that proliferate in vitro as plastic-adherent, spindle-shaped cells capable of producing fibroblast colony-forming units and displaying a specific pattern of cell surface antigens comparable to that of bone marrow mesenchymal stem cells (BM-MSCs) and other adult sources. They do not express the hematopoietic markers CD45, CD34, or CD14, but they do express variable levels of CD90, CD73, CD105, CD29, CD44, CD49d, CD49e, CD56, and CD166, and they are recognized by the monoclonal antibody against stromal precursor cells-1[2,3,5–7,10–12,20] (Table 2). These cells are also capable of differentiating toward one or more lineages, including osteogenic, adipogenic, chondrogenic, and vascular/endothelial.[2,5–7,10–13] Furthermore, recent reports suggest that, like the amniotic epithelial fraction, HAMSCs have multilineage differentiation potential.[22]

The immunologic profile of HAECs and HAMSCs reveals that they express low levels of major histocompatibility complex (MHC) class I surface antigens and reduced levels of the major components of the antigen processing machinery. They do not express MHC class II antigens,[2,13,15] the costimulatory molecules CD80 (B7-1), CD86 (B7-2), CD40, or CD40 ligand, in the presence or absence of interferon gamma (IFN-γ), one of the most potent known inflammatory cytokines.[2,15,16] They neither express the programmed cell death receptor 1 (PD1) (an inhibitory receptor that is normally expressed on activated T and B cells), nor its two ligands: programmed death ligands 1 and 2 (PD-L1 and PD-L2).[15,16,23] These two are typically upregulated by stimulation with IFN-γ.[15,16] AM-derived cells are also negative for the immunoglobulin-like transcript receptors 2, 3, and 4 (ILTR-2, ILTR-3, and ILTR-4).[15] There is some controversy about the expression of TRAIL, tumor necrosis factor alpha (TNF-α), and Fas-ligand (Fas-L), all members of the TNF family involved in the induction of apoptosis[14,15] (Table 3).

One of the unique characteristics of HAECs and HAMSCs is that they constitutively express the tissue-restricted, nonclassical human leukocyte antigen G (HLA-G).[5,8,24,25]

Table 1 Phenotypic characteristics of HAECs

Antigen	Expression
SSEA-3	+
SSEA-4	+
TRA 1-60	+
TRA 1-81	+
E-Cad	+
CD9	+
CD24	+
CD29	+
CD104	+
CD49e	+
CD49f	+
CD49d	+
CD44	+
CD49f	+
CD34	–
CD45	–
CD14	–
CD73	–
CD90	–
CD105	–

Note: Data from Ilancheran et al,[5] Wolbank et al,[13] Banas et al,[15] Chang et al,[16] Roubelakis et al.[20]
Abbreviations: E-Cad, E-Cadherin; HAEC, human amniotic epithelial cells; SSEA, stage-specific embryonic antigen; TRA, tumor rejection antigen; –, negative; +, positive.

Table 2 Phenotypic characteristics of HAMSCs

Antigen	Expression
CD34	–
CD45	–
CD14	–
CD73	+
CD90	+
CD105	+
CD29	+
CD44	+
CD49d	+
CD49e	+
CD56	+
CD166	+
STRO-1	+

Note: Data from Parolini et al,[2] Whittle et al,[3] Ilancheran et al,[5] Insausti et al,[6] Alviano et al,[7] Ilancheran et al,[10] Parolini et al,[11] Soncini et al,[12] Roubelakis et al.[20]
Abbreviations: HAMSC, human amniotic mesenchymal stromal cells; STRO, stromal precursor cells; –, negative; +, positive.

Table 3 Immunologic profile of HAECs and HAMSCs

Antigens	IFN-γ absent	IFN-γ present	References
HLA A-B-C	–, ±	–	2,13,15
HLA DR	–	–	2,13,15
HLA G	+	++	5,8,15,24,25
CD80 (B7-1)	–	–	2,15,16
CD86 (B7-2)	–	–	2,15,16
CD40	–	–	2,15,16
CD40L	–	–	2,15,16
PD-1	–	–	15,16,23
PDL1	–	+	15,16,23
PDL2	–	+	15,16,23
ILTR2	–	–	15
ILTR3	–	–	15
ILTR4	–	–	15

Abbreviations: HAEC, human amniotic epithelial cells; HAMSC, human amniotic mesenchymal stromal cells; HLA, human leukocyte antigen; ILTR, immunoglobulin-like transcript receptor; IFN-γ, interferon gamma; PD1, programmed cell death receptor 1; PDL1, programmed cell death receptor ligand 1; –, negative; +, positive; ±, weak positive; ++, strong positive.

Under physiological conditions, constitutive HLA-G expression is found in immune-privileged organs (eg, testis, ovary, and fetal cells) and is associated with tolerogenic properties via the interaction with inhibitory receptors. HLA-G has been shown to have important immunomodulatory functions.[24,25] It appears to be recognized mainly by ILTR, which are expressed by T and B lymphocytes, as well as by natural killer and dendritic cells, and abrogate activating signals received by these cells. Levels of HLA-G on cultured AM-derived cells are upregulated by exposure to IFN-γ.[15] In conclusion, these characteristics indicate that AM-derived cells are immune-privileged cells, able to survive in immunocompatibility-mismatched allogeneic transplant recipients.

In vitro effects of AM-derived stem cells on different cells of the innate and adaptive immunity

The maternofetal immune tolerance observed during pregnancy has inspired most of the studies done to investigate the immune properties of the human amniotic cells. These studies have demonstrated that human amniotic cells exhibit pleiotropic immune regulatory activities both in vivo and in vitro, which are mediated by complex mechanisms that inhibit the function of different immune cell subpopulations of the innate and adaptive immunity.

In vivo, several preclinical studies have reported that cells derived from the AM can engraft different tissues after xenogeneic and allogeneic transplantation into immunocompetent animals without eliciting an immune response, indicating that these cells are not immunogenic.[18,26–32] Additionally, AM has been applied in patients with ophthalmologic disorders,[33,34] burn injuries,[35] and chronic ulcers to improve wound healing.[36–39] In none of these cases acute rejection has been observed in the absence of immunosuppressive treatment.

In vitro, the mechanisms involved in immunosuppressive and immunomodulatory properties of the AM-derived cells have been evaluated on various target cells of the innate and the adaptive system (Figure 1). Some assays have been performed without separating the two types of cells (epithelial and mesenchymal).[16,17,19,40–43] Others have been performed on each population of cells.[13–15] Wolbank et al[13] demonstrated that, although epithelial and mesenchymal fractions show distinct morphology and marker expression, they have similar potency to modulate immunoreactions in vitro.

Innate immunity

Dendritic cells (DCs) have a fundamental role in antigen presentation to naïve T cells following DC maturation, which can be induced by proinflammatory cytokines and/or pathogen associated molecules. During maturation, immature DCs (iDCs) acquire the expression of costimulatory molecules and upregulate expression of MHC class I and class II molecules together with other cell-surface markers (such as CD11c and CD83).[44]

The immunomodulatory effects of AM-derived cells exerted on antigen-presenting cells have been demonstrated by their ability to block maturation of monocytes into DCs[41,42] (Figure 1). Monocytes exposed to DC differentiation and maturation conditions in the presence of AM-derived cells showed impaired development and failed to express CD1a, to upregulate the costimulatory molecules CD80, CD86, and CD83, and to increase surface expression of HLA-DR.[41] The addition of lipopolysaccharide to monocytes cultured toward iDC differentiation in the presence of AM-derived cells did not result in differentiation, even after removal of such cells.[41]

The mechanisms responsible for this inhibition remain to be elucidated, although several possible explanations have been proposed. It has been suggested that AM-derived cells inhibit DC differentiation by preventing cell cycle entry and inhibiting protein synthesis in stimulated monocytes. Monocytes induced in the presence of AM-derived cells showed almost complete arrest in the G_0 phase of the cell cycle.[41] HLA-G expressed on HAECs and HAMSCs has also been implicated to explain the tolerogenic DC, through the killing inhibitory receptor ILT pathway present in monocytes, macrophages, and DCs. It has been demonstrated that the tolerogenic DC induced through HLA-G leads to the induction of anergic and immunosuppressive regulatory T cells (Treg)[45,46] (Figure 1).

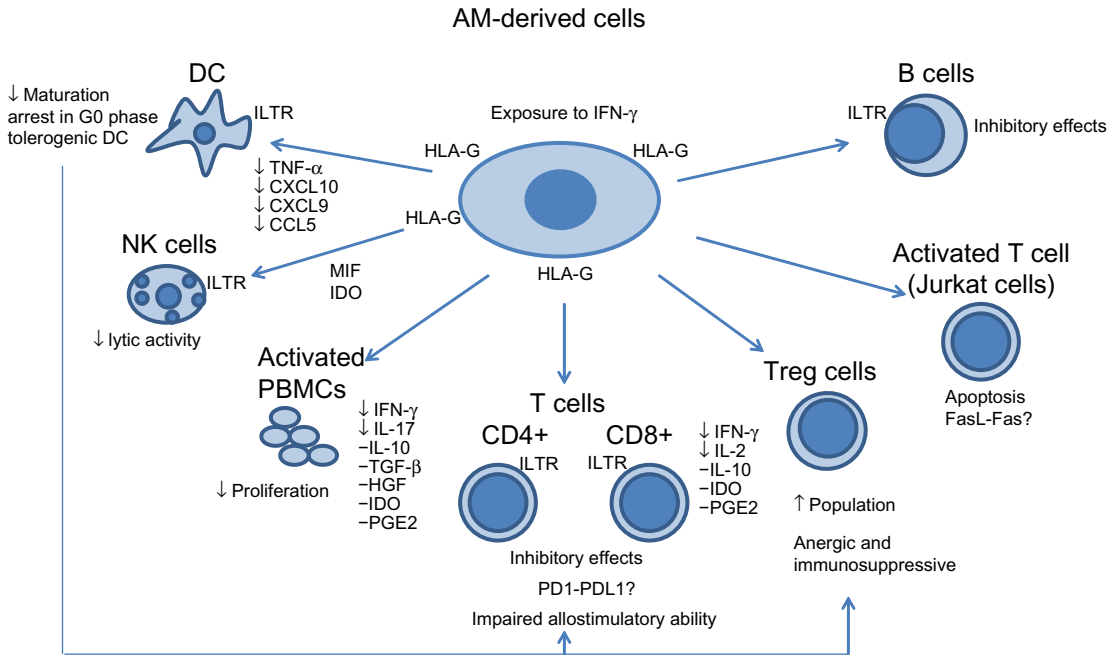

Figure 1 Proposed mechanisms of the in vitro interaction of AM-derived stem cells with different cells of the immune system.
Abbreviations: AM, amniotic membrane; DC, dendritic cell; FasL, fas ligand; G0, resting phase of the cell cycle; HGF, hepatic growth factor; HLA-G, human leukocyte antigen G; IDO, indoleamine 2,3-dioxygenase; IFN-γ, interferon gamma; IL, interleukin; ILTR, immunoglobulin-like transcript receptor; Jurkat cells, human acute lymphoblastic T cell leukemia, clone E6.1; MIF, migration inhibitory factor; NK, natural killer; PD1, programmed cell death receptor 1; PDL1, programmed cell death receptor ligand 1; PGE2, prostaglandin E-2; PBMC, peripheral blood mononucleated cell; TGF-β, transforming growth factor beta; TNF-α, tumor necrosis factor alpha; Treg cells, regulatory T cells.

Using a transwell system, Magatti et al observed that AM-derived cells were able to inhibit monocytes differentiation in the absence of cell-cell contact, suggesting the involvement of soluble factors.[41,42] They found that AM-derived cells produce high levels of the Th2-related cytokines CCL2, CXCL8, and interleukin 6 (IL-6), the latter known to be implicated in the inhibition of CD34+ cells and monocyte differentiation to DCs. Additionally, they found that AM-derived cells block the production of inflammatory cytokines TNF-α, CXCL10, CXCL9, and CCL5 in DC differentiation cultures.[41] All of these were considered as evidence of the AM-derived cells' immunosuppressive mechanisms, mediated by anti-inflammatory processes.

The block in the monocyte induced differentiation/maturation toward DC in the presence of AM-derived cells, also resulted in impaired allostimulatory ability on allogeneic T cells.[16,41,42] This effect, which persisted even after removal of AM-derived cells and complete reinduction of the monocytes toward DC differentiation, suggested an irreversible functional change of AM-derived cells on differentiating monocytes.[41] These findings, similar to those observed by other investigators using BM-MSCs, have been considered as proof that AM-derived cells are tolerogenic, at least in part, through a direct impact on DCs, leading to impaired T cell functions.[41,46,47]

Natural killer (NK) cells are important effector cells of innate immunity, and they have a key role in antiviral and antitumor immune responses owing to their cytolytic activity and production of pro-inflammatory cytokines.[48] The function of NK cells is tightly regulated by cell surface receptors that transduce either inhibitory or activating signals. NK cell-mediated lysis of target cells requires the expression of ligand(s) by the target cells that are recognized by the activating NK receptor, together with low-level to absent expression of MHC class I molecule by the target cell, which is recognized by the MHC class I specific inhibitory receptor of NK cells. The absence of MHC class I antigens on the HAECs provides a degree of immune privilege against cells of the adaptive immune system, but renders these cells potentially vulnerable to attack by NK cells. However, using K562, which is a well-established NK cells target, and MCF-7, a poor NK cells target, it was observed that placenta-derived MSCs were not lysed by NK cells.[16] Reverse transcription polymerase chain reaction and enzyme-linked immunosorbent assay analysis revealed that HAECs produce migration inhibitory factor, a potent inhibitor of macrophage migration and, coincidentally, a potent inhibitor of NK cell-mediated lytic activity.[14] Additionally, AM-derived cells may inhibit the interaction of HLA-G antigens and the killing inhibitory ILT receptors, which are expressed by NK cells as well as

by T and B lymphocytes[45–49] (Figure 1). However, it must be appreciated that activated, but not freshly isolated, NK cells have been reported to kill MSCs.[50]

Adaptive immunity

After T-cell receptor (TCR) engagement, T cells proliferate and exert several effector functions, including cytokine release and, in the case of CD8+ cells, cytotoxicity.[43] The proliferation of T cells stimulated with polyclonal mitogens, allogeneic cells, or specific antigen is inhibited by AM-derived cells.[13–17,19,42,51] According to some investigators, HAECs and HAMSCs inhibited proliferation of activated peripheral blood mononuclear cells (PBMCs) by phytohemagglutinin (PHA) or allogeneic cultured cells by cell-to-cell contact and, in a dose-dependent manner, in mixed lymphocyte reaction (MLR), as demonstrated by a decrease in proliferation with increasing amounts of AM-derived cells.[13,15] They found that in a transwell system, AM-derived cells were unable to suppress the proliferative response of activated PBMCs.[13,15] However, other investigators demonstrated that these effects on PBMCs were caused not only by cell-to-cell contact, but also by AM-derived cell culture supernatant or conditioned media from these cultures.[14,16,17,19]

Using fluid-derived MSCs, Sessarego et al showed that these cells could inhibit the proliferation of T cells when activated by a physiological dual stimulus, through TCR and CD28.[51]

Roelen et al analyzed the production of cytokines during the primary MLR, secondary MLR, and the mitogenic proliferation response, both in the absence and presence of fetal MSCs.[40] They observed that the production of cytokines by the cultures was affected by the addition of fetal MSCs. The cytokines/growth factors that were significantly increased by coculture with fetal MSCs were IL-2, IL-4, IL-7, IL-10, IL-15, IFN-γ (in secondary MLR), and VEGF (vascular endothelial growth factor). Using similar assays, Kang et al observed lower levels of IL-17 and IFN-γ production in the supernatant from cocultures of HAMSCs and PBMCs in the presence of mitogens, compared to the supernatant obtained from cultures of PBMCs alone.[19] However, the level of IL-10 and TGF-β production increased significantly in the supernatant obtained from the cocultures of HAMSCs and PBMCs.[19] These same authors observed that messenger (m)RNA expression of TGF-β, hepatic growth factor (HGF), indoleamine 2,3-dioxygenase (IDO) and cyclooxygenase 2 (COX-2) were induced more, not only in HAMSCs grown in the presence of PBMCs, but also in HAMSCs separated from PBMCs by transwells, compared to those grown without PBMCs.[19] MLR with the addition of neutralizing antibodies to two of the potentially inhibitory cytokines, IL-10 and TGF-β, in a transwell cultures could abrogate the inhibitory effect of placenta-derived multipotent MSCs (fetal in origin), indicating that both are important factors mediating the suppressive capacity of these cells.[16,40] Fetal MSCs are more effective in inhibiting T cells than maternal MSCs, probably due to higher IL-10 production by the fetal cells.[40]

Cultured, expanded, placenta-derived MSCs have similar inhibitory effects on peripheral blood CD4+ and CD8+ and umbilical cord blood CD4+ and CD8+ lymphocyte proliferation induced by mitogens (PHA) and allogeneic peripheral blood lymphocytes, often in a dose-dependent manner.[16,17] These cells in the transwell chamber system could suppress CD4+, CD8+ T cells, and PBMCs, as in the usual coculture system.[16,17] In these studies, Li et al assayed the levels of IL-2, IFN-γ, and IL-10 in the supernatant of MLR cultures at different time points.[17] They observed that the presence of placental MSCs increased the level of IL-10 (Th2 cytokines) and decreased levels of IL-2 and IFN-γ (Th1 cytokines) (Figure 1). Following the acceptance of that divergence of naïve T-cells into Th1 or Th2 effectors partly depends on the cytokines that they encounter;[52] the authors considered that IL-10 might play a role in MSCs' regulatory effect.[17]

There are also some indications that the immunosuppressive effect of placenta-derived MSCs involves Treg cells, which are CD4+ $CD25^{high}$ T cells capable of modulating tolerance in the immune response.[16,40,49] Recently, T cells with a regulatory phenotype were detected in the placenta, and there was a greater than threefold increase in the proportion of CD4+ $CD25^{high}$ T cells in lymphocyte proliferation assays stimulated with PHA after 3 days of coculture with placenta-derived MSCs.[16] Using the Treg marker, Foxp3, a threefold increase in $CD34^{+}$/$Fox3^{+}$ lymphocytes was also found when placenta-derived stem cells were added to MLR[16] (Figure 1).

The state of T-cell activation and differentiation also appear to be critical to the immunomodulatory effects that AM-derived cells may elicit. Banas et al observed that in contrast to the significant inhibition of primary immune responses in the presence of AM-derived cells, preactivated T cells driven by IL-2 were not affected by coculture with AM-derived cells.[15] They concluded that when naïve or memory T cells are stimulated, they are prone to inhibitory effects of AM-derived cells, whereas activated T cells may be less affected.[15] However, Li et al reported that HAECs factors induced apoptosis of activated T cells (Jurkat cells: human

acute lymphoblastic T-cell leukemia, clone E6.1).[14] Although the mechanism by which HAECs mediated induction of apoptosis of lymphocytes remains unknown, the authors hypothesized that HAECs mediate caspase-dependent killing through the interactions between Fas-L and Fas-positive cells[14] (Figure 1).

In conclusion, in vitro, at least three interrelated mechanisms have been identified in the interaction of AM-derived cells with different cells of the immune system, both when added in a contact assay or a transwell setting: 1) AM-derived cells are hypoimmunogenic, and they block the generation and maturation of antigen-presenting cells; 2) they are capable of modulating T cell phenotype, modulating the immune response in vitro, and in particular, they are able to inhibit allogeneic lymphocyte proliferation; and 3) they abolish the production of inflammatory cytokines and immunosuppress the local environment.

Mechanisms of immunosuppression by AM-derived stem cells

Although several studies have documented the immunosuppressive activities of AM-derived stem cells, the underlying mechanisms are only partially known. They have been mainly investigated in MSCs isolated from BM.

Some studies indicate that MSCs are not spontaneously immunosuppressive; that priming by inflammatory cytokines is essential for MSCs-mediated immunosuppression.[53,54] During an immune response, the inflammatory cytokines produced by T cells and antigen-presenting cells modulate the function of MSCs, leading to the production of growth factors, altered expression of surface molecules, and release of immunosuppressive factors. Several reports have demonstrated that at sites of tissue damage, MSCs produce growth factors, such as epidermal growth factor, fibroblastic growth factor, platelet-derived growth factor, VEGF, insulin-like growth factor-1, stromal cell derived factor-1, TGF-β, and HGF; release large amounts of chemokines, especially CCL2, CXCL9, CXCL10, and CXCL11; and increase the expression of adhesion molecules, such as intercellular adhesion molecules (ICAM)-1 and vascular cell adhesion molecules (VCAM)-1.[53–55]

Cell adhesion molecules, such as B7-H1, ICAM, and VCAM, may participate in immunomodulation. The immunomodulation by human umbilical cord mesenchymal stem cells (hUC-MSCs) is largely mediated by cell-cell contact via adhesion molecules, particularly B7-H1.[56] Moreover, the adhesion molecules, ICAM-1 and VCAM-1, mediated immunosuppression in mouse BM-MSCs induced by IFN-γ from activated T cells; this activity was abrogated by antibodies against ICAM-1 and VCAM-1.[56]

Levels of HLA-G on cultured AM-derived cells are upregulated by exposure to IFN-γ.[15] The expression of these antigens increased substantially in AM-derived cells (from 4% to 36% after 5 days of incubation) when they were titrated into MLR.[15] Some of the inhibitory effects of AM-derived cells could be exerted by the HLA-G antigens through the killing inhibitory receptor ILT-4 pathway, which directly interacts with HLA-G as well as with HLA class I molecules.[15] HLA-G has been observed to upregulate the expression of ILT-2 and ILT-4 on NK and T cells. Although the mechanism is not fully understood, HLA-G has been shown to induce apoptosis of activated CD8+ cells and to inhibit CD4+ cell proliferation.[15,45,46,49] Moreover, the production of soluble HLA-G5 by amniotic cells has been shown to suppress T cell proliferation and NK cell and T cell cytotoxicity and to promote the generation of regulatory T cells.[15,49]

Under some experimental conditions, the inhibition of T cell proliferation by amniotic cells requires engagement of the inhibitory surface protein PD1 by its ligand 1 (PD-L1). PD-L1 has been found to be expressed by the syncytiotrophoblast in early pregnancy and PD-L2 on all trophoblast populations throughout pregnancy.[23,57] Both seem to play key roles in T-cell mediated tolerance of the semi-allogeneic fetus, as binding of either ligand of the PD1 receptor (expressed on activated T and B cells) inhibited antigen-stimulated T-cell activation and cytokine production in vitro. It has been proposed that, in an early allogeneic environment in which pro-inflammatory cytokines may be present, amniotic cells may upregulate PD-L1 expression, which in turn may inhibit T-cell activation and proliferation.[15] In vitro PD-L1 is upregulated in placenta-derived MSCs after stimulation with IFN-γ.[16]

It is likely that through the synergistic action of the chemokines and adhesion molecules, immune cells accumulate in close proximity to the MSCs, where the high concentration of secreted factors can suppress immune cells effector functions.[53,54]

In response to immune cells, HAMSCs, as MSCs obtained from other sources, release some soluble factors, resulting in the suppression of proliferation and inhibition of the release of pro-inflammatory cytokines by immune cells; these molecules include IL-10, TGF-β1, HGF, IDO, and prostaglandin E-2 (PGE2)[16,17,19,44,53] (Table 4).

IL-10 is a cytokine that functions as a broad spectrum anti-inflammatory cytokine by inhibiting production of IL-1, TNF-α, and other pro-inflammatory factors.[53] IL-10 has also

Table 4 Primed AM-derived stem cells

Immunosuppressive factors	References
IL-10	53,57
TGF-β	16,19,49,57
IDO	16,19
PGE2	3,19,60

Abbreviations: AM, amniotic membrane; IDO, indoleamine 2,3-dioxygenase; IL, interleukin; PGE2, prostaglandin E-2; TGF-β, transforming growth factor beta.

been implicated in the inhibitory effect on T cell proliferation exerted by fetal MSCs at the fetomaternal interface.[57]

TGF-β is a potent anti-inflammatory cytokine that enhances the immunomodulatory properties of placenta-derived MSCs.[16] The possible participation of TGF-β in the immunomodulatory effect exerted by HAMSCs on PBMCs has been suggested by several investigators who observed a strong increase in the expression of TGF-β mRNA in HAMSCs after 3 days of IFN-γ treatment [16] and coculturing with leukocytes,[19] and an increased level of TGF-β in the culture supernatant obtained from HAMSCs and PBMCs cocultured for 3 days.[19] The immunosuppressive effect of placenta-derived MSCs has been abrogated with anti-TGF-β antibodies.[16,19,49,57]

IDO is another well-known immune-suppression factor constitutively expressed by placenta-derived stem cells.[16] It is a key regulator of placental immunotolerance during pregnancy that inhibits various immune cell populations, including T cell and NK cells. IDO catalyzes the rate-limiting step in the degradation of tryptophan, an essential amino acid, along the kynurenine pathway.[16,19,53] The resulting reduction in local tryptophan concentration and the production of tryptophan metabolites that are immunomodulatory are thought to contribute to the immunosuppressive effects of IDO-expressing cells. IDO mRNA and kynurenine production increased when HAMSCs and PBMCs were cocultured, suggesting that IDO was induced by coculturing and participated in immune modulation by HAMSCs.[19] In BM-MSCs, derived IDO was reported to be required to inhibit the proliferation of IFN-γ-producing Th1 cells and, together with PGE2, to block NK-cell activity.[58–60]

PGE2 is another inflammatory stimulus-induced, immunosuppressive molecule produced by MSCs.[53] It regulates the maturation and antigen presentation of DCs and inhibits T cell proliferation and cytokine production.[19,53] It is synthesized from arachidonic acid by COX-1 and COX-2 enzymes, which are constitutively expressed by MSCs, indicating that PGE2 is also constitutively expressed.[19] HAECs and HAMSCs produce PGE2 in culture, although the basal PGE2 output appears to be significantly greater in HAMSCs than in the epithelial cells.[3] The PGE2 production increased in HAMSCs when they were cocultured with PBMCs.[19] It has been reported that PGE2 is the most powerful immunomodulatory factor in hUC-MSCs because inhibition of PGE2 synthesis almost completely mitigates the immunosuppressive effect, whereas neutralization of TGF-β and IDO has little effect.[60]

In summary, it is possible that cell-cell contact and several immunosuppressive mediators produced by AM-derived cells, upon triggering by inflammatory factors, are involved in their immunomodulation properties. The importance of any specific mediator could vary depending upon the local microenvironment leading in a redundant system involving more than one mechanism. However, further studies are needed to clarify the specific role of such factors.

Potential use of AM-derived stem cells to treat inflammatory diseases

The data discussed here on the immunomodulatory properties of AM-derived stem cells are in accordance with those described for MSCs obtained from other sources such as bone marrow,[44,49,53,58–60] adipose tissue,[13,61,62] and cord blood.[49,63–65] However, mainly MSCs isolated from bone marrow have been extensively studied in animal disease models, such as graft versus host disease, experimental autoimmune encephalomyelitis (EAE), collagen-induced arthritis, inflammatory bowel disease, diabetes type 1, and systemic lupus erythematosus. The fact that AM-derived cells are plentiful, easily obtained from a normally discarded amnion tissue, and are not associated with any substantial ethical issues supports the use of HAECs and HAMSCs as a potential therapy to modulate pathogenic immune responses.

Here we present some studies on animal models of diseases with an inflammatory component where the AM-derived stem cells have been used as a therapy to modulate pathogenic immune responses.

Neurological disorders

HAECs have been used for the treatment of spinal cord injury in animal models. In this condition, the inflammation-mediated secondary injury plays an important role in many of the observed deleterious effects.[66] After transplantation of HAECs into the damaged areas of a contusion model of spinal cord injury in nonimmunosuppressed monkeys, cells survived up to 120 days in the transplanted environment, supported the growth of host axons, prevented the formation of glial scar, prevented death of axotomized neurons, and induced new collateral sprouting with no evidence of

inflammation or rejection. Furthermore, improved performance in locomotion tests was observed in treated animals compared to control animals.[27] HAECs transplanted into the injured spinal cord of rats survived during 8 weeks and integrated into the host spinal cord without immune rejection. Compared with the control group, HAECs promoted regeneration and spouting of the axons, improved the hind limb motor function of the rats, and inhibited the atrophy of axotomized cells.[28] In the same way, recently it has been demonstrated that HAECs transplanted into the spinal cord of T13 spinal cord hemisected rats suppressed mechanical allodynia and reduced the expression of the microglial marker, F4/80 proteins, known to be involved in spinal cord injury and inflammation.[67]

Stroke is another neurological disorder in which inflammation has been implicated as a major contributor to the secondary cell death cascade following on from the initial stroke episode. Cells from different sources have been proposed as a novel potential treatment to abrogate the inflammatory side effects observed in this disease.[68] Transplantation of HAECs and HAMSCs has been shown to exert beneficial effects in a nonimmunosuppressed rodent stroke model. Transplantation of AM-derived cells directly into the ischemic penumbra at day 1 or 2 poststroke ameliorated behavioral dysfunction, attenuated both motor and neurological deficits, and reduced infarct size associated with occlusion of the middle cerebral artery compared to the vehicle-infused stroke group.[29,30] Following the last behavioral test at day 14 poststroke, histology via Nissl staining revealed an increased number of healthy-looking cells in the ischemic penumbra compared to the vehicle-infused stroke group.[11,29]

In rats with intracerebral hemorrhage, the effects on brain edema and neurological functional recovery after transplantation of HAECs into the lateral ventricle have also been evaluated. The behavior of the animals and brain edema were evaluated after 28 days, and brain sections were made for morphological and immunohistochemical analyses with fluorescence microscopy. Transplanted HAECs were observed along the lateral wall and survived for at least 4 weeks. Around the injury site, activation of microglia was reduced. The water content of intracerebral hemorrhage rats decreased in the treatment group. The behavior test scores were improved in the treatment group compared with those in the control groups.[69]

Multiple sclerosis

Multiple sclerosis is a T cell-mediated autoimmune inflammatory disease of the central nervous system (CNS). HAECs have been examined for the therapeutic effect in an EAE (experimental autoimmune encephalomyelitis) mouse model of multiple sclerosis.[32,70] McDonald et al reported that intraperitoneal injection of HAECs suppressed symptoms and decreased CNS inflammation, demyelination, and axonal degeneration in the spinal cord and brain of a mouse model of multiple sclerosis.[32] They also found that HAECs reduced proliferation of T cells and decreased their secretion of proinflammatory cytokines.[32] More recently, Liu et al reported that intravenously administered HAECs reduced CD3+ T cell and F4/80(+) monocyte/macrophage infiltration and demyelination within the CNS of an EAE mouse.[70] HAECs immunosuppression was mediated by PGE2 and TGF-β, as it was demonstrated by the neutralization of TGF-β or PGE2 in splenocyte proliferation assays. Splenocytes from HAEC-treated mice showed a Th2 cytokine shift with significantly elevated IL-5 production.[70]

Liver disease

Transplantation of HAECs in a liver disease model of fibrosis in mice induced by administration of carbon tetrachloride (CCl4) led to hepatic engraftment observed at 4 weeks after transplantation.[31] CCl4-treated immunocompetent mice receiving single or double HAEC doses showed a significant, but similar, decrease in liver fibrosis area associated with decreased activation of collagen-producing hepatic stellate cells, and a decrease in hepatic protein levels of the profibrogenic cytokine, TGF-β1. CCl4 administration caused hepatic T cell infiltration and an increased number of hepatic macrophages compared to normal mice; both types of cells decreased significantly following HAECs transplantation. Treated mice had significantly lower hepatic protein levels of the chemokine monocyte chemoattractant protein-1 (MCP-1), than mice given CCl4 alone, and showed increased expression of genes associated with M2 macrophages, including YM-1, IL-10, and CD206, all associated with fibrosis resolution. The transplantation of HAECs resulted in a reduction of hepatocyte apoptosis and a decrease in inflammation and fibrosis.[31]

Lung injury

In an inflammatory lung injury model in mice, where damage was induced by administration of bleomycin, a potent stimulator of lung fibrosis, several assays have evidenced that HAECs modulated the host inflammatory response, reduced lung fibrosis, and prevented loss of lung function.[71–73] The effect was concomitant with a reduction in proinflammatory cytokines (TNF-α, IFN-γ, MCP-1, and IL-6 in

the mouse lung) and resulted in a decrease in inflammatory cells infiltration and an increase in the anti-inflammatory cytokine IL-10.[72,73] Lung tissue repair was promoted via paracrine-acting molecules, as demonstrated through the administration in similar animal models of conditioned medium (CM) generated from human amniotic mesenchymal tissue cells (AMTC). At day 14, lung fibrosis scores in AMTC-CM treated mice were significantly lower compared with mice of the bleomycin group in terms of fibrosis distribution, fibroblast proliferation, collagen deposition, and alveolar obliteration. No differences were observed between mice in the bleomycin group and mice treated with control medium. AMTC-CM treatment significantly reduced the fibrosis progression between the two observation time-points, emphasizing the importance of the MSC secretome.[74]

Conclusion

Results in vitro and from preclinical animal models suggest that human AM-derived cells have the capacity to strongly suppress immune responses, potentially induce peripheral tolerance, and reverse ongoing inflammatory damage. In the preclinical studies mentioned above, the investigators observed that the immunomodulatory effects of AM-derived cells blunt the inflammatory response and allow tissue remodeling after injury, resulting in reduced numbers of fibroblasts and less scarring in the different organs evaluated, such as the spinal cord, liver, and lung. In these studies, the effects were unrelated to the transdifferentiation of AM-derived cells in other cell types. Regardless of the type of injury, the therapeutic effect of AM-derived cells seems to depend on the release of trophic and anti-inflammatory molecules, as reported for BM-derived MSCs.[44] However, further research using AM-derived cells in animal models of inflammatory diseases is required to identify the detailed mechanisms responsible for their immunomodulatory effects and to validate the efficacy of these cells in immune-mediated illnesses, with the aim of translating these results into clinical studies in humans.

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Disclosure

The authors declare no conflicts of interest in this work.

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