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Published on: 25 Feb 2010 - Stem Cells and Development (Mary Ann Liebert, Inc. publishers 140 Huguenot Street, 3rd Floor New Rochelle, NY 10801-5215 USA)

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Abstract: Among the many cell types which may prove useful to regenerative medicine, mounting evidence suggests that human term placenta-derived cells will join the list of significant contributors. In making new cell therapy-based strategies a clinical reality, it is fundamental that no a priori claims are made regarding which cell source is preferable for a particular therapeutic application. Rather, ongoing comparisons of the potentiality and characteristics of cells from different sources should be made to promote constant improvement in cell therapies, and such comparisons will likely show that individually-tailored cells can address disease-specific clinical needs. The principle underlying such an approach is resistance to the notion that comprehensive characterization of any cell type has been achieved, neither in terms of phenotype nor risks-to-benefits ratio. Tailoring cell therapy approaches to specific conditions also requires an understanding of basic disease mechanisms and close collaboration between translational researchers and clinicians, to identify current needs and shortcomings in existing treatments. To this end, the international workshop entitled "Placenta-derived stem cells for treatment of inflammatory diseases: moving toward clinical application" was held in Brescia, Italy, in March 2009, and aimed to harness an understanding of basic inflammatory mechanisms inherent in human diseases with updated findings regarding biological and therapeutic properties of human placenta-derived cells, with particular emphasis on their potential for treating inflammatory diseases. Finally, steps required to allow their future clinical application according to regulatory aspects including good manufacturing practice (GMP) were also considered. In September, 2009, the International Placenta Stem Cell Society (IPLASS) was founded to help strengthen the research network in this field.

DOI: https://doi.org/10.1089/scd.2009.0404

placenta-derived cells: Disease mechanisms, cell biology, preclinical studies, and regulatory aspects at the round table. Stem Cells and Development, 19(2):143-154.
DOI: https://doi.org/10.1089/scd.2009.0404

STEM CELLS AND DEVELOPMENT
Volume 19, Number 2, 2010

DOI: 10.1089/scd.2009.0404

Toward Cell Therapy Using Placenta-Derived Cells: Disease Mechanisms, Cell Biology, Preclinical Studies, and Regulatory Aspects at the Round Table

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Among the many cell types that may prove useful to regenerative medicine, mounting evidence suggests that human term placenta-derived cells will join the list of significant contributors. In making new cell therapy-based strategies a clinical reality, it is fundamental that no a priori claims are made regarding which cell source is preferable for a particular therapeutic application. Rather, ongoing comparisons of the potentiality and characteristics of cells from different sources should be made to promote constant improvement in cell therapies, and such comparisons will likely show that individually tailored cells can address disease-specific clinical needs. The principle underlying such an approach is resistance to the notion that comprehensive characterization of any cell type has been achieved, neither in terms of phenotype nor risks-to-benefits ratio. Tailoring cell therapy approaches to specific conditions also requires an understanding of basic disease mechanisms and close collaboration between translational researchers and clinicians, to identify current needs and shortcomings in existing treatments. To this end, the international workshop entitled "Placenta-derived stem cells for treatment of inflammatory diseases: moving toward clinical application" was held in Brescia, Italy, in March 2009, and aimed to harness an understanding of basic inflammatory mechanisms inherent in human diseases with updated findings regarding biological and therapeutic properties of human placenta-derived cells, with particular emphasis on their potential for treating inflammatory diseases. Finally, steps required to allow their future clinical application according to regulatory aspects including good manufacturing practice (GMP) were also considered. In September 2009, the International Placenta Stem Cell Society (IPLASS) was founded to help strengthen the research network in this field.

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Mechanisms of Inflammatory Disease: Will an Increased Understanding Light the Path to Effective Cell Therapy-Based Treatments?

Inflammation is mediated by several cell types that patrol the organism in border areas where the body comes into contact with the environment. While it functions primarily to restrict dangerous events, the inflammatory response is Janus faced, encompassing not only protective/regenerative effects, but also deleterious outcomes [1,2].

Innate recognition, at the basis of inflammatory response initiation, takes place through receptors on macrophages, epithelial cells, and several other cells. Innate receptors recognize microorganism-derived molecular patterns (Toll-like receptors, scavenger receptors, lectin-like receptors) and endogenous stress-dependent molecules (inflammatory cytokines, complement). Upon receptor activation, cells initiate the defense response. Macrophages phagocytose and degrade foreign material, concomitantly producing inflammatory and toxic factors (cytokines, reactive oxygen and nitrogen species, prostaglandins, degrading enzymes) to destroy the invader and activate the adaptive immune response. IL-1 family cytokines and receptors are central to macrophage-dependent inflammation and the transition to adaptive immunity. However, the potent inflammatory effects of IL-1 must be tightly controlled to avoid excessive damage of surrounding host tissues.

The mechanisms governing inflammation are in delicate equilibrium, and pathological derangements can occur easily when this equilibrium is broken. An example is erroneous recognition of self molecules, causing chronic triggering of innate receptors. Indeed, most chronic inflammatory and autoimmune diseases are related to uncontrolled inflammation following recognition of endogenous molecules.

For optimal treatment of inflammatory diseases, precise timing in inhibiting inflammation is needed, to inhibit deleterious effects while promoting regenerative effects. The need for such fine-tuning is exemplified by the role of matrix metalloproteinases (MMP) after cerebral ischemia. Inhibition of MMP9 within hours after stroke reduces tissue injury and cerebral infarct size; however, MMP inhibition after 7 days impairs recovery by preventing angiogenesis, neuroblast migration, and brain remodeling [3,4]. Thus, timed "immunomodulation" is key to optimal treatment of inflammation.

Cell therapy has been proposed for treatment of chronic inflammation and immune alterations (multiple sclerosis, inflammatory bowel disease (IBD), arthritis, graft versus host disease) and ischemia (coronary/peripheral artery disease, stroke). Current evidence suggests that cell replacement is likely not the major mechanism by which cell therapy confers functional benefit. Despite reports of bone marrow-derived stem cells differentiating into neural cells or "fusing" with diseased neurons, the number of engrafted cells is low and likely insufficient to account for the observed functional improvements. Rather, increasing experimental data indicate that stem or progenitor cells, such as mesenchymal stromal cells (MSC), act beneficially by exerting trophic effects on host cells, reducing apoptotic cell death and stimulating angiogenesis. Indeed, administration of MSC after cerebral ischemia leads to increased angiogenesis, neurogenesis, synaptogenesis, and oligodendrogenesis, with axonal sprouting [5] either directly or by stimulating endogenous cells to secrete trophic and protective factors [5,6]. Concomitantly, the immunomodulatory effects of MSC, independently of the source, blunt the inflammatory response and allow tissue remodeling after injury, resulting in reduced numbers of fibroblasts and less scarring in the heart [7,8], lung [9], and kidney [10] and less astroglial scarring in the brain [7].

In developing therapeutic treatments, the administration route must be carefully considered. Administration can be local, such as intraparenchymal (eg, into muscle or brain) or intra-articular (eg, during arthritis), or systemic (eg, intra-arterial and intravenous). A limitation of intravenous delivery is that most administered cells are trapped in the lung and spleen. Emerging evidence indicates that after ischemic stroke the peripheral immune response is activated and immune cells migrate to the brain and contribute to cerebral injury [11,12]. Intravenous administration of hematopoietic stem cells and umbilical cord blood cells reduces cerebral ischemic injury and inflammation at least partly by interfering with splenic and lymphoid activation [13–15], suggesting that intravenous delivery might be preferable in pathologies involving lymphoid activation.

Although the anti-inflammatory and immunomodulatory effects discussed above pertain to stem or progenitor cells in general, accumulating evidence suggests that similar mechanisms might also accompany placenta-derived cells. Furthermore, the development of cell therapy approaches using these cells may also benefit from the fact that placental tissues harbor different cell types that may complement each other in a clinical setting (ie, amniotic epithelial cells of early embryological origin with multilineage differentiation potential, as well as cells with immunomodulatory properties [16]). Furthermore, aside from being easily procured in a painless and noninvasive manner, placental cells also offer additional advantages over stem cells from other sources such as bone marrow, which carry a risk of viral infection [17], and also show decreasing differentiation capacity with increasing donor age [18,19]. Finally, it is tempting to speculate that placenta-derived cells may also be preferable from an immunological point of view, given the unique role of this tissue in maintaining fetomaternal tolerance throughout pregnancy, and supported by the finding that placental cells show a greater capacity to down-regulate T-cell proliferation in vitro compared to bone marrow-derived cells (Parolini et al., unpublished data).

Here, we will provide an overview of the current state of the art regarding the potential of placenta-derived cells to treat inflammatory diseases, beginning with an update on the most recent findings on the characteristics of these cells as well as a detailed discussion of their immunomodulatory properties. Indications from preclinical studies supporting the use of these cells for treatment of a wide range of conditions will also be presented. Finally, aspects that will need to be addressed for GMP production of these cells for clinical application will be discussed.

Update on Characteristics and Handling Methods for Stem/Progenitor-Like Cells in the Human Placenta

The placenta is a fetomaternal organ consisting of 2 components: the maternal component, termed the decidua,

originating from the endometrium, and the fetal component, including the fetal membranes–amnion and chorion–as well as the chorionic plate, from which chorionic villi extend and make intimate contact with the uterine decidua during pregnancy.

Since the first studies on placenta-derived stem cells were reported, it has been recognized that, due to the complexity of the placental structure, there is a need for assigning proper terminologies to the different regions of this organ and to the various cell types that can be isolated from these regions. To this end, as reported by Parolini et al. [16], the First International Workshop on Placenta-Derived Stem Cells saw the following nomenclature proposed: human amniotic epithelial cells (hAEC), human amniotic mesenchymal stromal cells (hAMSC), human chorionic mesenchymal stromal cells (hCMSC), and human chorionic trophoblastic cells (hCTC). Furthermore, isolation protocols, phenotypic markers, and in vitro differentiation potential have been described for hAEC, hAMSC, and hCMSC [16].

Characterization of hAEC has shown that these cells express molecular markers of pluripotency, and can differentiate in vitro into cell types of all 3 germ layers [16]. More recently, comparative analysis of hAEC from amnion of early-stage pregnancies and from term amnion showed that expression of the stem cell-specific cell surface markers TRA1-60 and TRA1-81, and of the molecular markers of pluripotency Nanog and Sox2, are all significantly higher in fetal amnion, while expression of Oct-4 mRNA is similar between cells obtained either from fetal or term amnion [20].

hAMSC and hCMSC are defined as plastic-adherent cells that are capable of forming fibroblast colony-forming units and displaying a specific pattern of cell surface antigens comparable to that of bone marrow MSC ($CD90^+$, $CD73^+$, $CD105^+$, $CD45^-$, $CD34^-$, $CD14^-$, $HLA\text{-}DR^-$). These cells are also capable of differentiating toward one or more lineages including osteogenic, adipogenic, chondrogenic, and vascular/endothelial[16]. Furthermore, recent reports suggest that, like the amniotic epithelial fraction, the human amniotic mesenchymal region also harbors a multipotent side population showing multilineage differentiation potential [21].

Recent advances regarding the differentiation potential of placenta-derived cells have shown expression of major cartilage components by hAMSC after chondrogenic induction, with deposition of collagen II after implantation of these cells into the subfascial space of the abdominal muscle of mice [22]. Mesenchymal cells from the amniotic and chorionic membranes have also been recently shown to differentiate in vitro into a range of neuronal and oligodendrocyte precursors [23]. In addition, use of amniochorionic membrane as a scaffold has been proposed for improving osteogenic differentiation of chorionic membrane-derived cells (Mohr S. et al., submitted 2009). Meanwhile, it has been recently shown that hAEC display bifunctional hepatic differentiation potential in vitro, with the ability to differentiate into both parenchymal hepatocytes as well as cells expressing a molecular marker profile consistent with biliary cells and which form tubular 3D structures reminiscent of bile ducts when cultured on extracellular matrix [24]. However, discrepant results have been reported for osteogenic and adipogenic differentiation of hAEC and hAMSC [25,26], indicating the heterogeneous nature of these cell populations, and highlighting the need to develop better methods for selecting progenitor cells from placental tissues.

In exploring placental tissues as a source of progenitor cells, researchers have focused their attention mainly on cells derived from fetal tissue, in particular from amnion, chorion, and umbilical cord [16,27,28]. However, stem/progenitor properties of placental cells of maternal origin have also been described [29]. Recently, a comparative phenotypical study between bone marrow- and placenta-derived mesenchymal cells has underlined the fact that the cell types have a very similar cell surface marker profile; however, they differ in their expression of the chemokine receptors CCR1 and CCR3 [30], which are only present on placenta-derived cells; meanwhile, other molecules such as CD56, CD10, and CD49d have been shown to be more highly expressed on placenta-derived mesenchymal cells [31], and differences in proliferation potential have been also observed between these 2 cell types [29].

However, based on the lack of significant differences between these above-mentioned cell types, as well as the fact that placental cells are plentiful and easily procured, a good manufacturing practice (GMP)-compliant facility has been established for isolating and expanding human placenta-derived MSC in a first clinical trial setup [32].

As a potential source of MSC, placental tissues have also attracted recent interest in the hematopoiesis field. During mouse embryogenesis, the placenta has been newly unveiled as an important niche for hematopoietic stem cell (HSC) development, although the origin of placental hematopoiesis remains undefined. Recent advances in this field come from a study using $Ncx1^{-/-}$ embryos lacking a functional heart and circulation. Rhodes and colleagues [33] found hematopoietic progenitors with both myeloerythroid and lymphoid potential in the placenta of these embryos, as well as in dorsal aorta, yolk sac, and vitelline vessels, indicating that these cells arose in situ. Meanwhile, the potential role of human placenta in embryonic hematopoiesis has also been recently documented, together with development of procedures for processing and storing hematopoietic cells from placental tissue [34,35]. Specifically, $CD34^+CD45^{dim}$ cells isolated from human placenta were shown to contain myeloid and erythroid progenitors [34,35] and were capable of generating $CD56^+$ natural killer cells and $CD19^+CD20^+sIgM^+$ B cells in vitro [34]. More importantly, human placenta has been shown to contain bona fide HSCs throughout fetal development [36]. HSCs can be detected as early as gestational week 6 (either $CD34^+$ or $CD34^-$), and most strikingly at term. At weeks 16–20, HSCs are more enriched in the $CD34^+$ fraction. After storage in liquid nitrogen, the placental cells retain their HSC potential, suggesting this tissue as an important source for banking and clinical application [36].

Recent efforts have also been dedicated to optimizing isolation, culture, and preservation methods for placenta-derived cells. These include a study aimed at defining cell yields obtainable from the amniotic epithelial and mesenchymal regions [26], while others have proposed long-term expansion methods to allow thorough analysis of cellular material before use in cell-based therapies. Immortalized hAMSC have been established through ectopic expression of the human telomerase catalytic subunit, and compared to the parent population, the resultant cells displayed unaltered surface marker profile, morphology, karyotype, and

differentiation potential for up to 87 population doublings, as well as similar or reduced immunogenic/immunosuppressive properties [37]. Alternatively, it has also been shown that in vitro life span can be dramatically extended by optimizing expansion conditions. For example, when cultured with animal-free culture supplements such as human platelet lysate (PL), a suitable alternative to fetal calf serum (FCS) for MSC cultures [38,39], hAMSC have been shown to exhibit an increased proliferation potential and in vitro life span compared to cells cultured with FCS (Wolbank, unpublished data). However, although different culturing methods may influence cell behavior, it should be noted that in vitro culture itself can also cause some alterations, as highlighted by a study in which hAEC displayed reduced osteogenic potential associated with a phenotypic shift after culturing [25].

Finally, recent attempts to improve cryopreservation of placenta-derived cells have demonstrated that vitrification, which uses a high cryoprotectant concentration and does not require a programmable temperature-decreasing container, represents a fast preservation method. This has proved reliable and effective for long-term preservation of hAMSC, showing retention of surface marker expression and differentiation potential after thawing [40].

In summary, since the first studies on placenta-derived stem cells were published, much knowledge has been gained regarding the characteristics, handling methods, and potential of these cells. However, to maintain uniformity and clarity in the field, precise descriptions of the placental regions from which different cell populations are isolated are paramount, along with extensive phenotypic analyses of these cells.

Immunomodulatory Properties of Placenta-Derived Cells

Based on their role in maintaining fetomaternal tolerance during pregnancy, multiple reports have investigated the immunomodulatory properties of placenta-derived cells with the aim of validating their applicability in cell therapy-based treatments.

Like human bone marrow-derived MSC, cells derived from different placental regions are poor antigen-presenting cells due to their low or limited expression of MHC class II and co-stimulatory molecules [16,41]. Moreover, in vitro studies show that amniotic and chorionic membrane-derived cells not only fail to induce an allogeneic or xenogeneic immune response in mixed lymphocyte reactions, but also strongly suppress lymphocyte proliferation induced by mitogens or alloantigens, often in a dose-dependent manner [42–46]. Moreover, amniotic membrane-derived cells exert immunomodulatory effects on antigen-presenting cells, as demonstrated by their capacity to block maturation of monocytes into dendritic cells (DC), preventing expression of the DC marker CD1a and reducing expression of HLA-DR, CD80, and CD83. This monocyte maturation block also resulted in impaired allostimulatory ability of these cells on allogeneic T cells [47].

The immunomodulatory properties of placenta-derived cells may involve direct cell-to-cell contact [48], although equally compelling evidence implies a mechanism based on secretion of soluble factors, as demonstrated by immunomodulatory effects of these cells on lymphocytes even when the 2 cell types were separated by a semipermeable membrane (transwell system) [44]. Although the factors involved remain to be identified, it has been proposed that these may include indoleamine 2,3-dioxygenase (IDO), transforming growth factor β (TGF-β), and interleukin-10 (IL-10) [41,49,50]. Along this line, a recent comparative study demonstrated that at the fetomaternal interface, fetal MSC have a stronger inhibitory effect on T-cell proliferation compared with adult MSC, which is probably related to higher IL-10 production by the fetal cells [50].

Despite these interesting in vitro findings, some caution must be taken when extrapolating such results toward an in vivo setting. In particular, in vitro cell proliferation or stimulation experiments usually investigate interplay between placenta-derived cells and a "single" specific cell population (eg, DC, T cells), whereas in vivo, "multiple" distinct cell populations act together in initiating, maintaining, or suppressing immune reactions. In this context, it is unlikely that the placental cell:T-cell ratios used in in vitro experiments (eg, 1:1 to 1:10) would be paralleled in vivo. Furthermore, isolation and expansion protocols for placental cells might also influence their immunomodulatory properties. For example, expansion of amniotic mesenchymal cells without prior removal of the HLA-DR$^+$ subpopulation [44] may result in partial abrogation of the immunosuppressive effects of these cells in vitro or in vivo.

Several preclinical studies have already reported prolonged survival of human placenta-derived cells after xenogeneic transplantation into immunocompetent animals including rats [45,51–54], swine [45], and bonnet monkeys [55], with no evidence of immunological rejection. Furthermore, co-transplantation of cord blood and placental MSC has been shown to result in enhanced cord blood cell engraftment and improved homing of CD34$^+$ cells [56,57]. Migration to various organs and specific differentiation have also been observed after in utero transplantation of human placenta-derived MSC into fetal rats [58].

Although clear immunosuppressive effects have been observed for placenta-derived cells in vitro, as well as promising results (ie, survival) following transplantation in vivo, it should be noted that in a recent study, murine placenta-derived cells failed to survive following transplantation under the kidney capsule of allogeneic hosts due to recognition and rejection by the recipients' immune system [59]. Such immune-mediated rejection, which has also been observed with bone marrow MSC in an allogeneic setting [60–63], constitutes a major challenge for the development of future clinical applications [64].

Preclinical Evidences and Functional Mechanisms Supporting the Utility of Placenta-Derived Cells for Treating Inflammatory Diseases

In 1910, Davis was the first to report the use of fetal membranes in skin transplantation [65], prompting subsequent applications that demonstrated the utility of these membranes for treating other conditions including leg ulcers [66,67] and burns [68,69], as well as for applications in ophthalmology [70–72]. One century later, although fetal membranes in toto continue to be applied therapeutically in some settings, the focus of scientific investigations has turned to

the various cell populations present in these membranes, with accumulating evidence now lending support to the hypothesis that placental cells may be useful for treating a range of pathologic conditions.

Ocular surface disorders

The first clinical applications of amniotic membrane were reported for treatment of ocular surface disorders in the 1940s, and after a hiatus, use of this membrane was reintroduced to the field in 1995 by Kim and Tseng [73]. Today, use of the stromal matrix of the amniotic membrane, rather than of cells derived from it, continues to represent the main strategy by which placental tissues are applied in ophthalmology.

Use of the amniotic membrane in this field is based on several mechanisms mediated by various cytokines and enzymes, ultimately conferring beneficial effects including enhanced epithelialization and wound healing, suppression of inflammation and fibrosis, and inhibition of angiogenesis. In this context, it is noteworthy that the angiogenic profile secretome largely depends on the preparation method of amniotic membrane, which should therefore be considered when selecting an amniotic product for clinical application [74].

The anti-scarring properties of amniotic membrane are mediated by the presence of fetal hyaluronic acid in the stromal matrix, which suppresses TGF-β signaling, cell proliferation, and myofibroblastic differentiation of normal corneal and limbal fibroblasts, as well as of normal conjunctival and pterygium fibroblasts [75]. The amniotic stromal matrix also suppresses expression of inflammatory cytokines originating from the ocular surface epithelia, including IL-1α, IL-1β [76], and IL-8 [77], and up-regulates the expression of IL-1 receptor antagonist [76]. Such suppression of inflammation is paramount for preventing corneal and conjunctival scarring, neovascularization, and fibrosis. Several clinical applications in ocular surface reconstructive surgery have now been developed based on these properties [73].

The most important ophthalmology-based application of amniotic membrane is as a temporary bandage after acute chemical burns of the ocular surface [78]. In this scenario, the membrane serves as an anti-inflammatory agent during acute phases of chemical and thermal burns, limiting corneal and limbal inflammation and angiogenesis [79] and reducing symblepharon formation. A possible anti-inflammatory mechanism of the amniotic membrane may be trapping of inflammatory cells infiltrating the ocular surface after a chemical burn, whereby as these cells are caught in the membrane, they undergo apoptosis [80]. This hypothesis is in accordance with a study showing that both epithelial and mesenchymal cells of the amniotic membrane express Fas ligand (CD95), a cell surface receptor that mediates apoptosis [81].

Additionally, various other studies report significant reductions in inflammation when transplantation of amniotic membrane is applied during treatment of ocular surface disorders, including pterygium surgery [82], fornix reconstruction, and symblepharon repair [83], reconstruction of deep corneal ulcers and persistent epithelial defects in autoimmune and inflammatory disorders of the ocular surface [84,85], and in treatment of chronic pseudophakic corneal edema [86].

Neurological disorders

Neurological disorders represent a significant burden to western societies, highlighting the need to develop effective therapies. Cell replacement therapy has been proposed as a basis for new treatment strategies for a broad range of neurological diseases; however, the paucity of suitable cell types has so far hampered the development of this promising therapeutic approach [87]. In this context, placenta-derived cells have been investigated for their potential to confer beneficial effects in a range of neurological disorders.

In preclinical studies using animal models of Parkinson's disease [52,88–90] and ischemia [53], hAEC have been found to offer neuroprotection and functional recovery. The observed therapeutic effects are likely mediated by secretion of diffusible factors, including neurotransmitters [91–93] and many neurotrophic and growth factors [94,95].

The potential utility of placenta-derived cells has also been investigated for treatment of spinal cord injury (SCI), a condition in which inflammation-mediated "secondary injury," rather than the primary physical force, has been implicated for many of the devastating effects observed [96]. Therefore, counteracting these cell death cascades with a suitable therapy at the earliest possible time postinjury would likely translate into a successful treatment. Similarly, the tissue damage caused by the host's immune response following injury may be suppressed by the anti-inflammatory properties of placental cells, which further make them attractive candidates for SCI treatment. Indeed, hAEC transplantation has been shown to produce beneficial effects after transection SCI in bonnet monkeys and rats [55,97] with functional improvement observed in rats. Similarly, when cotransplanted with neural stem cells, AEC have been shown to enhance recovery after contusive SCI in rats [51]. Despite these promising data, however, there still appear to be critical gaps in the knowledge that would be required to allow commencement of clinical trials using placental stem cells for SCI repair. For example, of the many placental cell types showing stem cell features [16,98,99], only hAEC have been tested for SCI repair to date and much work is still required to fully prove their efficacy and the mechanisms underlying their functional benefits in this setting. Meanwhile, a study in which hAMSC were transplanted into an experimental model of SCI showed chondrogenic differentiation of these cells (Sankar et al., unpublished data). This could be due to release of TGF-β by host cells as part of the inflammatory reaction following SCI [100,101], which is also known to be a powerful inducer of stem cell chondrogenic differentiation [102]. Therefore, as for all applications in which placenta-derived cells have been proposed for therapy, use of these cells for SCI treatment clearly requires further validation.

Stroke is another serious neurological disorder representing a current unmet medical condition of significance worldwide, and for which placenta-derived cells may offer new hope for therapy. In the United States, stroke is the third leading cause of death and the primary cause for disability.

Because inflammation is a major contributor to the secondary cell death cascade following the initial stroke episode, transplanted cell-mediated abrogation of such inflammatory deleterious side effects should directly alter stroke progression. A major caveat for this anti-inflammatory mechanism to effectively mitigate cell therapy and stroke outcome is demonstrating robust and stable secretion of

anti-inflammatory factors by transplanted cells at the appropriate timing postinjury. Although inflammation is shown to exacerbate stroke, early pathological inflammatory cues, such as stromal derived factor-1, serve as a migratory guide for transplanted cells to reach the ischemic tissue [103]. These time-dependent positive and negative effects of inflammation may be circumvented by direct intracerebral transplantation of cells into the ischemic penumbra; however, in the acute setting of stroke, a minimally invasive peripheral cell administration may be more practical. Thus, the challenge for cell therapy to reconcile the double-edged sword feature of inflammation is to find the optimal therapeutic window when elevated inflammatory migratory signals can direct cell migration toward the ischemic brain and thereafter for the cells to subsequently suppress inflammation.

Cell therapy has been proposed as a novel treatment for acute [104,105], subacute [106,107], and chronic stroke [108–110]. Transplantation of human placenta-derived cells has been shown to exert beneficial effects in a rodent stroke model. Specifically, transplantation of hAEC or hAMSC at Day 2 poststroke attenuated both motor and neurological deficits associated with occlusion of the middle cerebral artery at days 7 and 14 compared to the vehicle-infused stroke group. Following the last behavioral test at Day 14 poststroke, histology via Nissl staining revealed transplantation of hAEC or hAMSC at Day 2 poststroke increased the number of healthy looking cells (>75% of the intact brain) in the ischemic penumbra compared to the vehicle-infused stroke group. These positive behavioral and histological effects were achieved when 400,000 human placenta cells were transplanted directly into the presumed ischemic penumbra in the absence of immunosuppression [111].

That placenta-derived cells display transplantable cell properties including their ability to secrete anti-inflammatory factors [112] will benefit from optimizing the timing and route of cell delivery after transplantation.

Pulmonary fibrosis

In a mouse model of bleomycin-induced lung injury, treatment with a mixture of fetal membrane-derived mesenchymal and epithelial cells from either human or mouse have both been shown to cause a reduction in severity and extent of lung fibrosis [9]. These antifibrotic effects were observed at Day 14 after bleomycin instillation, by which time maximal lung fibrosis is observed [113,114] and notably, these effects were seen regardless of the cell source (allogeneic or xenogeneic) or administration route (systemic: intravenous or intraperitoneal; local: intratracheal).

Treatment with placenta-derived cells also resulted in evident reductions in the numbers of infiltrating neutrophils in the lungs of bleomycin-injured mice at Day 14, which could be partly responsible for the observed reduction in fibrosis, as the presence of neutrophils is known to be associated with poor prognosis in idiopathic pulmonary fibrosis in humans [115]. Whether the transplanted fetal membrane-derived cells released soluble factors that acted to down-regulate neutrophil recruitment remains to be elucidated, although this possibility is supported by reports that soluble factors released by these cells can inhibit T-cell proliferation [44] and dendritic cell differentiation and function [47] in vitro, while these cells also display anti-inflammatory effects in clinical settings [116].

Although both allogeneic or xenogeneic cells were detectable in the lungs of bleomycin-treated mice through DNA microchimerism and immunohistochemistry until Day 14, previous studies have shown that only a very small percentage of placenta-derived cells engraft and survive long term after transplantation [45]. Further studies are therefore warranted to elucidate the mechanisms of action of placenta-derived cells in this model. In addition, the recent indication that MSC from both mouse bone marrow and human umbilical cord blood produce factors that stimulate proliferation and matrix production by lung fibroblast cells [117], which could potentially exacerbate existing fibrotic damage, suggests that caution should also be exercised when proposing placental mesenchymal cells for treatment of pulmonary fibrosis. Thus, although promising, the data discussed above represent preliminary evidence that would require further validation in order to verify whether transplantation of placenta-derived cells does indeed represent a viable treatment option for pulmonary fibrotic disease.

Critical limb ischemia

Recently, Pluristem Therapeutics Inc. has investigated 3D expanded human term placenta-derived cells, termed PLX-PAD, for their potential to treat critical limb ischemia [118], whereby cells were administered via local intramuscular injection 5 h after induction of hind limb ischemia in mice through a previously established method [119]. During a follow-up period of 21–28 days, blood flow in ischemic limbs of cell-treated mice was significantly elevated compared to non-cell-treated mice, and this was accompanied by a significant decrease in the rate of cell necrosis in these animals. Furthermore, immunohistochemical analysis of tissues from limbs of cell-treated animals demonstrated a statistically significant increase in the number of new capillaries supplying the limb, while reduced levels of nitrotyrosine, an indicator of oxidative stress, and also of VCAM-1, an indicator of endothelial inflammation, were also observed in cell-treated animals compared to controls. Importantly, no treatment-related adverse effects were reported in PLX-PAD-injected mice. Based on these results, PLX-PAD is now being assessed in clinical trials for critical limb ischemia in the United States and European Union.

Inflammatory bowel disease

Chronic relapsing and remitting inflammation of the intestinal tract is the hallmark trait of ulcerative colitis (UC) and Crohn's disease (CD), collectively termed inflammatory bowel diseases (IBD). In genetically susceptible individuals an aberrant immune response, probably triggered by commensal bacteria or luminal antigens, is accompanied by impairments in tissue repair processes, ultimately leading to loss of tissue architectural organization, ineffective ulceration healing, and fibrosis [120,121].

Current therapies, ranging from anti-inflammatory drugs to immunosuppressive regimens, are often inadequate to control IBD and possess severe long-term side effects. As for other inflammatory-based diseases, cell therapies based on the immunomodulatory properties of MSC have been proposed for IBD and are currently under evaluation in phase III clinical trials for IBD manifestations using bone marrow- and adipose tissue-derived MSCs [120].

Recently hAMSC were shown to possess trophic effects upon intestinal epithelial cells, stimulating architectural organization and polarized differentiation (Lanzoni et al., unpublished data). These findings suggest that hAMSC may be useful for treating IBD; while their angiogenic potential may aid in ameliorating perfusion and healing, the paracrine activity of these cells may be beneficial in inducing ulcer re-epithelialization. Finally, their immunomodulatory effects may facilitate in restoring a correct balance between inflammatory cell activation and suppression in the intestinal mucosa, thereby preventing further damage.

Liver-based metabolic diseases

Several preclinical studies reported to date provide promising evidence regarding the potential of human amniotic membrane-derived cells to perform hepatic functions in vivo. The first such evidence came from a study whereby hAEC that had been transduced with the β-galactosidase gene were transplanted into the livers of SCID mice, resulting in detection of integrated transplanted α-fetoprotein- and albumin-positive cells in the hepatic parenchyma at both 1 and 2 weeks after cell injection, suggesting that hAEC could serve as transgene carriers after transplantation into the liver [90]. Later, it was shown that after transplantation of human amniotic membrane into the peritoneum of SCID mice, human albumin could be detected in the sera and peritoneal fluid of these animals from Day 1 until Day 7 (duration of the study) [122]. In another study, transplantation of hAEC into immunodeficient mice resulted in detection of human α-1 antitrypsin circulating in the serum of recipient animals, confirming that hAEC can perform this important hepatic function in vivo [123].

More recently, it has been shown that 2 weeks after transplantation of hAEC into the livers of SCID/Beige mice that had been pretreated with CCL4 in oil, human cytokeratin-positive cells could be detected in the bile ducts of these animals, with some bile ducts appearing to be completely humanized (Strom et al., unpublished data).

These studies provide compelling evidence in support of the bifunctional hepatic potential of hAEC in vivo, with demonstration of differentiation toward both parenchymal hepatocytes and cells with characteristics of bile ductular epithelial cells, thereby supporting the potential of hAEC as a useful tool for liver regeneration in the future.

Cardiac ischemia

Even though their ability to differentiate toward cardiomyocytes is still debated [8,54], several lines of evidence suggest that transplantation of isolated amniotic and chorionic cells can improve cardiac function. For example, Ventura et al. have reported improved myocardial function for up to 4 weeks after intramyocardial injection of fetal membrane-derived cells into infarcted rat hearts [8].

In other studies, injection of rat amnion-derived cells into syngeneic animals with an acute infarcted left ventricular myocardium following permanent ligation of the proximal left coronary artery prevented ventricle dilatation, while contractile function was maintained between 2 and 6 weeks after transplantation. Histological assessment revealed that the amniotic cell-treated myocardium had reduced scar areas and fibrosis, with increased left ventricle myocardial wall thickness [124].

Finally, application of a human amniotic membrane fragment onto the left ventricle of rats that had undergone ischemia through left anterior descending coronary artery ligation has been shown to significantly reduce postischemic cardiac dysfunction [125]. Echocardiographic assessment of morphological and functional cardiac parameters performed over a 3-month period demonstrated that membrane-treated rats showed higher preservation of cardiac dimensions and improved cardiac contractile function in terms of higher left ventricle ejection fraction, fractional shortening, and wall thickening [125]. In this study, no engraftment of amniotic cells was detected in host cardiac tissues, again supporting the hypothesis also suggested by other reports that the beneficial effects observed are likely due to paracrine secretion by amniotic cells of soluble factors that promote protection and regeneration of host tissues, rather than differentiation of the transplanted cells themselves.

Regulatory Considerations: European and US Regulations for Transferring Cell Processing and Banking Into GMP

European situation

Despite the fact that the first cellular attempts with bone marrow transplantation resulting in transient grafting were published in the year 1957 by E.D. Thomas et al. [126], resulting in the first successful syngeneic bone marrow transplantation [127] and the first reporting of a successful hematopoietic cell transplantation using placental (cord) blood stem cells by E. Gluckman in the year 1989 [128], no binding European Community legislation existed until the EU amended Directive 2001/83/EC concerning medicinal products for human use through the Directive 2003/63/EC, to also cover somatic cell therapy (SCT) and gene therapy (GT) medicinal products. Additionally, principles and guidelines of GMP for medicinal products for human use and investigational medicinal products for human use were published with Directive 2003/94/EC.

Only 1 year later, on March 31, 2004, the Cells and Tissues Directive (2004/23/EC) was adopted, ensuring the quality and safety of human cells and tissues. The scope of this Directive covers donation, procurement, testing, processing, preservation, storage, and distribution of human cells and tissues. Hence, all human cells and tissues that are used for application in the human body are covered, with the exception of blood and blood-derived products, human organs and any organs, tissues or cells of animal origin. Therefore, this Directive clearly applies also to hematopoietic peripheral blood, umbilical cord (blood) and bone marrow stem cells, reproductive cells (eggs, sperm), placental tissue, and cells thereof, as well as adult and embryonic stem cells. Directive 2004/23/EC is further supported by 2 technical directives, 2006/17/EC (requirements for donation, procurement and testing) and 2006/86/EC (traceability requirements, notification of serious adverse reactions and events, and certain technical requirements for the coding, processing, preservation, storage). Both aim to ensure adequate staffing and training, appropriate laboratory facilities, and standard operating procedures, controlled by a quality management system. The main pillar of the system is based upon quality assurance, which sets specifications for production, quality control, and release. It defines responsibilities and personnel

qualification and controls compliance with approved procedures and qualified equipment and clean rooms. Depending on the type of product, certain prerequisites regarding premises and equipment need to be fulfilled, including defined pressure cascades, temperature, and humidity levels in the clean rooms. Furthermore, environmental monitoring and alerting are mandatory (microbiology, airborne particles) and essential for product release.

The production of a GMP-conforming cell or tissue product represents a multilevel process, starting with donation and transportation. Each cell or tissue donor needs to sign an informed consent form. Additionally, the donor's medical history as well as the donation itself and transport and delivery need to be recorded in writing. In addition, the donor is tested with regard to infectious agents.

Preparation and storage as second level of a production process need to be performed under defined conditions regarding equipment and clean room, using GMP-conforming produced reagents only. Besides an obligatory written documentation of preparation, the product must be packaged, labeled, and stored adequately.

Once all quality control results are available (eg, virology, microbiology, cell count, CFUs, viability, genetic stability, etc.), the cell or tissue product will be evaluated, released, and provided for clinical application.

In November 2007, the EU published Regulation (EC) No 1394/2007 on Advanced Therapy Medicinal Products (ATMP) (valid for placenta-derived cell product such as PLX-PAD, Pluristem, Israel), amending Directive 2001/83/EC and Regulation (EC) No. 726/2004, which has been enforced starting from December 30, 2008. ATMP are divided into 3 classes: tissue engineered (TE), SCT, and GT medicinal products. Cells and tissues will be considered "engineered" if they have been subjected to "substantial manipulation," such as cell expansion, selection, or functional activation, or if their intended use differs from the function that the cells normally carry out in the donor tissue. A list of procedures that are not considered substantial manipulation is provided in Annex I of the regulation. TE medicinal products are intended for regeneration, repair, or replacement of damaged tissues, whereas SCT medicinal products are defined as autologous, allogeneic, or xenogeneic cells manipulated in order to promote their functions toward treatment, prevention, or diagnosis of a disease. However, cell therapy with ex vivo genetically modified cells will be considered a GT medicinal product and will therefore be regulated under the specific set of rules for this class. All ATMP-containing human cells and tissues shall also refer to Directive 2004/23/EC for the donation, procurement, and testing of cells and tissues for therapy.

US situation

In the United States, the regulation of human cells and cellular-based products is a tiered approach with risk and intended use determining the level of regulation. Lower-risk products, such as privately banked human umbilical cord blood, peripheral blood, or cells that are intended for homologous use (defined as repair, reconstruction, replacement, or supplementation of a recipient's cells or tissues with HCTP that performs the same basic function in the recipient as the donor), autologous use, allogeneic use in first or second degree blood relatives, or for reproductive use, are regulated solely under Section 361 of the Public Health Service Act (PHS Act; see 21 CFR 1271.10 for qualifying criteria), and thus do not require premarket approval. Higher-risk products that are more than minimally manipulated, such as culture-expanded, encapsulated, or genetically modified cell populations, are regulated as biological products and subject to Sections 351 and 361 of the PHS Act and the Food Drug & Cosmetic Act (FD&C Act). Premarket approval is required.

All human cells and cellular-based products are subject to 3 sets of regulations under 21 CFR 1271: Establishment Registration, Donor Eligibility, and Current Good Tissue Practice (CGTP). These rules are intended to prevent the introduction, transmission, or spread of communicable disease. While the aim of the Donor Eligibility regulations is to reduce the risk that donors harbor infections that could be transmitted to recipients, CGTPs reduce the risk of the spread of communicable disease by governing the recovery, processing, storage, labeling, packaging, and distribution of these products. Biological products are subject to the FD&C Act, in addition to the PHS Act, since most biologics also meet the definition of a drug (Section 201(g)(1)). Cells and cellular-based products regulated as biological products must, therefore, be manufactured in accordance with both CGTPs (21 CFR 1271) and Current GMP (CGMP; 21 CFR 210 and 211) (eg, pertaining to the placenta cell products PDA001, Celgene Corporation and PLX-PAD, Pluristem). The 2 sets of regulations are intended to supplement each other. If a regulation in 21 CFR 1271 conflicts with a requirement in parts 210 or 211, then the regulation more specifically applicable to the product will supersede the more general. Other important regulations that biological products are also subject to, but are not limited to the 21 CFR 600 Biological Products regulations, include 312 Investigational New Drug Application, and 201.57(a) for labeling and 314.81.b.3.i for advertising and promotion.

General challenges for GMP-conforming processing and banking of placenta-derived stem cells

Besides the application, implementation, inspection, and certification processes for GMP conformity, some additional challenges need to be met. Facility and equipment as well as personnel and their training cause increased expenses. All processes become more time-consuming not only because of associated necessary clothing and working rules but also because of more extensive testing and documentation, training, and maintenance.

Furthermore, procedures need to be upscaled from laboratory to production size, for example, large-scale bioreactors, providing a robust and relatively inexpensive platform for cell culture growth and assuring stable and qualified cell production processes. The use of bioreactors (eg, as applied for PLX-PAD, Pluristem) allows for online culture control, optimization, standardization, scale-up, and a "hands-off" operation making the end product dependable, predictable, and with minimum risk of contaminants, therefore suitable for human use and therapeutic applications.

Conclusions

Besides having an essential and unique role in fetal development and preparation for life outside of the womb, mounting evidence now suggests that the placenta may also hold the

key to treating several conditions that arise during life itself whereby tissue or organ function has been compromised due to disease or injury. Whether the beneficial effects of placenta-derived cells are due to differentiation of the transplanted cells themselves or to paracrine actions on the surrounding host tissue in order to reduce inflammation and promote regeneration remains to be fully elucidated, although current evidence seems to lend greater support to the latter of these hypotheses. In any case, the promising data obtained to date constitute compelling evidence regarding the potential utility of these cells for clinical application. Further studies to validate the efficacy of placental cells for treating a wide range of conditions, as well as the development of strategies for GMP-conforming production of these cells, represent fundamental steps that will be required in the future to allow translation of the promising findings that have been discussed here into effective therapeutic approaches.

Acknowledgments

We are indebted to Fondazione Cariplo for providing continuous and generous financial support for research into placenta-derived stem cells at Centro di Ricerca E. Menni, Brescia, Italy. We sincerely thank Celgene Corporation, Cryo-Save, and Areta International for sustaining the costs of the international Workshop "Placenta-Derived Stem Cells for Treatment of Inflammatory Diseases: Moving Toward Clinical Application," held in Brescia, Italy, March 13 and 14, 2009.

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Received for publication October 14, 2009
Accepted after revision November 28, 2009
Prepublished on Liebert Instant Online November 30, 2009

This article has been cited by:

1. H. Abdulrazzak, D. Moschidou, G. Jones, P. V. Guillot. 2010. Biological characteristics of stem cells from foetal, cord blood and extraembryonic tissues. *Journal of The Royal Society Interface* **7**:Suppl_6, S689-S706. [CrossRef]