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Immunogenicity and Immune-Modulating Properties of Human Stem Cells

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1. Introduction

The future use of stem cell-based therapeutic applications in regenerative medicine is regarded as promising. In addition to autologous and allogeneic transplantation procedures, various innovative methods have been designed to generate patient-histocompatible stem cells from which lineage-specific cell progenies could be obtained (reviewed in Nehlin & Barington, 2009). Immunological aspects of the transplanted cells as well as the recipient need to be considered in order to predict the outcome of clinical cell therapies. Undifferentiated stem cells show initially a low degree of immunogenicity leading to weak immune responses when introduced into non-histocompatible hosts. In addition, stem cells possess immune-modulating properties that confer the capacity to withstand a cytotoxic response in a foreign host. The nature and significance of these strategies will be described in detail along this chapter.

Many valuable contributions dealing with immunogenicity and immunological tolerance have been possible by means of mouse embryonic and multipotent stem cells. However, this overview will explore in-depth the immunological features and clinical uses of two types of human stem cells, embryonic stem cells (Figure 1) and multipotent mesenchymal stem cells (Figure 2 & 3) that allow them to be considered in transplantation procedures.

2. Basic principles of antigen presentation, the adaptive immune response and immune histocompatibility

The highly polymorphic classical "Major Histocompatibility Complex" (MHC) class I protein family consists of extracellular, membrane spanning, alpha chains HLA-A, -B, and -C (Human leukocyte antigens) acting as ligands for T-lymphocyte receptors (TcR) expressed on T lymphocytes, the killer-immunoglobulin-like receptors (KIR) on Natural Killer (NK) cells and for certain members of the leukocyte immunoglobulin-like receptor (LILR / ILT / LIR) family. The non-classical MHC class I protein family include less polymorphic HLA members such as HLA-E, HLA-F, HLA-G, HLA-K and HLA-L (Li & Raghavan, 2010).

A major function of HLA molecules is presentation of intracellularly produced self and non-self peptides. During intracellular infection, pathogen-derived (non-self) peptides are presented by virtually all nucleated cells in the body to TcR of cytotoxic CD8^{+} T lymphocytes leading to killing of infected cells. Endogenous (self) peptides are also presented, but usually T cells with specificities to self-peptides are eliminated in the thymus.

The introduction of allogeneic stem cell-derived tissues into an immunocompetent recipient is likely to result in T-cell-mediated rejection by either of two mechanisms. One is indirect allorecognition, where polymorphic donor-derived peptides are presented for recipient T cells, and the other is direct allorecognition, where polymorphic variants of donor MHC are recognized by recipient T cells. The latter is by far the strongest and because MHC is extremely polymorphic, this mechanism constitutes a major hurdle for allogeneic stem cell transplantation (Afzali et al., 2008; Gökmen et al. 2008; Hornick, 2006; Ingulli, 2010).

T-cell activation is dependent on binding of antigen-presenting MHC class I (signal 1) and non-antigen-specific co-stimulatory molecules CD40, CD80 and CD86 expressed by donor cells to their ligands on T cells (signal 2)(Jenkins, 1994). Presentation of peptides by MHC class I to TcR in the absence of co-stimulation induces anergy or apoptosis of T cells, abortive proliferation or tolerance-inducing immunoregulation (Ford & Larsen, 2009; Pearl et al., 2011).

Two types of T cells, cytotoxic and regulatory, have been implicated in immune responses in general and in relation to stem cell transplantation. Cytotoxic or cytolytic T cells (CTL or T_C) are activated T lymphocytes (usually CD8 positive) that can induce the killing of target cells, be it infected or allogeneic cells. CTL can induce apoptosis of targets cells by two independent mechanisms: release of cytotoxins such as perforin, granzymes and granulysin, or by binding of FasL (CD95L) to Fas (CD95) molecules expressed on the target cell (Brown, 2010).

Regulatory T cells (T_{reg}) comprise several specialized subsets of T cells that are able to control immune responses and promote and maintain immune tolerance in an antigen-specific way. Presence of T_{reg} has been associated with tolerance whereas their deficiency or defective function has been correlated with autoimmunity in many experimental models (Roncarolo et al., 2011).

The degree of histocompatibility at the HLA-locus can be determined by serologic or genomic tissue typing. In case of discrepancy for HLA class II antigens, the resulting incompatibility may be measured through Mixed Lymphocyte Reactions (MLR). Peripheral blood mononuclear cells (PBMC) from two different individuals (HLA mismatched allogeneic setting) are co-cultured for 4-6 days. One of the cell populations (stimulators) is irradiated to avoid its proliferation. The non-irradiated cells (responders) will start proliferating due to direct allorecognition, and this is measured through uptake of ^3H-(tritiated) thymidine. Suppression of T cell proliferation is often measured by adding unmodified or modified stem cells to the MLR (Aggarwal & Pittenger, 2005; Le Blanc et al., 2003a; Wonderlich et al., 2006).

MHC class II molecules and co-stimulatory molecules are primarily expressed on professional antigen-presenting cells (APC) such as B cells, monocytes and dendritic cells. MHC class II consists of three classes of HLA-II antigens: HLA-DP, -DQ and -DR presenting peptide antigens derived from extracellular proteins to CD4+ T helper cells to elicit an immune response. Because MHC class II expression is cell-type specific and mainly restricted to thymic epithelial cells and bone marrow-derived antigen presenting cells it is not expected to be a problem in many stem cell applications (Handunnetthi et al., 2010).

Natural killer (NK) cells participate in the innate immune response as they are capable of killing tumor cells and virally-infected cells. NK cells express a large number of cell surface receptors named Killer-cell Immunoglobulin-like Receptors (KIR) and NKG2 (CD94) that have classical and non-classical class I antigens as their ligands , respectively, and deliver either activating or inhibitory signals. The relative balance of these signals regulates NK cell

activity (Orr & Lanier, 2010) and absence of HLA class-I expression therefore may lead to killing by NK cells. This is of relevance for stem cell therapy because some stem cells lack classical HLA class I expression all together and because more differentiated cells may fail to express alleles recognized by the NK cells of the recipient.

Organ transplantation is a routine therapeutic strategy for patients with end-stage organ failure (Newell, 2011) and bone marrow-derived hematopoietic stem cells are used to treat several hematopoietic malignancies and rare immunodeficiencies (Nehlin & Barington, 2009). Clinical experience through more than three decades with these treatments clearly demonstrates the relevance of both T cells and NK cells in transplantation. Allogeneic transplantation of organs often results in the development of either a) graft-versus-host-disease (GvHD) or b) immune rejection. GvHD is a condition where donor cells within a transplant launch an immune reaction against the recipient cells (Menendez et al., 2005; Shlomchik, 2007). The host can also build an acute and/or chronic rejection against antigens perceived as non-self by the recipient's immune system leading to destruction of the graft. Those antigens can belong to MHC, minor histocompatibility complex (mHC) or AB0 blood groups (Bradley et al., 2002; Charron et al. 2009; Feng et al., 2008; Shlomchik, 2007; Spencer et al., 2010).

3. Immunological tolerance at the fetal-maternal interface

A classical example of immune tolerance is found during pregnancy. The maternal immune system allows for the successful development of a semi-allograft consisting of a fetus and a placenta that express both maternal (self) and paternal (non-self) antigens. Cytotoxic adaptive immune responses are diminished, bypassed, or even abrogated (Leber et al., 2010). Immunological processes such as innate immunity remain intact to continue to provide host defense against infection and to interact with fetal tissues to promote successful placentation and pregnancy.

The trophoblasts are the cells that form the outer layer of the blastocyst, that develop into a large part of the placenta, and have a crucial role in the implantation of the developing embryo by interactions with the decidua, the lining of the maternal uterus containing endometrial stromal cells. The placenta and fetal trophoblast cells are directly exposed to maternal blood and tissues. The trophoblast layer protects the inner cell mass (ICM) from attack by cytotoxic components of the maternal immune system dedicated to destroying foreign tissues. The inner cell mass consists of a group of cells inside the primordial embryo that will eventually develop into defined fetal structures (Mor & Abrahams, 2009).

In humans, trophoblast cells differentiate from the trophoblast shell that surrounds the post-implantation embryo into two main lineages, villous trophoblast and extravillous trophoblast. Primary villous trophoblast cells do not express β2-microglobulin or any HLA class I or HLA class II molecules, whereas extravillous trophoblast cells express HLA-C, HLA-G and HLA-E, but not HLA-A, HLA-B or HLA-DR molecules in a normal pregnancy. Villous trophoblasts are in contact with the systemic immune system, whereas extravillous trophoblasts interact with the local mucosal immune cells. When extravillous trophoblasts were exposed to interferon-gamma (IFN-γ), a pro-inflammatory cytokine, they could not induce the expression of HLA-A, -B, -DR or up-regulate significantly HLA-G (Apps et al., 2009). A comprehensive overview of MHC expression at the fetal-maternal interface was recently reported (Tilburgs et al., 2010).

Human embryonic tissues possess a range of proteins and mechanisms that efficiently counteract and prevent maternal cytotoxic T cell attack and thereby provide protection and immune privilege to the fetus (Clark, 2005; Fändrich, 2002; Mor & Abrahams, 2009; Parhar et al. 1989; Petroff & Perchellet, 2010; Rebmann et al., 2010; Rizzo et al., 2011a; Verloes et al., 2011).

Knowledge of the immunological tolerance mechanisms taking place at the feto-maternal interface in mouse and rat models have contributed to extend such findings to human cells. Attention has focused on a group of pluripotent stem cells known as human embryonic stem cells (hESC), derived from the ICM of 6-8 days pre-implantation blastocysts obtained from *in vitro* fertilization procedures (Thomson et al., 1998), because they could potentially be used in cell therapy and regenerative medicine (Figure 1; Nehlin & Barington, 2009). However, the use of hESC may be limited by immunological incompatibility between the donor and the recipient as explained in the next sections.

4. Immunogenicity of human embryonic, multipotent and reprogrammed stem cells

4.1 General considerations

Ever since the development of the techniques that allowed researchers to establish blastocyst-derived hESC, there has been a great deal of interest in their potential use in regenerative medicine (Thomson et al., 1998). To satisfy clinical requirements, a number of matters need to be addressed including 1) the precise control of differentiation towards the tissue or cell-type of choice without remnant undifferentiated hESC; 2) safety issues pertaining the risk of transplanting undifferentiated hESC cells that could result in teratoma formation, the possible presence of genetic modifications as a result of *ex-vivo* culture, and immunogenicity concerns (see below); 3) the development of xenogeneic-free culture conditions and 4) potential ethical conflicts (Nehlin & Barington, 2009; section 6.1).

In-depth studies of the molecules and mechanisms responsible for the low grade of immunogenicity and allograft tolerance of cultured hESC in non-histocompatible recipients have been relatively few compared with bone marrow stem cell studies (section 4.3). The immune-privileged status of hESC resembles the tolerance properties exhibited by the developing embryo in the feto-maternal interface (section 3; English & Wood, 2011; Grinnemo et al., 2008a; Menendez et al., 2005). In comparison, multipotent stem cells such as bone marrow stromal cells still retain immune-privileged properties even after further differentiation (Le Blanc & Pittenger, 2005).
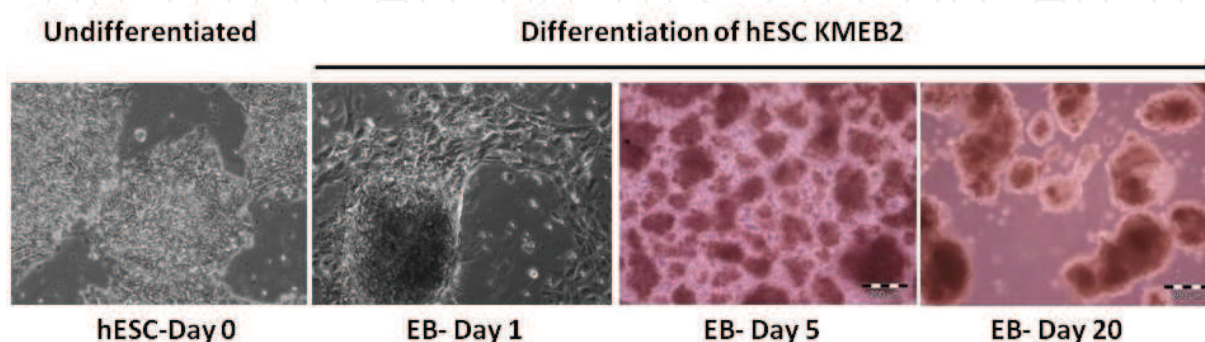

Fig. 1. Early differentiation of hESC

Phase contrast photographs of hESC KMEB2 (Harkness et al., 2010) growing on feeder-free Matrigel in an undifferentiated state (day 0), followed by differentiation towards embryoid body formation at days 1, 5 and 20 (left to right panels). Magnification: 100x

Traditionally, hESC have been isolated from the ICM of the blastocyst stage of the early developing embryo (Thomson et al., 1998). Also, in recent years, donor-specific hESC were obtained from single blastomeres at the 8-cell stage of embryogenesis or generated by somatic cell nuclear transfer (SCNT). Pluripotent stem cells with features resembling the hESC state have been generated by parthenogenetic activation of unfertilized oocytes and genetic reprogramming (Jopling et al. 2011; Nehlin & Barington, 2009; Yamanaka & Blau, 2010). The immunological aspects regarding hESC and hESC-like pluripotent stem cells are addressed below.

4.2 MHC class I expression in human embryonic stem cells, early stem cell progenitors and induced pluripotent stem cells
4.2.1 Expression studies
Immunogenicity concerns focus on the presentation of antigens and expression of MHC class I proteins, as well as expression of molecules that make hESC potential targets of cytotoxic responses by T and NK cells.

Almost all cellular studies dealing with HLA class I expression have relied on a single antibody (W6/32) targeting simultaneously HLA-A, -B, -C and cross-reacting with HLA-E and –F (Uchanska-Ziegler & Ziegler, 2007). Indirect immunofluorescence assays showed that human pre-implanted embryos expressed neither HLA class I, II antigens nor β2-microglobulin (Desoye et al., 1988). However, a later study using immunocytochemistry showed expression of HLA-G and MHC class I (Jurisicova et al., 1996). The status of expression of several immunogenicity markers on the surface of several hESC lines was later semi-quantified by flow cytometry: MHC class I proteins were expressed at very low levels, and increased moderately upon *in vitro* or *in vivo* differentiation. However, no cell surface staining of HLA-G or MHC class II was apparent in undifferentiated or differentiated hESC. Treatment of hESC *in vitro* with IFN-γ, a cytokine secreted during the course of an immune response, resulted in high-level expression of MHC class I (Drukker et al., 2002; Grinnemo et al., 2006). Another study showed that hESC express both MHC class I and β2-microglobulin at low levels on the cell surface regardless of using mouse feeder cells or feeder-free cultures. Upon IFN-γ stimulation, both MHC class I and β2-microglobulin were strongly up-regulated and this effect was considerably enhanced after cells had been pre-treated with retinoic acid, a differentiation stimulator (Draper et al., 2002).

The low rate of MHC class I expression was explained by the low expression of components of the antigen-processing machinery (APM) such as β2-microglobulin and tapasin, without detectable expression of TAP1, TAP2, LMP2, and LMP7 (Cabrera et al., 2007; Suarez-Alvarez et al., 2010).

Few studies exist where human MHC class I expression is followed during the differentiation process. Recently, an extensive study showed that hESC-derived embryoid bodies displayed significantly higher expression of HLA-B, HLA-E,–F and β2-microglobulin compared to the undifferentiated cells. Expression of NK cell receptor NKG2D ligands (MICA, MICB) was evident in all undifferentiated pluripotent stem cells lines analyzed, and their expression was maintained after differentiation. MHC expression was subject to epigenetic control in hESC. It was shown that methylation of histone H3K9me3 repressed the tapasin gene in undifferentiated cells whilst HLA-B and β2m acquired the histone

H3K4me3 modification during the differentiation to embryoid bodies. Absence of HLA-DR and HLA-G expression was regulated by DNA methylation (Suarez-Alvarez et al., 2010).

The expression of the HLA-A allele A*02 was found to be moderate in hemangioblasts or hematoendothelial precursors derived from hESC cell line H9, that exist transiently in early embryonic development, and express markers of immature hematopoietic and endothelial cells (CD31, CD34, VE-cadherin, Flt-1) and mature differentiated cells (CD45, CD33, CD146). However, HLA-A02* expression increased dramatically as cells were differentiated into endothelial or hematopoietic stem cells (Basak et al., 2009). Differential regulation of HLA-A,-B,-C alleles in multipotent stem cells has been revealed (Isa et al., 2010; section 4.3) and similar findings have been evaluated in embryonic and early hematopoietic precursors (Sabir et al., in preparation).

hESC can not only be obtained from the inner cell mass of day 5-6 developing embryos, or earlier, but can also be generated by SCNT, parthenogenesis, or by reprogramming adult somatic cells to generate inducible pluripotent stem cells (iPS cells; Jopling et al., 2011; Nehlin & Barington, 2009; Yamanaka & Blau, 2010). Parthenote-derived hESC show equivalent phenotypes to hESC in the undifferentiated state and can differentiate as demanded, but no data on the expression of molecules conferring immunogenicity is yet known (Harness et al., 2011), and is also unknown for SCNT-derived hESC.

Reprogrammed iPS cells resemble hESC in many ways, with their capacity of self-renewal, and pluripotency state. One of their main features is that they can be obtained from essentially any somatic, fully differentiated adult cells and converted to pseudo-hESC from which one can derive cells and tissues that are genetically compatible with the donor of the original cells. Such strategy, although seemingly expensive, could represent a favorable method to avoid any kind of immunological rejection. Thus, exponential interest in generating such cells has led to characterize the expression of various immunogenicity-associated molecules. Of particular interest, HLA-B, -C, -E, and β2-microglobulin mRNA levels were reduced in iPS cells compared to parental fibroblasts, whereas HLA-A, -G and MHC class II expression was absent. The mRNA levels of APM components TAP-1, TPN, LMP2 and RFX5 plummeted during the reprogramming process to iPS (Suarez-Alvarez et al., 2010).

However, a recent report showed that expression of MHC class I in iPS cells by flow cytometry is slightly higher in reprogrammed undifferentiated hESC than in ICM-derived hESC (Pearl et al., 2011). It is unclear if such difference would account for immunological discordance taking into account that iPS cells are expected to be histocompatible since the donor and recipient of iPS cells is the same individual. A drawback is represented by the genetic tools used to reprogram somatic cells to iPS cells, because they could raise immunogenicity concerns (Nehlin & Barington, 2009; Yamanaka & Blau, 2010).

4.2.2 Cellular and immunological studies

hESC underwent minimal killings when incubated with NK cells. NK cytotoxicity is mediated by engagement of NK lysis receptors, NKp30, NKp44, NKp46, and CD16. All of their ligands, except the NKp44 ligand, were absent on hESC and were not induced after IFN-γ treatment (Drukker et al., 2002).

Undifferentiated hESC were shown to possess immune-privileged characteristics and when transplanted into immune-competent mice, they did not elicit an immune response. Moreover, the inhibitory effect of hESC on alloreactive T cells was mediated by direct cell

membrane interactions rather than by secreted factors. Even slightly differentiated hESC-derived progenitors within cell aggregates known as embryoid bodies (Figure 1), did not induce proliferation of allogeneic T cells (Li et al., 2004). In contrast, a more recent study found that cellular extracts from hESC could indeed retain the immunoregulatory properties of intact cells e.g. inhibiting the function and maturation of monocyte-derived dendritic cells (Mohib et al., 2010).

In xenotransplantation and allotransplantation settings, when hESC were transplanted into various strains of immunocompetent mice and monitored during one month, the cells were totally eliminated (Drukker et al., 2006). In contrast, when a hESC-derived graft was transplanted into immune deficient mice lacking T, B or NK cells, it was found that T-cell deficient animals failed to reject the hESC-derived graft. The lack of NK cells or B cells did not interfere with vigorous hESC rejection, indicating that T cells play a pivotal role in hESC immune rejection. MHC class I molecules were expressed at low levels while MHC class II, and co-stimulatory proteins CD80 and CD86 were not expressed. The low immunostimulatory capacity of hESC was verified by transplanting undifferentiated or differentiated hESC into a mouse model, in which mice were pre-conditioned to carry PBMC from human origin. After one month, only a minute alloresponse was observed while control adult grafts were totally rejected. If MHC class I expression was induced, an increase in the alloresponse took place. These findings suggested that the use of immunosuppressants could be reduced in the case of hESC-derived transplants compared to solid organ transplantions (Drukker et al., 2006).

hESC-derived cells were found to be capable of long-term hematopoietic engraftment when transplanted into non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice (Tian et al., 2006). hESC were shown to be immunologically inert when transplanted into the myocardium of immunocompetent mice and did not inhibit immune responses judged by an increase in lymphocyte infiltration with positive histological staining for CD11b, CD3, CD4 and CD8 during direct or indirect antigen presentation, and they were acutely rejected in a xenogeneic setting (Grinnemo et al., 2006), similar to previous findings (Drukker et al., 2006).

The earlier interpretations that hESC are immune privileged were contradicted by a new study whereby the fate of transplanted hESC were traced by bioluminescent imaging in immune-competent host mice. Graft infiltration by host immune cells occurred within 5 days, and already after 10 days, there was no evidence of hESC left. When hESC were transplanted into immune-deficient (NOD/SCID) host mice, the transplants expanded in number after only 10 days and teratoma formation was evident at 42 days. Rejection was demonstrated to be mediated predominantly by CD4+T cells and it was delayed when immunosuppressive therapies commonly used in the clinic were applied, such as tacrolimus and sirolimus (Swijnenburg et al., 2008).

Individual hESC may survive allotransplantation due to low immunogenicity compared to mature, adult cells, thereby escaping from NK cell- or T cell-mediated cytotoxicity. However, during differentiation MHC class I expression often increases and the presence of certain cytokines such as interferons may strongly induce its expression. In such cases, antigen disparities between donor hESC and recipient cells will lead to rejection, unless prevented (section 6.1). Alloreactive T cells are the major effectors of graft rejection and these cells usually prevent the generation of teratomas in immunocompetent animals injected with allogeneic ESC.

Recently, the immunogenicity of inducible pluripotent stem (iPS) cells was examined in the mouse model. In contrast to derivatives of mouse embryonic stem cells, abnormal gene

expression in some cells differentiated from iPSC could induce T-cell-dependent immune responses in syngeneic recipients. Therefore, the immunogenicity of patient-specific iPS cells should be evaluated before any clinic application of these autologous cells into the patients (Zhao et al., 2011).

One of the factors used in reprogramming procedures towards generating iPS cells is OCT4, a transcription factor that plays a key role in the pluripotency program. Most healthy individuals harbor OCT4-specific CD4^{+} memory T cells indicating a lack of immune tolerance to this antigen (Dhodapkar et al., 2010).

4.3 MHC class I expression in human multipotent mesenchymal stem cells

Human mesenchymal stem cells (hMSC) are multipotent stem cells with a fibroblast-like morphology and the capacity to self-renew *in vivo* that are distributed across virtually every tissue in the body. The "potency" of such cells is not restricted to skeletal tissues (bone, cartilage, fibrous tissue, fat, and myelosupportive stroma) but also includes non-skeletal mesodermal derivatives such as heart, endothelial cells and striated muscle. In contrast, osteogenic, stromal, or skeletal stem cells are multipotent CD146^{+} cells found in the bone marrow stroma that can differentiate into bone, cartilage, fibrous tissue, adipose tissue, and myelosupportive stroma but not to skeletal muscle, other mesoderm-derived tissues, and non-mesodermally derived tissues (Figure 2; Bianco et al., 2010; Nombela-Arrieta et al., 2011).
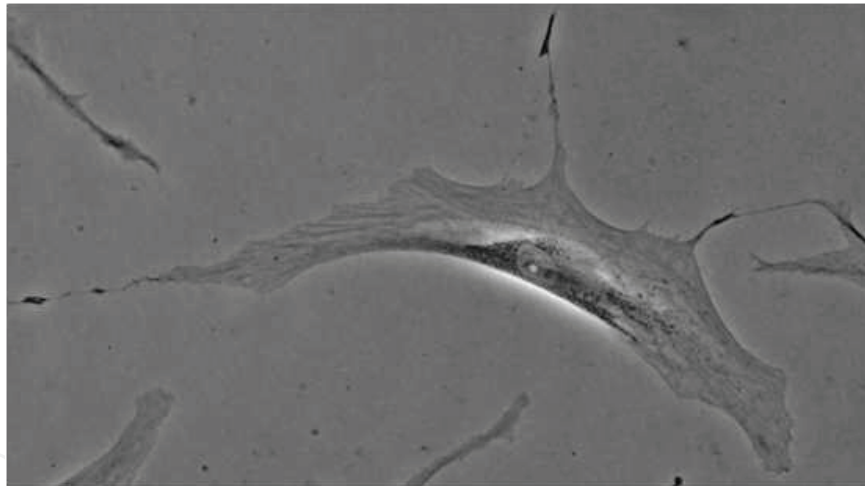

Fig. 2. Phase contrast photograph of a human bone marrow-derived stromal cell (hMSC). Magnification: 100x

Recently, CD146 expression was shown to differentiate between perivascular versus endosteal localization of non-hematopoietic bone marrow stem cell populations and this localization correlated with CD146 being expressed during normal oxygen conditions (normoxia) and absent during hypoxia (Tormin et al., 2011).

hMSC are an attractive source of stem cells for use in tissue engineering such as bone regeneration and cartilage repair, due to their differentiation capacity, their relative availability and their immune privilege properties (section 6.2; Niemeyer et al., 2006; Nombela-Arrieta et al., 2011).

An early study described the presence of various immunologically relevant markers such as neutral endopeptidase CD10, aminopeptidase CD13, neural cell adhesion molecule CD56, a

typical NK cell marker, and MHC class I in hMSC isolated from fetal, mature, and geriatric individuals (Young et al. 1999). In another study, hMSC isolated from the bone marrow stroma were also shown to constitutively express MHC class I and even lymphocyte function-associated antigen (LFA)-3 antigens whereas MHC class II and intercellular adhesion molecule (ICAM)-1 antigens were only expressed upon IFN-γ treatment and CD80, CD86, or CD40 co-stimulatory molecules were not expressed at all. Moreover, hMSC failed to stimulate allogeneic PBMC or T-cell proliferation in mixed lymphocyte reactions (Tse et al., 2003).

In yet another study, undifferentiated hMSC were shown to express significant levels of MHC class I expression and no MHC class II expression, and when hMSC were differentiated into adipocytes, osteoblasts, and chondrocytes, they were shown to express lower levels of MHC class I, but still not MHC class II. Both undifferentiated hMSC and differentiated hMSC were not immunogenic as they did not stimulate allogeneic lymphocytes in co-culture experiments. Upon IFN-γ treatment, MHC class II expression increased dramatically in hMSC, but despite this, the inhibitory effect on lymphocyte alloreactivity persisted (Le Blanc et al., 2003b). Several terminally differentiated cell types like neurons, hepatocytes, skeletal and cardiac muscle cells failed to constitutively express HLA class I (Fleming et al., 1981).

hMSC can process and present HLA class I-restricted viral or tumor antigens to specific CTL with a limited efficiency, likely because of some defects in APM components such as lack of expression of LMP7, LMP10, and ERp57. However, they are protected from CTL-mediated lysis through a mechanism that is partly sHLA-G-dependent (see section 5.2.2.3; Morandi et al., 2008).

While hESC and the inner cell mass in blastocysts have been shown to express very low levels of HLA class I, lineage-committed stem cells like mesenchymal stem cells (MSC) have a much higher expression similar to that of lymphocytes. Constitutive expression of HLA class I is largely restricted to cells of the lymphoid organs, the epithelia and the lining of small vessels. HLA-A, -B- and -C are equally expressed in blood leukocytes and regulated primarily at the level of transcription through promoter elements that are conserved among the HLA genes. Using quantitative multicolour flow cytometry and allele-specific antibodies targeting classical MHC class I on muscle satellite cells, bone marrow stromal cells and adipose-derived stem cells, as well as in PBMC, we found high cell-surface expression of HLA-A whereas HLA-B and -C alleles were strongly down-regulated. IFN-γ stimulation of stem cells during 48-72h was required to induce full HLA-B protein expression. The major contributor to repression of HLA-B and -C in stem cells during basal, non-induced conditions may be a post-transcriptional mechanism leading to translational attenuation in stem cells. Since different HLA alleles have variable affinities for intracellularly-generated peptides, the lack of HLA-B and -C expression can influence antigen presentation and the resulting immune response (Isa et al., 2010).

5. Immune-modulating properties

5.1 Immune-modulating properties of human embryonic stem cells

As explained previously in section 3, embryonic tissues are endowed with powerful immune-protecting mechanisms. However, relatively little is known about molecules expressed by hESC of the ICM exerting immune tolerance functions, especially considering that such intrinsic hESC properties could perhaps be used in regenerative transplantation protocols.

hESC have limited antigen presentation capacity because of low MHC class I cell surface expression levels and the complete lack of MHC class II molecules and co-stimulatory molecules (Drukker et al., 2006; Grinnemo et al., 2006).

Among known immune tolerance molecules (section 5.2), ICM-derived hESC expressed the tolerogenic HLA-G molecule at the mRNA and protein levels, but it underwent down-regulation in ICM cells during blastocyst growth (Rizzo et al., 2011a; Verloes et al., 2011). Several immune-modulating proteins found in hMSC are also expressed in hESC (section 5.2; Nehlin et al., Isa et al.; Sabir et al., in preparation).

5.2 Immune-modulating properties of human mesenchymal stem cells

hMSC are capable of down-regulating allogeneic immune responses by a number of strategies that are illustrated in Figure 3. These strategies will be explained in detail below. Immunosuppression by MSC is species-specific, indicating that the valuable contributions from mouse studies in this area cannot necessarily be extrapolated to the human scenario (Ren et al. 2009). Thus, here we will explore the most relevant findings in the field of hMSC-mediated immunosuppression that have recently gained much attention.
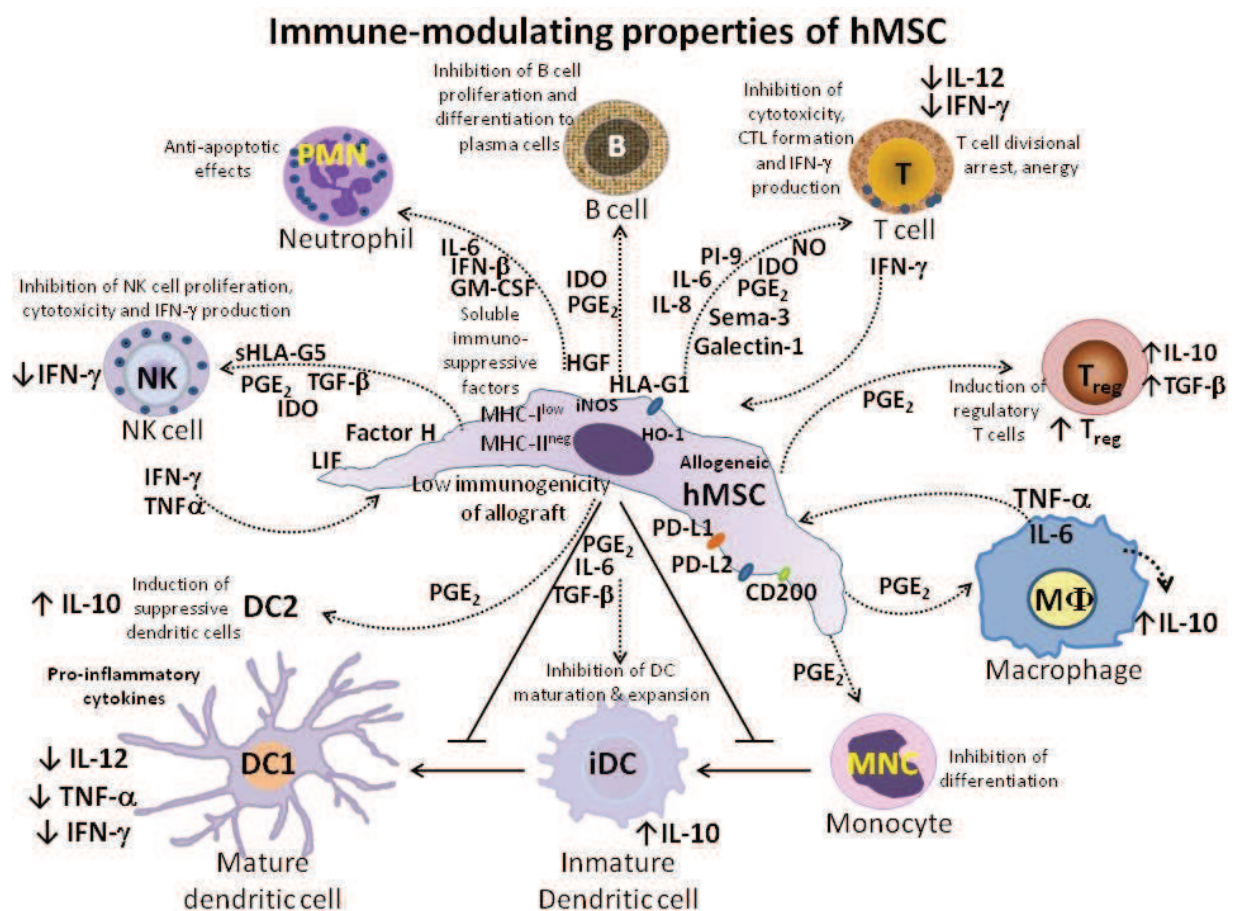

Fig. 3. Illustration depicting the main immune-modulating properties of human mesenchymal stem cells.

hMSC exhibit potent immune-modulating properties which could be useful in numerous clinical applications (section 6.2; Barry et al., 2005; English et al., 2010; Hoogduijn et al., 2010;

Le Blanc & Pittenger, 2005; Nauta & Fibbe, 2007; Rasmusson, 2006; Siegel et al., 2009; Sotiropoulou & Papamichail, et al., 2007; Trento & Dazzi, 2010). The immunomodulatory properties of MSC derived from adult human tissues including bone marrow (BM), adipose tissues (AT), umbilical cord blood (CB), and cord Wharton's jelly (WJ) were shown to be comparable (Yoo et al., 2009; Najar et al., 2010). CD4+ and CD8+ T-lymphocytes were equally targeted by hMSC of different origins, and the effects included prevention of lymphocyte activation as well as the suppression of T-cell proliferation regardless of the stimuli used to activate the lymphocytes (Najar et al., 2010).

Many different pathways mediating hMSC immunotolerance have been suggested such as suppression of T and B cell proliferation both by cell-mediated and soluble factors (Siegel et al., 2009; Uccelli et al., 2008).

Multiple cell-cell interactions and the secretion of soluble factors determine the grade of immunomodulatory capacity by hMSC. Adapted from Aggarwal & Pittenger 2005; Barry et al. 2005; Le Blanc & Ringden, 2007; Nasef et al., 2008; Nauta & Fibbe, 2007; Nemeth et al., 2009; Rasmusson, 2006; Uccelli et al. 2008, and many references in the text.

5.2.1 Origins of the hMSC immunosuppression: cell-cell interactions

Multiple interactions take place between hMSC and cells of the innate and adaptive immune system (Uccelli et al., 2008; Shi et al. 2011). The co-culture of hMSC with purified blood subpopulations in mixed lymphocyte reactions (MLR)(see section 3) has yielded valuable information as regards to hMSC-mediated immuno-modulatory mechanisms of action.

Several studies have explored the effect of hMSC on T cell populations. An early report indicated that hMSC were capable of inhibiting allogeneic T cell responses *in vitro* (Klyushnenkova et al., 1998). Autologous or allogeneic hMSC strongly suppressed T-lymphocyte proliferation, without induction of apoptosis, by both cellular as well as non-specific mitogenic stimuli and was likely due to the production of soluble factors (Di Nicola et al., 2002).

An in-depth analysis was later carried out by Rasmusson et al., 2003, where it was shown that hMSC inhibit T cells in the early activating phase of the allograft reaction, but not in the effector phase. When hMSC were added early in the MLR, they inhibited cytotoxicity, presumably by preventing the formation of active CTL. When MSC were added on day 3 to the 6-day MLR, little effect on cytotoxicity was observed, indicating that hMSC did not appear to inhibit activated CTL and even NK cells (Rasmusson et al., 2003).

Proliferation of allogeneic CD3+ T cell populations was suppressed by hMSC, in a dose-dependent, genetically unrestricted manner, regardless of being pre-treated or not with IFN-γ (Klyushnenkova et al., 2005). hMSC suppressed the proliferation of both CD4+ and CD8+ T lymphocytes, as well as of NK cells. The suppressive activity of hMSC was not only cell-contact dependent but required further the presence of IFN-γ produced by activated T cells and NK cells (Krampera et al., 2006; Pradier et al., 2011).

hMSC altered as well the cytokine secretion profile of naive and effector T cells: T helper 1 (Th1) and 2 (Th2), and NK cells to induce a more anti-inflammatory or tolerant phenotype, increased the proportion of T_{reg} and decreased the secretion of IFN-γ from NK cells induced by IL-2 (Aggarwal & Pittenger, 2005).

hMSC inhibited T-cell proliferation triggered either by allogeneic, mitogenic or antigen-specific stimuli. Interestingly, hMSC inhibited T-cell proliferation by inducing apoptosis once T cells were CD3-mitogenically activated, but had no effect on resting T cells (Plumas et al., 2005). hMSC were able to inhibit proliferation of not only resting thymocytes but also

dividing thymocytes cultured in the absence of trophic factors. hMSC could support T cell survival in a quiescent G_0 state without inducing apoptosis, but T cells still regained their activation capacity once immunosuppression was not longer present. These results suggested that hMSC are endowed with the intrinsic capacity of promoting survival of T cells in a resting state. The protective effect of hMSC targets mainly the "death receptor" pathway of apoptosis, as suggested by the down-regulation of Fas receptor and Fas ligand on TCR activated T cells (Benvenuto et al., 2007).

hMSC were shown to target T-cell proliferation but not their effector function (cytotoxicity). This could be explained by inducing T cells to the G_0/G_1 cell cycle phase, in part through inhibition of cyclin D expression and up-regulation of p27[kip1] (Giuliani et al., 2011; Ramasamy et al., 2008; Siegel et al., 2009). According to a recent study, T cell inhibition by hMSC was not due to the soluble HLA-G5 isoform, but to the surface expression of HLA-G1, as shown by the need of cell-cell contact and by the use of neutralizing anti-HLA-G antibodies (Giuliani et al., 2011; section 5.2.2.3).

hMSC down-regulated alloantigen-induced lymphocyte expansion, especially that of CD8+T cells and of NK lymphocytes, decreased in a dose-dependent manner alloantigen-specific cytotoxic capacity mediated by either CTL or NK cells and favoured the differentiation of regulatory/suppressive CD4+ T-cell subsets co-expressing CD25 and/or CTLA4. More effective suppressive activity on MLR-induced T-cell activation was observed when hMSC were used as third-party, rather than autologous, with respect to MLR-responder cells. These findings support the use of MSC to prevent immune complications related to both hematopoietic stem cell and solid organ transplantation and to the theory that hMSC are universal suppressors of immune reactivity (Maccario et al., 2005; Spaggiari et al. 2009).

Another report showed that the immunosuppressive effect of hMSC targets T cell proliferation of different subpopulations but their effector function or cytotoxicity was not affected in the presence of hMSC at different ratios (Ramasamy et al., 2008).

The significance of the hMSC:T or hMSC:NK co-culture ratios has been investigated in MLR. At low concentrations, hMSC supported rather than inhibited mitogen-stimulated PBMC. Higher concentrations of hMSC not only suppressed alloreactive effector cells, but greatly reduced lymphocyte proliferation caused by potent T-cell mitogens, in autologous or allogeneic PBMC, meaning that the responses were independent of MHC (Le Blanc et al., 2003a). Consistent with this early study, using purified CD3+ T cells only, hMSC were shown to allow T-cell proliferation at a lower MSC:T-cell ratio (1:40) whereas an optimal inhibitory effect was shown when the target (hMSC): effector (T cell) ratio was 1:4 or 1:8 (Najar et al., 2009).

IL-15–stimulated NK cells from 4-day co-culture with hMSC were tested in cytotoxicity assays. When the hMSC:NK cell ratio was low (up to 1:10), hMSC altered the phenotype of NK cells and suppressed their proliferation, cytokine secretion, and cytotoxic capacity against T-cell specific peptide-HLA class I-complexes presented on cancer cells (Sotiropoulou et al., 2006). Some of these effects required cell-to-cell contact, whereas others were mediated by soluble factors (see below), suggesting the existence of diverse mechanisms for MSC-mediated NK cell suppression. On the other hand, hMSC from HLA-mismatched individuals are susceptible to lysis by activated NK cells (Selmani et al., 2009; Sotiropoulou et al., 2006).

Ex vivo-isolated human NK cells become activated upon interaction with bone marrow stromal cells, releasing high amounts of IFN-γ and TNF-α cytokines. These effects depend

on the LFA1/ICAM1 interaction and the NKp30 receptor (Poggi et al., 2005). hMSC inhibited the surface expression of NKp30 and NKG2D activating NK receptors that are involved in NK-cell activation and target cell killing, and no cell surface expression of the NKp44 activating receptor (absent in resting NK cells and expressed upon cell activation) occurred in NK cells cultured with hMSC (Spaggiari et al., 2008).

The effects of hMSC on monocytes and dendritic cells were also examined. hMSC could suppress CD14+ monocyte differentiation into dendritic cells (DC), the most potent antigen-presenting cells (APC)(Jiang et al., 2005), as well as maturation of APC (Beyth et al., 2005) and altered the cytokine secretion profile of dendritic cells (Aggarwal & Pittenger, 2005). Mature DC treated with hMSC decreased expression of antigen-presenting and co-stimulatory molecules, and down-regulated IL-12 secretion (Jiang et al., 2005). Similar results were shown where hMSC strongly inhibited the differentiation of alloantigen-induced monocytes to immature dendritic cells (DC1)(Maccario et al., 2005).

Allogeneic hMSC did not affect B lymphocyte proliferation during allo-stimulation with PBMC in mixed lymphocyte cultures at the the B-cell/hMSC ratio of 1:10 (Krampera et al., 2006). However, another study reported that hMSC inhibited B-cell proliferation by induction of cell cycle arrest at the G_0/G_1 phase. The differences were likely due to the cell ratios used, where maximum inhibition of B-cell proliferation was observed at the B-cell/hMSC ratio of 1:1, detected at a 1:2 ratio and non-measurable at 1:5 and 1:10 ratios. Also, hMSC inhibited B-cell differentiation because IgM, IgG, and IgA production was significantly impaired. CXCR4, CXCR5, and CCR7 B-cell expression, as well as chemotaxis to CXCL12, the CXCR4 ligand, and CXCL13, the CXCR5 ligand, were significantly down-regulated by hMSC, suggesting that these cells affect the chemotactic properties of B cells. B-cell co-stimulatory molecule expression and cytokine production were unaffected by hMSC (Corcione et al., 2006). hMSC were able to suppress allo-specific antibody production *in vitro*, and may therefore help overcome a positive cross-match in sensitized transplant recipients (Comoli et al., 2008).

hMSC also inhibited invariant Natural Killer T (iNKT) and γδ T cell expansion from peripheral blood mononuclear cells. Such inhibition was neutralized by indomethacin, a non-steroid anti-inflammatory drug that inhibits the function of the prostaglandin E_2 molecule (see below, section 5.2.2). iNKT and γδ T have protective and regulatory immune functions in common because they are involved in defense against infectious organisms, tumor rejection, autoimmune disease pathogenesis, and maintenance of transplant tolerance (Prigione et al., 2009).

hMSC have also been shown to influence human polymorphonuclear neutrophil (PMN) responses in co-culture experiments, exerting anti-apoptotic effects that sustained and amplified the functions of PMN in response to toll-like receptors TLR3- and TLR4-triggering, that may consequently contribute to inflammatory disorders. The biological effects exerted on PMN by TLR3-activated bone marrow-derived hMSC are mediated by the combined action of interleukin 6, interferon-β (IFN-β), and granulocyte macrophage colony-stimulating factor (GM-CSF), while those exerted by TLR4-activated BM-MSC mostly depend on GM-CSF (Cassatella et al., 2011). The key hMSC-derived soluble factor responsible for neutrophil protection from apoptosis was IL-6, (Raffaghello et al., 2008; see section 5.2.2.4).

Finally, an interesting study showed that the Stro-1-enriched population of hMSC isolated from the bone marrow elicits a significantly (~10 times) more profound dose-dependent inhibition of lymphocyte proliferation in mixed lymphocyte reactions than hMSC in general, suggesting its use in allogeneic transplantation (Nasef et al., 2009).

5.2.2 Origins of the hMSC immunosuppression: secreted factors

Even though the presence of hMSC in mixed lymphocyte cultures elicits stronger immunosuppressive effects than only hMSC-free culture supernatants, evidence suggests that secreted factors released to the media might account for a significant part of the hMSC-derived immunosuppression (Najar et al., 2009; Nasef et al., 2008a; Rasmusson et al. 2003). Soluble immunomodulatory factors such as indoleamine 2,3-dioxygenase (IDO), prostaglandin E2 (PGE_2), sHLA-G, transforming growth factor (TGF-β), interleukins IL-1β, IL-6, IL-8, IL-10, IL-11, hepatocyte growth factor (HGF), IFN-γ, stromal cell-derived factor-1 (SDF-1) are all secreted by hMSC (Di Nicola et al., 2002; Barry et al., 2005; Giuliani et al., 2011; Najar et al., 2009; Nasef et al., 2008a; Wu et al., 2010).

Secretion of pro-inflammatory and anti-inflammatory cytokines, chemokines, growth factors and prostaglandins by hMSC under resting and inflammatory conditions has been investigated (Hoogduijn et al., 2010; Wu et al., 2010). Exposure of hMSC to pro-inflammatory cytokines such as IFN-γ, tumour necrosis factor (TNF)-α and interleukin (IL)-6 enhances the immunosuppressive capacity of hMSC, suggesting that the use of pre-conditioning to pro-inflammatory conditions, improve hMSC properties for clinical immune therapy (Crop et al., 2010; Ryan et al., 2007).

Supernatants from hMSC exert suppression of T cell activity (Najar et al., 2009; Nasef et al., 2008a), in contrast to what has been found in hESC, where cell-cell contact is essential for immune tolerance (Li et al., 2004). The mechanisms by which some of these factors produced by hMSC act upon the immune system will be briefly explained below:

5.2.2.1 IDO

hMSC express the kynurenine pathway (KP) which is the central route that accounts for the degradation of the essential amino acid tryptophan to kynurenine and subsequently nicotinamide adenine dinucleotide (NAD+). KP has roles in antimicrobial activity, modulation of immune responses and in the creation of a tryptophan-depleted milieu that promotes immunosuppression (Croitoru-Lamoury et al. 2011; Munn et al. 1998). The KP pathway's first and rate limiting enzyme, indoleamine 2,3-dioxygenase (IDO), has two isoforms IDO1 and IDO2. hMSC-secreted IDO catabolizes tryptophan necessary for rapid T cell proliferation. IFN-γ induces the expression of IDO in hMSC, enabling them to inhibit T cell proliferation and modulate the function of major cell populations involved in both the innate and adaptive immune systems, including APC, NK cells, T- and B-cells (Djouad et al., 2007; Meisel et al., 2004; Uccelli et al., 2008). IDO inhibits the proliferation of activated T and NK cells (Krampera et al., 2006; Spaggiari et al., 2008). Also, IFN-γ-induced IDO activation in hMSC leads to their impaired proliferation and an alteration of their differentiation capacity (Croitoru-Lamoury et al., 2011).

When IDO inhibitors were used, however, it was shown that proliferation of PBMC was not restored in mixed lymphocyte cultures with hMSC. Insulin-like growth factor (IGF)–binding proteins were shown to contribute to the inhibitory mechanism of hMSC on PBMC proliferation, and this effect was independent of IFNγR1 signaling and IDO expression (Gieseke et al., 2007).

The mechanism of how MSC-mediated immunosuppression varies among different species has been investigated by Ren et al. (2009). Immunosuppression by hMSC is mediated by IDO whereas mouse MSC (mMSC) utilize nitric oxide under the same culture conditions. When the expression of IDO and inducible nitric oxide synthase (iNOS) were examined in

hMSC and mMSC after stimulation with the pro-inflammatory cytokine IFN-γ in combination with TNFα, IL-1α, or IL-1β, it was shown that hMSC expressed extremely high levels of IDO, and very low levels of iNOS, whereas mMSC expressed abundant iNOS and very little IDO. Chemokines induced by IFN-γ and TNF-α also were released by MSC of mouse or human origin for optimal immunosuppression to attract immune cells to the vicinity, including T cells, which express the chemokine receptor CXCR3 (Ren et al., 2009).

Toll-like receptors (TLR) expressed on bone marrow-derived hMSC enhanced their immunosuppressive phenotype independent of IFN-γ, due to the production of immunosuppressive kynurenines by IDO1. Induction of IDO1 by TLR involved an autocrine IFN-β signaling loop, which was dependent on protein kinase R (PKR), but independent of IFN-γ (Opitz et al., 2009).

5.2.2.2 PGE$_2$

Prostaglandin E$_2$ (PGE$_2$) is, like all prostaglandins, a 20-carbon oxygenated lipid-signaling molecule, with pro-inflammatory functions, involved in producing swelling, redness and pain. Prostaglandin synthesis begins with the release of arachidonic acid from phospholipids by phospholipase A2, and arachidonic acid is then oxygenated by cyclooxygenase (COX) enzymes (COX1 and COX2) expressed by hMSC to form prostaglandin H2 (PGH2), from which PGE$_2$ and other prostaglandins are formed by specific enzymes. In the case of PGE$_2$, they are known as PGE$_2$ synthase enzymes: microsomal PGE$_2$ synthases 1 and 2 (mPGES1 and mPGES2) and cytosolic PGES (cPGES). PGE$_2$ plays a role in many immune functions, including the activation of B lymphocytes and the induction of T$_{reg}$ cells. PGE$_2$ inhibition by indomethacin partially restored the proliferation of T cells in presence of MSC from human or murine origin (Aggarwal & Pittenger, 2005). In the clinic, the production of PGE$_2$ has been targeted with inhibitors of COX-2 function to treat a range of painful and inflammatory conditions.

PGE$_2$ can be produced by many cells and influence the function of a wide array of immune cells, including T cells, B cells, NK cells, macrophages and dendritic cells. Inhibition of PGE$_2$ synthesis by COX inhibitors restored to a great extent *in vitro* T cell proliferation, while blocking other known hMSC-secreted inhibitors did not have the same effect. hMSC inhibited activated T cells proliferation and pro-inflammatory cytokines production. Thus, PGE$_2$ appears to be a dominant secreted molecule involved in hMSC-induced suppression of an *in vitro* alloresponse (Yañez et al., 2010) and hMSC-mediated blocking of monocyte-derived DC maturation (Spaggiari et al., 2009). Also, PGE$_2$ and IDO represent key mediators of the hMSC-induced inhibition of NK cells (Spaggiari et al., 2008).

When hMSC were co-cultured with DC, high levels of PGE$_2$ were detected. PGE$_2$ blockade with indomethacin allowed maturation of plasmocytoid-DC but not myeloid-DC, and allowed T lymphocyte proliferation but did not restore pro-inflammatory cytokine secretion (Yañez et al., 2010).

hMSC reduced the expression of MHC class II, CD40, and CD86 co-stimulatory molecules on mature DC, which was responsible for a decrease in T-cell proliferation. The differentiation of bone marrow progenitors into DC was partly inhibited when cultured with conditioned supernatants from hMSC, and this effect was associated, at least in part with the secretion of IL-6 from hMSC. Suppression of T-lymphocyte activation was partially counteracted by anti-IL-6 but no enhanced effects were found by IL-6 and PGE$_2$ together suggesting that PGE$_2$ may act through the induction ofg IL-6 secretion (Djouad et al., 2007).

When mast cells (MC) are co-cultured with mMSC to allow cell-to-cell contact, mMSC suppressed mast cells degranulation, pro-inflammatory cytokine production, chemokinesis and chemotaxis. These inhibitory effects were dependent on up-regulation of COX2 in mMSC and were facilitated through the activation of EP4 receptors on MC. Whether a similar mechanism applies to hMSC remains to be investigated (Brown et al., 2011).

5.2.2.3 HLA-G and LIF

HLA-G is a non-classical MHC class I molecule, which is expressed in both membrane-bound and soluble isoforms. HLA-G expression is also claimed to be associated with embryo implantation, the protection of the allogeneic fetus from the maternal immune system, and placentation (section 3; Rebmann et al., 2010; Rizzo et al., 2011a).

HLA-G protein expression was found to be constitutive in hMSC and the level was not modified upon stimulation by allogenic lymphocytes in hMSC-mixed lymphocyte reaction assay (Nasef et al. 2007). Furthermore, hMSC secrete the soluble isoform HLA-G5 (sHLA-G5), which inhibits NK cell-mediated cytotoxicity and IFN-γ secretion and suppresses allogeneic T cell proliferation and expansion of CD4+CD25highFOXP3+ T_{reg} cells (Morandi et al., 2008; Selmani et al., 2009). A summary of HLA-G functions was recently presented (Menier et al., 2010). The HLA-G1 isoform, not the sHLA-G5 form, has recently been found to be crucial for the inhibition of T-cell proliferation (Giuliani et al., 2011).

Leukemia inhibitory factor (LIF) is a secreted glycoprotein cytokine that can inhibit the proliferation of myeloid leukemic cell lines and has several functions in hematopoietic expansion of bone marrow progenitors, pregnancy and in the humoral and cellular immune response. LIF and HLA-G expression in hMSC-mixed lymphocyte reactions is coordinated. When LIF was inhibited by a neutralizing antibody, HLA-G was not expressed (Nasef et al., 2008b).

5.2.2.4 Cytokines and chemokines

An early study showed that when hMSC were prevented from cell-cell contacts in transwell experiments, T-lymphocyte proliferation was significantly reduced. Soluble factors such as TGF-β1 and hepatocyte growth factor (HGF) were proposed as mediators of T-cell suppression (Di Nicola et al., 2002).

Among the secreted pro-inflammatory and anti-inflammatory cytokines, chemokines and prostaglandins characterized in hMSC supernatants under resting conditions is worth mentioning ICAM-1, IL-6, IL-8, CCL-2 and TIMP-2 (Wu et al. 2010). IFN-γ, or type II interferon, is a cytokine that is critical for innate and adaptive immunity against viral and intracellular bacterial infections and for tumor control. IFN-γ is not itself produced by hMSC, but originates from activated T and NK cells (Hoogduijn et al., 2010). IFN-γ is also known to play an important role in the induction of immune-modulatory molecules such as IDO (Krampera et al., 2006; see above). IFN-γ inhibited proliferation and altered human and mouse MSC neural, adipocytic and osteocytic differentiation via the activation of IDO (section 5.2.1; Croitoru-Lamoury et al., 2011).

Upon activation, CTL produce cytokines important for their effector functions such as IFN-γ and TNF-α. Incubation with peptide pulsed hMSC did not lead to any detectable induction of IFN-γ and TNF-α secretion by specific CTLs (Rasmusson et al., 2007).

TGF-β1 secreted by hMSC is an immunosuppressive factor capable of inhibiting NK-cell proliferation, cytotoxicity, and cytokine production and of downregulating the expression of

activating receptors 2B4 and NKG2D in NK cells. PGE_2 and TGF-β1 had an additive inhibitory effect on NK-cell proliferation (Sotiropoulou et al., 2006).

5.2.2.5 Galectins and Sema-3A

Galectins are a family of β-galactoside binding proteins that bind not only to glycan structures expressed by host cells but can recognize β-galactoside carbohydrates on many pathogens. Galectins are considered as soluble pathogen recognition receptors. Within the immune system, galectins are expressed by virtually all immune cells, either constitutively or in an inducible fashion (Sioud et al., 2010; Sioud, 2011). Galectins-1 and -3 have been found to be main regulators of hMSC immunosuppressive function, and are constitutively expressed and secreted by human bone marrow MSC. Interfering RNAs abrogated their suppressive effect on allogeneic CD4+ and CD8+ T cells (Gieseke et al., 2010; Sioud et al., 2010; 2011). hMSC derived galectin-1 significantly modulated the release of cytokines involved in GvHD and autoimmunity, such as TNF-α, IFN-γ, IL-2 and IL-10. hMSC promote a shift from a pro-inflammatory Th1 toward a more anti-inflammatory Th2 T-cell response (Gieseke et al., 2010).

Galectin-1 is a homodimeric galactose-binding lectin with a single carbohydrate-recognition domain that binds to the neuropilin-1 receptor (NP-1) expressed on T cells. The main ligand of NP-1 is semaphorin-3A (Sema-3A) that arrests T cells in the G_0/G_1 phase. Galectin-1 and semaphorin-3A (Sema-3A) are two soluble factors highly expressed by hMSC capable to inhibit T-cell proliferation through neuropilin-1 (NP-1). Blocking the interaction to NP-1 abolished hMSC immunosuppression (Lepelletier et al., 2010).

5.2.2.6 PD-1 ligands

One of the well known co-stimulatory pathways is the programmed death (PD-1) pathway, which plays an important role in delivering inhibitory signals that regulate T cell activation, immune tolerance and immune-mediated tissue damage. PD-1 receptor expression is inducible on T cells, NK cells and activated monocytes. PD-1 interacts with the two ligands; PD-L1 (B7-H1; also called CD274) and PD-L2 (B7-DC also called CD273) which are transmembrane glycoproteins belonging to the B7 IgG superfamily (Keir et al. 2008; Petroff & Perchellet, 2010). The interaction leads to signalling via PD-1 receptor and deactivation of the immune cells such as T, B, NK, DC and macrophages, etc. While PD-L1 is widely expressed on low quantities in many cell types including trophoblasts, PD-L2 is more restricted to the myeloid cell types such as monocytes, DC and macrophages (Francisco et al., 2010; Petroff & Perchellet, 2010). hMSC express both PD-L1 and PD-L2 as well as several splice variants but their precise role in the induction of immune tolerance remains to be defined (Isa et al., in preparation). IFN-γ plays a critical role in triggering the immunosuppresion by mouse MSC through the up-regulation of PD-L1 (Sheng et al. 2008). Interestingly, the PD-L ligands may play a critical role in maintaining tolerance to the developing fetus (Petroff & Perchellet, 2010).

5.2.2.7 CD200

CD200 is a transmembrane glycoprotein involved in immune-modulation, such as graft rejection, autoimmune diseases, spontaneous fetal loss, inflammatory disorders and malignancy. The interaction of CD200 and CD200R on T cells results in inhibition of de-granulation and cytokine production which mediate immune regulation through a direct and/or indirect action on activated T-cells via DC (Gorczynski, 2005). CD200 is expressed in

hMSC suggesting that it might exert immune suppressive effects on T cells (Delorme et al., 2008; Larsen et al., in preparation).

5.2.2.8 Factor H

hMSC constitutively secrete factor H, which potently inhibits complement activation, and its production is increased by pro-inflammatory cytokines, but suppressed by IDO and PGE_2 inhibitors. Factor H is the primary fluid phase complement regulator and it is mainly produced by hepatocytes in the liver. Complement is a pivotal part of the innate immunity whose primary roles are fighting infection and clearing out immune complexes. Excessive complement activation can lead to e.g. graft rejection (Tu et al. 2010).

5.2.2.9 Serpins

Serpins are a large family of proteins that control proteolytic cascades or have other cellular functions such as storage, hormone carrier proteins or tumor suppression. Serpins that inhibit the cytolytic enzyme granzyme B are expressed in the cytoplasm and nuclei of CTL and in cells of immunoprivileged sites, such as the placenta, testis, ovaries, and brain. In the mouse, one serpin known as serine protease inhibitor 6 (SPI6) is required to protect CTL from granzyme B-mediated death and facilitates the survival of virally infected cells and tumors. SPI6 helped mouse MSC to escape from host immune attack (El Haddad et al., 2011). The human orthologue of SPI6 is serpin B9 or PI-9/CAP-3, the only known intracellular inhibitor of granzyme B. PI-9 is expressed in many human cell types, but also in cancer cell lines where PI-9 is thought to protect them from granzyme B attack (Rousalova et al. 2010). The presence and potential role of PI-9 in hMSC remains to be investigated.

5.2.2.10 Nitric oxide

An important secreted factor that participates in suppression of T-cell proliferation is nitric oxide (NO) mediated by NO inhibition of transcription factor Stat5 phosphorylation. NO is produced by NO synthases (NOS), of which there are 3 subtypes: inducible NOS (iNOS), endothelial NOS, and neuronal NO. In the presence of a direct interaction between T cells and mouse MSC, there was a high level of NO production accompanied by a strong suppression of T-cell proliferation. The presence of T cells induced the expression of iNOS in mouse MSC (Sato et al., 2007). hMSC also express iNOS but at lower levels than in mouse MSC (Ren et al., 2009).

5.2.2.11 Heme-oxygenase 1

Heme oxygenases (HO) are rate-limiting intracellular enzymes that degrade heme to biliverdin, carbon monoxide, and free divalent iron. The stress inducible form, HO-1, has been described as an immunosuppressive molecule and mediator of the IL-10 anti-inflammatory cytokine. hMSC inhibited allogeneic PBMC proliferation by 60% in MLR experiments and HO-1 inhibition with SnPP completely abolished the immunosuppressive effect of hMSC (Chabannes et al., 2007). hMSC were shown to induce, in a HO-1–dependent fashion, formation of IL-10+ T_{reg} 1 and transforming growth factor-β+ Th3 T_{reg}-subsets in alloreactive and TCR-activated lymphocytes, and IL-10 production. HO-1 was down-regulated by soluble factors produced in the MLR and its functions were replaced by molecules such as COX-2. Two of HO-1 metabolic products, the heavy-chain ferritin and bilirubin, have been linked to T_{reg} activation and expansion (Mougiakakos et al., 2011).

6. Strategies leading to control of immunogenicity and immunotolerance of stem cells as means to overcome immune host responses in non-histocompatible transplantations

The first successful clinical organ transplantation was performed by Joseph E. Murray in 1954 between identical monozygotic and did not require immunosuppression (Murray et al., 2001). Successful transplantations between genetically diverse individuals require immunosuppression to suppress rejection by the recipient's immune system. In spite of improved immunosuppressants (see below, section 7), chronic rejection is still the leading cause of graft failure and happens in >50% of solid organ transplants within 10 years (Orlando et al., 2011a). During the first year after transplantation, the survival rates of the grafts are well above 90%, but the long-term survival of the graft is compromised (Li & Yang, 2009; Meier-Kriesche et al., 2004). Thus, immunological tolerance does not establish in practice.

Acute rejection usually manifests around the time of engraftment and the incidence ranges between 20 and 70%, depending on the extent of histocompatibility mismatches, the age of the recipient and the intensity of preparative regimens, while chronic rejection resembles autoimmune disorders and involves B-cell dysregulation (Busca, 2011).

Creation of custom-made bioengineered organs, where the cellular component is exquisitely autologous and have an internal vascular network will theoretically overcome the two major hurdles in transplantation, namely the shortage of organs and the toxicity deriving from lifelong immunosuppression. Advances in transplantation of custom-made organs have been described (Orlando et al., 2011b). The uses of hESC and hMSC in regenerative therapies will be described in section 6.1.

6.1 Uses of human embryonic stem cells in clinical practice

Adult stem cells have a limited lifespan and cannot be expanded endlessly to satisfy the numbers needed in clinical practice (Mason & Dunnill, 2009; Nehlin & Barington, 2009; Rayment & Williams, 2010). The earliest achievement regarding the isolation and culture of hESC from blastocysts (Thomson et al., 1998) suggested that their future use in regenerative medicine was possible.

However, despite their low immunogenicity, hESC are still immunogenic and immunosuppression or tolerance induction is needed for sustained engraftment in allogeneic transplantation protocols. Therefore, the focus of many laboratories has been to try to defeat potential immunological barriers against hESC. Immunogenicity concerns represents a challenge of future stem cell therapy approaches (Drukker, 2004; Drukker & Benvenisty, 2004; Chidgey et al., 2008; Charron et al., 2009; Fairchild et al., 2004; 2007).

Ideally, the use of self stem cells would be the most encouraging path but many technical issues need to be solved before it can be brought to clinical practice (Ahrlund-Richter et al., 2009; Bongso et al., 2008; Chidgey et al., 2008; Ginty et al., 2011, Nehlin & Barington, 2009; Yamanaka & Blau, 2010). Several strategies involved in sustaining antigen-specific tolerance through interplay between T_{reg} and DC could prolong acceptance of hESC-derived tissues with minimal use of immunosuppressants (Lui et al., 2009; Lui et al., 2010). However, given the promise of induced pluripotency (Yamanaka & Blau, 2010), stem cell transplantation tolerance protocols may well be displaced (Fairchild, 2009).

Clinical immunotolerance could be achieved by a) mixed hematopoietic chimaerism or b) co-stimulatory blockade. Mixed chimerism involves ablation of the host immune system,

followed by its reconstitution with a mixture of host and donor T cell-depleted bone marrow. Reconstitution of the host immune system allows new emerging T cells to perceive the transplanted bone marrow cells as being "self", while donor-reactive T cells are eliminated in the thymus or differentiate into T_{reg}, leading to chimaeric hosts with both donor and self blood cells. The host needs no immunosuppression as it has become tolerant to the donor tissue (Ilstad & Sachs, 1984). The use of this approach has unfortunately been hampered by the toxic side effects inherent to conventional bone marrow transplantation protocols, the ablative regimen used and the use of immunosuppressants to avoid rejection and graft-versus-host disease (Pilat & Wekerle, 2010).

Approaches to facilitate immune tolerance by manipulation of transplantable hESC have been conceived previously such as development of a universal hESC line blocking HLA expression, somatic cell nuclear transfer (SCNT) and creation of a HLA genotyped hESC cell bank. Advantages and disadvantages have been discussed in detail (Boyd & Fairchild, 2010; Bradley et al., 2002; Cabrera et al. 2006; Chidgey et al. 2008; Drukker, 2008; Nehlin & Barington, 2009; O'Rourke et al. 2008).

A promising approach to achieve tolerance is to block co-stimulation during allogeneic transplantation. Co-stimulation blockade with anti-CD40L/anti-LFA-1 and CTLA4Ig blocking antibodies to induce tolerance to hESC transplanted into testicle, an immune-privileged environment, and heart was examined in immunocompetent and severe combined immunodeficient (SCID) mice. hESC injected into the testis of SCID mice and costimulation blockade treated C57BL/6 mice developed into teratoma in all animals, and were surrounded by CD4+CD25+Foxp3+ T cells, inducing tolerance to the grafts, while in the control treated mice, no surviving hESC were found. Thus, co-stimulation blockade induced tolerance to hESC in the immune-privileged environment of the testis (Grinnemo et al., 2008).

When hESC treated with co-stimulation blockade were transplanted into the hearts of SCID mice, hESC developed teratoma-like formations, whereas immune-competent mice exhibited loss of all hESC cells by 1-2 months due to lymphocytic infiltrates. However, if the co-stimulation blockade was repeated ~3 weeks after the initial transplantation, some surviving hESC-derived cells could be monitored 2 months later. Isolation of T_{reg} from intramyocardial transplanted recipients treated with co-stimulation blockade demonstrated specificity toward undifferentiated hESC and down-regulated naive T-cell activation toward hESC. hESC-specific T_{reg} developed to hESC transplanted to the heart. Thus, transplantation success in co-stimulation blockade treated mice was similar to that seen in SCID mice (Grinnemo et al. 2008b).

Recently, a successful co-stimulatory blockade protocol was created, by simultaneous blocking of CTLA-4, CD40L and IRF-1 using blocking antibodies during a short period of 6 days. Experimental mice were transplanted with e.g. transgenic human iPS or hESC, and their fate was examined using bio-imaging. The donor cells were able to survive and grow, and after 54 days, there was no evidence of rejection (Pearl et al., 2011). This finding suggested that allogeneic hESC transplantation with a brief co-stimulation blockade of leukocyte co-stimulatory pathways could be feasible. Unfortunately, the long-term effects of such procedures with respect to the risk of infections are not yet known.

The idea of creating a cell bank composed of donor HLA-typed hESC lines representing different haplotypes matching those of a large population that would help to reduce the risk of graft rejection and satisfy unmet clinical needs was envisioned (Taylor et al. 2005). However, a perfect 6/6 match between donor and recipient in terms of classical MHC class I

HLA-A,-B and –C alleles would not be enough to prevent rejection. Allograft survival is inversely correlated with the degree of mismatch between the donor's and the recipient's MHC antigens. The ideal transplant that carries lessened risk of rejection is the one where a perfect 12/12 match is ensured, whereby in addition to identical HLA-A,-B,-C between donor and recipient, also identical MHC class II proteins HLA-DR, -DP, and –DQ are sought (Loiseau et al. 2007). It appears unrealistic to build a stem cell bank of the required magnitude in order to match all HLA genotypes world wide. Also, even minor histocompatibility antigens may pose a risk (Bradley et al., 2002; Charron et al. 2009; Feng et al., 2008; Shlomchik, 2007; Spencer et al., 2010).

To assess if hMSC could be used to induce tolerance to co-transplanted hESC, ligation-induced myocardial infarction was performed in immunocompetent rats and 3 weeks later the hearts were injected with either hMSC, hESC or both. Co-transplantation of hESCs and hMSC provided better preservation of left ventricle function compared with single-cell treatment alone. The lack of clear evidence for an immunosuppressive or tolerogenic action of hMSC suggested that the benefits observed were mediated by synergistic trophic effects that enhanced repair of injured host tissue (Puymirat et al. 2009).

hESC could also be used in therapies to inhibit tumor progression. Supernatants from hESC have been shown to reduce the clonogenicity and tumorigenesis, as well as to increase apoptosis in aggressive cancer lines (Postovit et al., 2008).

The world's first clinical trial involving the use of hESC-derived cells is ongoing. A cell line known as GRNOPC1 contains hESC-derived oligodendrocyte progenitor cells that have demonstrated remyelinating and nerve growth stimulating properties leading to restoration of function in animal models of acute spinal cord injury. A phase I study has been initiated to assess safety and tolerability in a paralyzed patient with spinal cord injury. Each transplant recipient will be immune-suppressed from the time of injection with low-dose tacrolimus for 46 days, at which time the immune suppression will be tapered and withdrawn at 60 days (www.geron.com/GRNOPC1Trial/).

6.2 Uses of human mesenchymal stem cells in clinical practice

The first published human clinical study reporting positive results with hMSC was in a young boy with acute lymphoblastic leukemia in third remission that had received a transplant of blood stem cells from an HLA-A, HLA-B, HLA-DR1 identical, unrelated, female donor, but after 70 days, developed grade IV GvHD unresponsive to conventional therapy. Transplantation of his mother's hMSC exerted such a strong immunosuppressive effect *in vivo* that the patient made a remarkable recovery (Le Blanc et al., 2004).

After this successful case, the profound inmuno-modulatory and anti-inflammatory effects of hMSC have been exploited for clinical applications in a number of clinical trials to treat diseases such as hematological malignancies, autoimmune diseases such as Crohn's, type 1 diabetes, rheumatoid arthritis, systemic lupus erythematosus, neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis and multiple sclerosis, In addition, hMSC are included in prospective studies to prevent GvHD, treat refractory GvHD, cancer, stroke, acute myocardial infarction, critical limb ischemia, acute tubular necrosis, and use in plastic surgery, bone and cartilage tissue engineering, wound healing, dental pulp regeneration, heart transplantation, insulin-dependent diabetes mellitus, etc. (Abdi et al., 2008; Aggarwal & Pittenger, 2005; Dazzi et al., 2007; Doeppner & Hermann, 2010; Kan et al., 2007; Le Blanc & Ringden, 2007; Niemeyer et al., 2006; Sasportas et al., 2009; Sato et al., 2010; Singer & Caplan, 2011; Trento & Dazzi, 2010; Tögel &

Westenfelder, 2007; Vanikar et al., 2010; Wu et al., 2010). Several clinical trials where bone marrow hMSC have been used in the treatment of ischemic heart disease such as in clinical refractory angina, ischemic cardiomyopathy with left ventricular dysfunction, and end-stage heart failure have yielded promising results (Fuh & Brinton, 2009). Prochymal, a commercial hMSC line, underwent a successful clinical trial to treat myocardial infarction, giving insights into the preparation of hMSC (Hare et al., 2009). More recently, another study demonstrated that it was safe to treat patients with stable coronary artery disease, with autologous hMSC, showing significant improvement in left ventricular function, exercise capacity and clinical symptoms (Friis et al., 2011).

A summary of pre-clinical models and clinical trials where *in vitro* expanded hMSC were used and where the biological properties of hMSC were explained in detail have recently been reported (English et al., 2010; Garcia-Gomez et al., 2010; Parekkadan & Milwid, 2010; Salem & Thiemermann, 2010; Shi et al., 2011; Singer & Caplan, 2011). hMSC supported engraftment and survival of unrelated human donor hematopoietic stem cells infused into sublethally irradiated NOD-SCID mice (Maitra et al. 2004). The biological effects of MSC in mouse and rat pre-clinical models of disease have also been reported (Uccelli et al., 2008).

hMSC have the capacity to migrate (homing) and integrate into damaged tissues and provide immunomodulatory effects by paracrine (soluble factors) and/or cell-cell contact that is regulated by the inflammatory microenvironment. Local or systemic infusions are now being successfully used to co-transplant hMSC with other parenchymal cells, such as hepatocytes or islet cells, to enhance the engraftment and function of such cells in an immunoprotected fashion. These findings may extend future prospects of the clinical application of MSC into broader applications (Uccelli et al., 2008; Yagi et al. 2010). After *in vivo* administration, MSC induce peripheral tolerance and migrate to injured tissues, where they can inhibit the release of pro-inflammatory cytokines and promote the survival of damaged cells (Uccelli et al. 2008).

hMSC are easily isolated from bone marrow, fat and other tissues, and are readily propagated *in vitro*. Transplanted/injected MSC have been shown to migrate to a variety of organs and tissues, but they preferentially undergo homing to sites of inflammation and pathology for tissue remodeling and repair. Transplanted allogeneic MSC can be detected in recipients at extended time points, indicating a lack of immune recognition and clearance (Singer & Caplan, 2011). Because tumor microenvironments also appear to be a target of hMSC homing, there are various controversies surrounding these interactions regarding clinical outcomes (Kidd et al., 2008). hMSC have been used to secrete recombinant cytokine tumor necrosis factor apoptosis ligand (TRAIL) to induce apoptosis of glioma cells *in vivo* (Sasportas et al., 2009). Another study has shown that hMSC exhibit innate anti-tumor effects against human pancreatic carcinoma cells implantated in SCID mice and can serve as delivery vehicles for IFN-β for the treatment of pancreatic cancer (Kidd et al., 2010). Also of great concern is the potential tumorigenicity of hMSC. Although malignant transformation of primary hMSC has not been noted to date in clinical trials using hMSC, expansion *in vitro* for extended periods of time could confer the risk of chromosomal instability and malignant transformation as was reported for mMSC (Tolar et al., 2007).

The strong immunosuppressive activity of MSC has been exploited to attempt treating GvHD (Dazzi & Marelli-Berg, 2008). The clinical experience with hMSC for the treatment of GvHD is encouraging but incomplete. Results of clinical trials utilizing hMSC for the treatment of acute and chronic GvHD have been summarized (Kebriaei & Robinson, 2011). Longer follow-ups of current clinical trials are necessary to determine whether any long-

term unwanted effects are associated with the use of hMSC to treat GvHD (Kebriaei & Robinson, 2011).

Clinical trials involving hMSC for the treatment of GvHD have been recently described (Sato et al. 2010). Infusion of hMSC expanded *in vitro*, irrespective of the donor, might be effective therapy for patients with steroid-resistant, acute GvHD (Le Blanc et al., 2008). hMSC appear capable of suppressing acute GvHD without increasing systemic infections (Sato et al., 2010). Donor hMSC significantly inhibited the proliferation of alloactivated recipient T cells before and after kidney transplantation suggesting that the application of hMSC in solid organ transplantation may facilitate graft acceptance and function (Crop et al., 2009).

Steroid-refractory GvHD may also be treated with hMSC infusions but hMSC have been almost impossible to detect after infusion when administered *in vivo*, and thus little is known regarding their migration, their mechanism of action, or their persistence (Paczesny et al., 2009). Advances in the immune reconstitution after hematopoietic stem cell transplantation (HSCT), a widely used method in cancer treatment have been reviewed elsewhere (Cavazzana-Calvo et al., 2009). One trial example involved co-transplantation of *ex vivo*-expanded donor hMSC with CD34+ cells from a relative in children with a hematological malignancy, leading to a reduced risk of graft failure in haploidentical hematopietic stem cell- transplant recipients (Ball et al., 2007). However, relapse of the underlying disease, GvHD, or severe opportunistic infections, account for the majority of deaths following HSCT. Approaches such as immune reconstitution, withdrawal of the immunosuppression, chemotherapy or novel drugs with or without donor lymphocyte infusions, and even second allogeneic stem cell transplantation are considered (Kröger, 2011; Seggewiss & Einsele, 2010). Cellular therapy including adoptive transfer of *ex vivo*–expanded immunomodulatory cells such as T_{reg} cells, NK/T_{reg} cells, donor-derived NK cells, and hMSC and adoptive transfer of allogeneic T cells specific for viral or tumor antigens appears promising to improve immune reconstitution after transplantation (Peters et al., 2009; Seggewiss & Einsele, 2010).

Immune responses against hMSC has also been studied in a kidney transplant setting. Donor hMSC and kidney recipient immune cells (PBMC) isolated at various time points after kidney transplantation were used in MLR assays. Donor hMSC significantly inhibited cytotoxic effector cells of the recipients isolated before transplantation. Allogeneic hMSC were susceptible to lysis by cytotoxic CD8+ T-cells and NK cells, while autologous hMSC were lysed by NK cells only. NK cell-mediated lysis was inversely correlated with the expression of HLA class I on MSC. PBMC isolated 3, 6 and 12 months after donor kidney transplantation showed increasing lysing ability against donor hMSC. Even 12 months after kidney transplantation, CD8+ T cell-mediated lysis of donor hMSC persisted, indicating that there was no evidence for desensitization against donor hMSC. Therefore, controlling the immunogenicity conferred by the HLA expression status, the survival over time of hMSC and avoidance of lysis by cytotoxic immune cells are important for the efficacy of MSC therapy in organ transplantation (Crop et al., 2009; Crop et al., 2011).

Treatment with allogeneic hMSC or the conditioned medium restored alveolar epithelial fluid transport and lung fluid balance in an *ex-vivo* perfused human lung preparation injured by *E. coli* endotoxin, and keratinocyte growth factor played a crucial role in this effect (Lee et al., 2009).

Also, the use of inducible T_{reg} or T regulatory type 1 cells are promising candidates for stem cell therapy because of their immunomodulatory activities such as ability to secrete

suppressive cytokines and cell-to-cell contact-dependent killing of target myeloid cells mediated by granzyme B and perforin. Such properties are helpful not only to inhibit GvHD after allogeneic hematopoietic stem cell transplantation, but also in other transplantation settings, or to re-establish tolerance in autoimmune or allergic diseases (Peters et al., 2009; Roncarolo et al., 2011).

Although shown in a mouse sepsis model, it was found that injections of bone marrow mMSC protected cells from damage in affected vital organs and had reduced vascular permeability, one of the deadliest consequences of sepsis. The therapeutic effect was mediated by reprogramming of the macrophage after direct contact with MSC, resulting in macrophage-dependent production of IL-10 and lower expression of TNFα and IL-6. PGE$_2$ from MSC after activation of Toll-like receptor 4 by bacterial lipopolysaccharide (LPS) was responsible for the reprogramming (Nemeth et al., 2009). Also shown in a mouse model very recently, it was found that intramyocardial delivery of c-kit$^+$ bone marrow cells after myocardial infarction induces endogenous progenitor-derived cardiomyocyte renewal and improves ventricular function, an effect not observed with MSC (Loffredo et al., 2011).

Recently, methods have been developed to identify hMSC with the highest immunosuppressive capacity based on soluble HLA-G production (HLA-G5) in IL-10-treated bone marrow hMSC. A decreased positivity for CD90 is associated with loss of immunosuppressive capacity (Rizzo et al., 2011b).

Pre-clinical screening before allogeneic stem cell therapy is possible if isolated hMSC can undergo a cytotoxicity assay by means of mixed lymphocyte cultures and the subsequent measurement of their proliferation potential (Koppula et al., 2009).

hESC-derived hMSC demonstrated having immunosuppressive effects towards T and NK cells, similar to natural hMSC suggesting another origin from which hMSC can be obtained for therapy (Yen et al., 2009). As mentioned previously, the possibility of reprogramming self-cells into hESC from which one could develop the cell type and numbers needed for a given therapy, would be an ideal situation.

Many challenges need to be assessed in the future as regards the characterization and quantities of stem cells necessary for regenerative medicine which may vary by several orders of magnitude depending on the conditions to treat and their needs among the millions of people afflicted worldwide by a number of degenerative illnesses. Follow-up studies to understand the long-term *in vivo* effects of allogeneic transplantations are needed (Mason & Dunnill, 2009; Rayment & Williams, 2010). Also, optimization of the growth conditions to preserve hMSC immunomodulatory properties are merited (Samuelsson et al., 2009).

Although hMSC exhibit immune privileges as explained above, allogeneic hMSC infused intravenously into the host without immunosuppression or chemotherapeutic conditioning may still lead to adverse effects that require treatment. The purpose of deliberately induced immunosuppression in a host recipient is to prevent rejection during transplantation of non-histocompatible (allogeneic) cells, tissues or organs, and to treat GvHD. However, the use of immunosuppresants increases the vulnerability of the individual to opportunistic infections, nephrotoxicity, cancer and even accelerated aging (Li & Yang, 2009; Nehlin and Barington, 2009). Several families of immunosuppressants have been developed such as glucocorticoids, cytostatics, therapeutic monoclonal antibodies, and many others (Duncan & Wilkes, 2005). In the case of allogeneic hematopoietic stem cell transplants, even though response rates were reported to be more than 60%, long-term survival still remains sub-optimal, mainly due to the detrimental side effects of infectious complications, progressive GvHD and relapse due to the underlying malignancy (Busca, 2011).

7. Conclusion

hESC are pluripotent stem cells with low immunogenicity and immune-modulating properties conferred by molecules intrinsic to the cells themselves, but also found secreted in the microenvironment. Many studies remain to ascertain the role of the various components acting upon immune system cells in allogeneic settings. This is especially merited if off-shelf cell lines from hESC banks will be used in the future.

In-depth immunological characterization of iPS cells is required to envision their use in auto-transplantation protocols and clinical trials to reveal if any potential incompatibility might arise as a result of the reprogramming process. Reprogrammed cells (iPS) will offer great clinical advantages once potential hurdles are fully sorted out.

Multipotent stem cells such as hMSC are endowed with multiple immune-regulatory properties acting on different cells of the immune system that protect them from cytotoxic effects. The regulation and quantification of the hMSC-dependent immune-suppression properties both *in vitro* and *in vivo* in long-term transplantation studies await further analyses. The therapeutic uses of hMSC are becoming widespread in a number of clinical conditions, but its place in future medicine still needs to be clarified.

8. Acknowledgement

9. References

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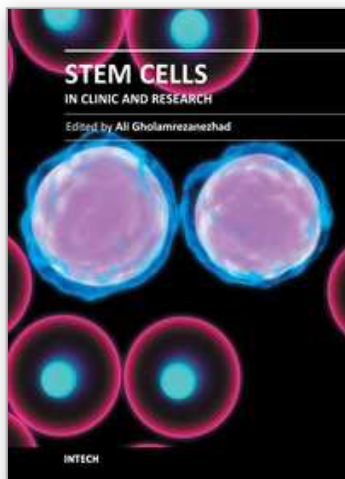
Stem Cells in Clinic and Research
Edited by Dr. Ali Gholamrezanezhad

Based on our current understanding of cell biology and strong supporting evidence from previous experiences, different types of human stem cell populations are capable of undergoing differentiation or trans-differentiation into functionally and biologically active cells for use in therapeutic purposes. So far, progress regarding the use of both in vitro and in vivo regenerative medicine models already offers hope for the application of different types of stem cells as a powerful new therapeutic option to treat different diseases that were previously considered to be untreatable. Remarkable achievements in cell biology resulting in the isolation and characterization of various stem cells and progenitor cells has increased the expectation for the development of a new approach to the treatment of genetic and developmental human diseases. Due to the fact that currently stem cells and umbilical cord banks are so strictly defined and available, it seems that this mission is investigationally more practical than in the past. On the other hand, studies performed on stem cells, targeting their conversion into functionally mature tissue, are not necessarily seeking to result in the clinical application of the differentiated cells; In fact, still one of the important goals of these studies is to get acquainted with the natural process of development of mature cells from their immature progenitors during the embryonic period onwards, which can produce valuable results as knowledge of the developmental processes during embryogenesis. For example, the cellular and molecular mechanisms leading to mature and adult cells developmental abnormalities are relatively unknown. This lack of understanding stems from the lack of a good model system to study cell development and differentiation. Hence, the knowledge reached through these studies can prove to be a breakthrough in preventing developmental disorders. Meanwhile, many researchers conduct these studies to understand the molecular and cellular basis of cancer development. The fact that cancer is one of the leading causes of death throughout the world, highlights the importance of these researches in the fields of biology and medicine.

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