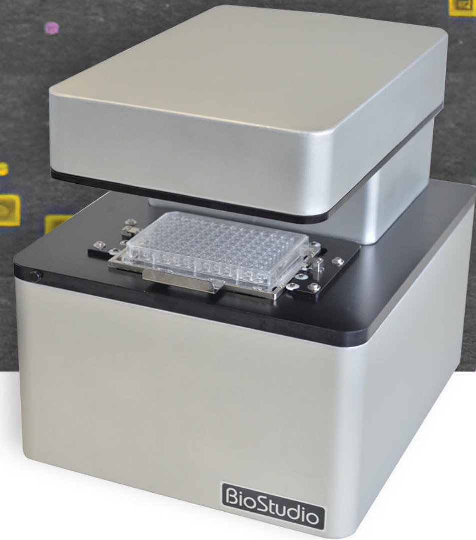

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Concise Review: Combining Human Leukocyte Antigen G and Mesenchymal Stem Cells for Immunosuppressant Biotherapy

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Key Words. Mesenchymal stem cells • Immunosuppression, HLA-G • Transplantation tolerance

ABSTRACT

Both human leukocyte antigen G (HLA-G) and multipotential mesenchymal stem/stromal cells (MSCs) exhibit immunomodulatory functions. In allogeneic tranplantation, the risks of acute and chronic rejection are still high despite improvement in immunosuppressive treatments, and the induction of a state of tolerance to alloantigens is not achieved. Immunomodulatory properties of MSCs and HLA-G in human allogeneic tranplantation to induce tolerance appears attractive and promising. Interestingly, we and others have demonstrated that MSCs can express HLA-G. In this review, we focus on the expression of HLA-G by MSCs and discuss how to ensure and improve the immunomodulatory properties of MSCs by selectively targeting MSCs expressing HLA-G (MSCs$^{HLA\text{-}G+}$). We also discuss the possible uses of MSCs$^{HLA\text{-}G+}$ for therapeutic purposes, notably, to overcome acute and chronic immune rejection in solid-organ allogeneic transplantation in humans. Since MSCs are phenotypically and functionally heterogeneous, it is of primary interest to have specific markers ensuring that they have strong immunosuppressive potential and HLA-G may be a valuable candidate. STEM CELLS *2013;31:2296–2303*

Disclosure of potential conflicts of interest is found at the end of this article.

INTRODUCTION

Mesenchymal stem cells (MSCs), also referred to as multipotent mesenchymal stromal cells, are able to form colony forming unit fibroblasts and to proliferate extensively in vitro [1]. MSCs can be isolated from different tissues including bone marrow (BM), umbilical cord blood, adipose tissue, liver, and muscle. Native MSCs (in situ MSCs) are still poorly characterized despite increasing papers describing their localization and characteristics [2–4]. In contrast, cultured MSCs are largely described in numerous papers. These cultured MSCs are generally considered to have at least a minimal phenotype: CD105+CD73+CD90+ and CD45−CD14− [5]. As well, they can differentiate into osteoblasts, chondroblasts, or adipocytes in vitro and in vivo [6].

In addition to their multipotential abilities, cultured MSCs can home to sites through cytokine/chemokine gradients [7, 8]. However, in in vivo context the inflammation is required to attract MSCs. After systemic injection, data show that MSCs are preferentially distributed to their tissue of origin although thereafter, few are found [9, 10]. MSCs also support the regeneration of damaged tissue by secreting various growth factors, cytokines, and antioxidants [11, 12]. Among the trophic factors secreted, several are anti-inflammatory or immunosuppressive. Hence, one of the most interesting properties of MSCs is their aptitude to modulate the immune response. In this review, we focus on cultured bone-marrow-derived MSCs (BM-MSCs) because they are the best-characterized population [13], they have been used in several reported clinical trials, and, in general, their immunosuppression potential does not differ greatly from that of MSCs from other origins [14]. In addition, to date, the immune properties of native MSCs are unknown.

Regenerative medicine and tissue transplantation represent the only therapeutic options for many patients with terminal organ failure. Allogeneic tissue transplantation is used worldwide, although acute and chronic immune rejection are still common despite immunosuppressive treatments [15]. During the last three decades, the development of multiple immunosuppressive treatments has greatly reduced immune rejection

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Correspondence: Frederic Deschaseaux, Ph.D., Stromalab, UMR UPS/CNRS 5273, EFS-PM, U1031 INSERM, BP 84225, 31432 Toulouse Cedex 4, France. Telephone: +33-5-61-32-34-99; Fax: +33-5-62-17-09-05; e-mail: frederic.deschaseaux@efs.sante.fr Received August 15, 2012; accepted for publication July 10, 2013; first published online in STEM CELLS *EXPRESS* August 6, 2013. © AlphaMed Press 1066-5099/2013/$30.00/0 doi: 10.1002/stem.1494

Table 1. Selected active clinical trials involving BM-MSCs

Transplantation/ autoimmunity	Conditions	Intervention	Phase	Sponsor	Status	Identifier
Cell transplantation	HSCs	BM-MSCs infusion	Expanded access	Osiris Therapeutics	Completed	NCT00826046
Cell transplantation	HSCs	BM-MSCs infusion	1 and 2	UMC Utrecht	Recruiting	NCT00827398
Cell transplantation	HSCs	BM-MSCs infusion	2	University Hospital of Liege	Recruiting	NCT00603330
Solid-organ transplantation	Kidney	BM-MSCs infusion	1 and 2	Leiden University Medical Center	Recruiting	NCT00734396
Solid-organ transplantation	Kidney and liver	BM-MSCs infusion	1 and 2	University Hospital of Liege	Recruiting	NCT01429038
Solid-organ transplantation	Kidney	BM-MSCs infusion	1 and 2	Mario Negri Institute for Pharmacological Research	Recruiting	NCT00752479
Autoimmunity	Crohn's disease	BM-MSCs infusion	1 and 2	Leiden UMC	Recruiting	NCT01144962
Autoimmunity	Crohn's disease	BM-MSCs infusion	1 and 2	Osiris Therapeutics	Recruiting	NCT00482092
Autoimmunity	Multiple sclerosis	BM-MSCs infusion	1	Cleveland Clinic Foundation	Recruiting	NCT00813969
Autoimmunity	Systemic lupus erythematosus	BM-MSCs infusion	1 and 2	Nanjing Medical University	Recruiting	NCT00698191

Clinical trials with MSCs used as immunosuppressant cell biotherapy undertaken in the United States, European Union, and China to (a) prevent graft-versus-host disease in allogeneic hematopoietic stem-cell transplantation, (b) prevent acute and chronic rejection in allogeneic solid-organ transplantation, and (c) control autoimmune diseases.
Abbreviations: BM-MSCs, bone-marrow-derived multipotential mesenchymal stem/stromal cells; HSC, hematopoietic stem cell.

[15]. However, the problem has not been completely solved and existing immunosuppressive drugs have multiple side effects [16, 17]. Therefore, MSCs are of particular interest and are being extensively investigated in regenerative medicine, even in an allogeneic context, because of their immunomodulatory properties.

Cultured allogeneic MSCs are widely used in clinical trials in hematopoietic stem cell (HSCs) infusion [18–20], in solid-organ transplantation in an allogeneic context [21] and for treating autoimmune diseases such as Crohn's disease [22] and type I diabetes mellitus (Tables 1, 2) [23]. However, some in vivo data on the immunomodulation capacity of human MSCs are controversial, and results are difficult to analyze because isolating MSCs from adult tissue often results in selecting a heterogeneous population and the culture conditions can vary [24–26]. Therefore, a specific marker must be targeted according to the therapeutic use or for potency assays. For example, for using MSCs as immunosuppressant cell biotherapy in allogeneic tissue transplantation or in regenerative medicine, a biomarker assessing for immunosuppressive function is of great interest.

HLA–G: A Key Immunomodulatory Molecule Expressed by MSCs

How MSCs can induce immunosuppression is a complex matter involving MSCs reacting to their environment, which ultimately shapes the response through multiple immunosuppressive pathways [27]. MSCs inhibit T-cell proliferation in vitro independently or dependently through cell contact, and a similar mechanism of action occurs with natural killer (NK) cells and dendritic cells (DCs) [27]. Indeed, MSCs can express membrane-bound ligands to immunoinhibitory receptors, such as programmed death ligand 1 or 2 as well as soluble immunosuppressive molecules such as prostaglandin E2 (PGE2) [28]. However, more attention has been focused on soluble immunosuppressive molecules secreted by MSCs (Table 3). Indeed, a number of immunosuppressive molecules expressed and secreted by MSCs, such as transforming growth factor β, PGE2, interleukin 10 (IL-10), indoleamine-

Table 2. Selected active clinical trials involving BM-MSCs: MSC markers used in clinical trials

Identifier	Markers used
NCT00826046	Positive for CD105, CD73, CD29, CD44, CD71, CD90, CD106, CD120a, CD124, CD166
	Negative for CD45, CD34, CD14
	CFU-f
	Osteo-chondro-adipocyte differentiation potentials
	TNFR1
	Inhibition of IL-2Rα activated T cells
NCT00827398	Positive for CD73, CD90, CD105
NCT00603330	Positive for CD73, CD90, CD105
	Negative for CD45, CD34, CD14, CD31, CD80, CD3, HLA-DR
	Osteo-chondro-adipocyte differentiation potentials
	Inhibition of T cell proliferation
NCT00734396	Positive for CD73, CD90, CD105
	Negative for HLA-DR, CD31, CD45, CD80
NCT01429038	See NCT00603330
NCT00752479	Positive for (>70%) CD73, CD90, CD105
	Negative for (<10%) CD14, CD34, CD45
	Osteo-chondro-adipocyte differentiation potentials
	Allo-immune response (secretion of IFNγ)
NCT01144962	See NCT00734396
NCT00482092	See NCT00826046
NCT00813969	Not shown
NCT00698191	Positive for (90%) CD73, CD90, CD105, CD29
	Negative for (<2%) CD45, CD34, CD14, CD79, HLA-DR

Abbreviations: CFU-fs, colony forming unit fibroblast; HLA, human leukocyte antigen; IFN, interferon.

Table 3. MSC immunosuppressive factors

	Effects on immune cells	
(a) MSCs soluble factors		
HGF	T-cell inhibition	[29]
HLA-G5	T-cell inhibition/Treg expansion-NK inhibition	[30]
IDO	T-cell inhibition-NK inhibition	[31]
IGF	T-cell inhibition	[32]
IL1RA	T-cell inhibition-MF inhibition	[33]
IL6	T-cell inhibition-DCs inhibition	[34]
LIF	T-cell inhibition/Treg expansion	[35]
PGE2	T-cell inhibition-NK inhibition	[36]
(b) MSCs membrane-bound factors		
Jagged 1	T-cell inhibition	[37]
HLA-G1	T-cell inhibition/Treg expansion	[30]
PDL1/PDL2	T-cell inhibition	[28]
CD54	Treg expansion	[38]
CD58	Treg expansion	[38]

MSCs can exert their immunomodulatory functions through multiple factors. They are (a) soluble factors secreted in the milieu and (b) membrane bound; in this case, a direct interaction between MSCs and immune cells is required.
Abbreviations: HGF, hepatocyte growth factor; HLA-G5, human leukocyte antigen G; IDO, indoleamine-2,3-dioxigenase; IGF, Insulin-like growth factor; IL1RA, Interleukin 1 receptor antagonist; IL6, interleukin 6; LIF, Leukemia inhibitory factor; MSC, mesenchymal stem cell; NK, natural killer cell; PGE2, prostaglandin E2.

2,3-dioxigenase (IDO), hepatocyte growth factor, and human leukocyte antigen G (HLA-G) have been extensively described as modulating T-cell proliferation and function, NK cytotoxicity, and DC maturation. Generally, cultured MSCs appear to be poorly able to elicit an immune response but well able to suppress it. Presumably, all the immunosuppressive functions of MSCs can be mediated by any of the previously described factors depending on the context (e.g., inflammation status), but certainly, key molecules play a central role in the immunosuppressive functions and finding these molecules is of primary interest.

By analogy, pregnancy establishes cellular and molecular mechanisms allowing for immunotolerance toward fetal antigens. This is a reliable model of true tolerance resulting from evolutionary selection. At the feto-maternal barrier, trophoblasts express and secrete multiple immunosuppressive molecules such as PGE2, IL-10, IDO, and HLA-G [39]. Yet, among all these immunosuppressive factors, HLA-G has emerged as a true key immunosuppressive molecule with the ability to prevent the rejection of the semiallogeneic fetus by the maternal immune system [40]. As well, a decrease in HLA-G level is a risk factor of pre-eclampsia [41]. Therefore, the mechanisms MSCs use to induce immunosuppression appear to be similar to those used by trophoblasts at the decidual placenta [42]. We, and others, have hypothesized that HLA-G might be an important pathway node playing a significant role in MSC immunosuppressive functions (Fig. 1) whatever the tissue of origin (BM, adipose tissue, placenta) [30, 43, 44].

HLA-G was first described as a key molecule inducing materno-fetal tolerance [45], tumor escape, and allograft transplantation acceptance [46, 47]. HLA-G molecules belong to the nonclassic HLA-class I molecules (HLA-Ib), along with HLA-E and HLA-F, as opposed to the classical HLA-class I molecules (HLA-Ia), with HLA-A, HLA-B, and HLA-C [48]. HLA-G mediates its immunosuppressive functions by interacting with immunoreceptor tyrosine-based inhibition motif-bearing receptors such as Ig-like transcript 2/leukocyte immunoglobulin-like receptor, subfamily B/CD85 (ILT2/LILRB1/CD85j) as well as ILT4/LILRB2/CD85d and killer Ig-like inhibitory receptor (KIR)2DL4/CD158d (Fig. 2A). The expression of ILT4 and KIR2DL4 is restricted to myeloid and NK cells, respectively, whereas ILT2 is expressed by all immune cells [47]. Therefore, HLA-G molecules can have inhibitory effects in all immune cells. HLA-G binding to its receptors occurs through phosphorylation and recruitment of the Src homology 2 domain-containing tyrosine phosphatases SHP-1 and SHP-2. The phosphatases subsequently dephosphorylate and thereby inactivate key molecules involved in cellular activation such as AKT, mammalian target of rapamycin, and signal transducer and activator of transcription [49]. Also, HLA-G can exert its immunosuppressive functions (a) directly by inducing CD8+ T-cell apoptosis [50] or by arresting T- and B-lymphocyte cell-cycle progression at the G0/G1 phase or (b) indirectly by inducing regulatory T cells or immature DCs expressing IL-10 [51]. In contrast to classical HLA-class I, HLA-G molecules show low polymorphism and are expressed in a limited number of healthy tissues. In adults, HLA-G is expressed within immune-privileged tissues such as cornea and by cell precursors such as erythroid and endothelial precursors [47]. In addition, we recently observed the expression of HLA-G within osteoblastic cells in the growth plate of bones and in callus postfracture: once secreted by osteoblastic cells, HLA-G was able to suppress osteoclastogenesis and was regulated by an osteogenic molecular pathway [52].

HLA-G has seven different isoforms, including four membrane-bound forms (HLA-G1 to -4) and three soluble forms (HLA-G5 to -7) derived from alternative splicing of the primary *HLA-G* transcript [53]. The membrane-bound HLA-G1 and soluble HLA-G5 isoforms are typically expressed in adults [54]. We, and others, have shown that MSCs express HLA-G (mainly HLA-G5) and such MSCs can secrete HLA-G during allogeneic challenge in vitro [30, 43]. Actually, HLA-G proteins derived from MSCs suppress T- and NK-cell proliferation and function. Moreover, HLA-G secreted by MSCs induces the expansion of $CD4^{+}CD25^{+}FoxP3^{+}$ regulatory T cells [30]. We, and others, have described a tightly regulated amplification loop of immunosuppression induced by MSCs that involves HLA-G and IL-10, which act in interdependently. As IL-10 upregulates HLA-G expression, HLA-G drives T cells toward a Th2-type of differentiation with an increase in IL-10 expression [55, 56]. For instance, we found that both IL-10 and HLA-G5 are required for full MSC-mediated immunosuppression [30]. This finding agrees with IL-10 being required to induce HLA-G+ T-reg cells and DCs [51, 57]. Despite lack of data showing the molecular pathway linking HLA-G and IL-10, these results strongly suggest that both molecules may act in synergy [30].

The expression of HLA-G can be directly regulated by different mechanisms. HLA-G expression by MSCs can be positively modulated by IL-10 (see above) and leukemia inhibitory factor. HLA-G can also be regulated by Indian Hedgehog (HH) during the osteoblastic differentiation, notably, through the binding of HH signaling transducer Glioma-associated oncogene (GLI) to *HLA-G* promoter [52]. Other molecules (glucocorticoid, interferon β [IFNβ]) were found to regulate HLA-G expression in immune cells [58]. However, the mechanisms seem to be cell type-specific and need to be evaluated in MSCs.

Of note, the immunosuppressive functions of MSCs are not represented by a single immunosuppressive molecule but rather a complex interdependent immunosuppressant network, itself depending on the inflammatory status of the MSC

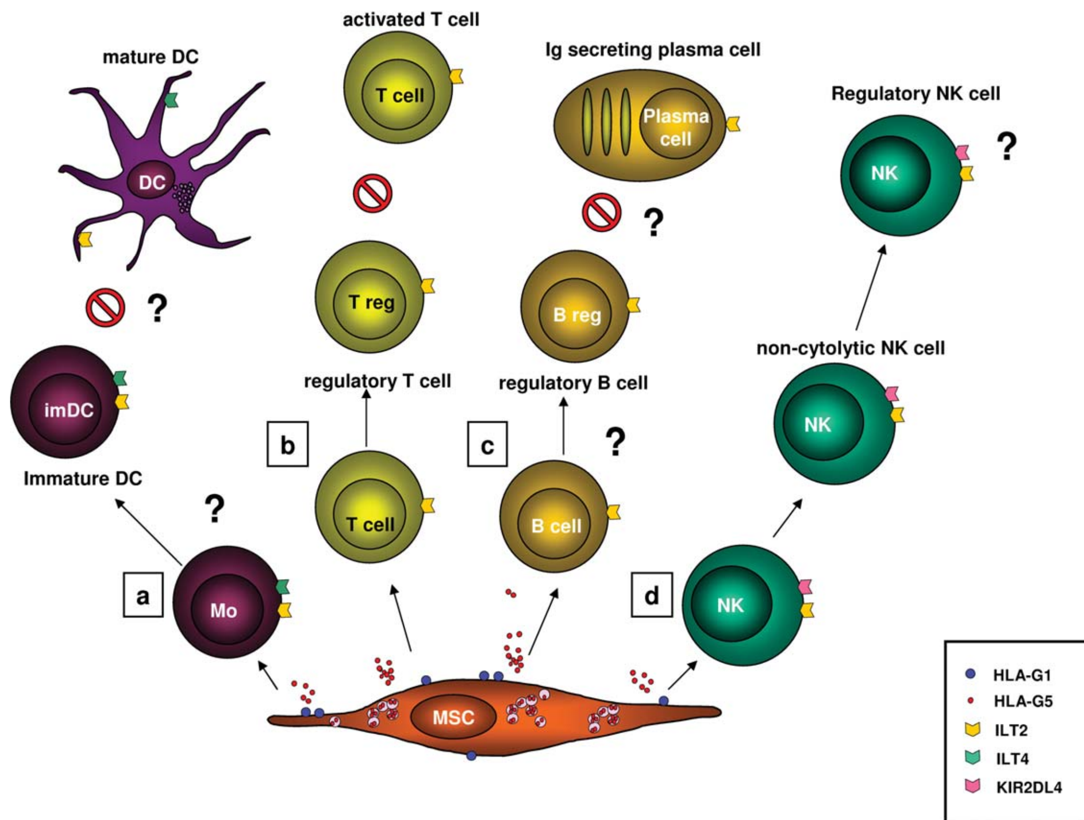

Figure 1. Putative interactions between MSCs expressing HLA-G (MSCs$^{HLA\text{-}G+}$) and cells from the innate and adaptive immune system expressing HLA-G receptors. Through ILT2 expressed on all immune cells, ILT4 expressed on myeloid cells, and KIRD2L4 expressed on NK cells, HLA-G secreted by MSCs can regulate the components of the innate and adaptive immune systems. **(A):** HLA-G impairs DC maturation originating from Mo. However, HLA-G secreted by MSCs impairing DC maturation is still a putative mechanism. **(B):** HLA-G inhibits T-cell alloproliferation and induces CD4+CD25+Foxp3+ regulatory T cells [30]. **(C):** HLA-G secreted by MSCs inhibits B-cell proliferation and function or induces regulatory B-cell activity (manuscript in preparation). **(D):** HLA-G secreted by MSCs inhibits NK cytotoxicity [30]. HLA-G also promotes regulatory NK and T-cells through trogocytosis [44]; however, whether this process occurs with MSCs expressing HLA-G remains unknown. Abbreviations: DC, dendritic cell; HLA-G, human leukocyte antigen G; ILT2, Ig-like transcript 2; KIR, killer Ig-like inhibitory receptor; NK, natural killer cell; Mo, monocyte; MSC, mesenchymal stem cell.

microenvironment [59]. Therefore, evaluating the respective role of the different immunosuppressive molecules according to external cues would be of interest. For instance, we found that as compared with HLA-G, PGE2 or IDO had no effect on inhibiting T-cell activation by MSCs in our experimental context of allogeneic antigen stimulation [30]. In addition, MSCs need to be activated by IFNγ (priming of MSCs) to induce the expression and function of IDO but not HLA-G. Yet the function and expression of IDO and HLA-G5 are not mutually influenced [60]. Of note, the priming of MSCs by IFNγ and TNFα raises some differences between BM-MSCs and adipose-derived MSCs (AD-MSCs) because IDO expression is particularly high in AD-MSCs; hence, IDO was described as a key immunosuppressive molecule in human primed AD-MSCs [61]. Therefore, evaluating an immunosuppressive molecule as a biomarker of the immunomodulation capacities of MSCs is crucial. Of interest is whether a variation in immunosuppressive protein expression also varies the immunomodulation potential of MSCs. Some data support HLA-G as biomarker like for fetal tolerance and pre-eclampsia. Indeed, during in vitro expansion, MSCs show decreased content of intracytoplamic HLA-G, which is concomitant with decreased MSC inhibitory function [30, 62]. Moreover, Rizzo et al. recently demonstrated that a heterogeneous population of MSCs stimulated with IL-10 showed an increased number of MSCs expressing both the membrane-bound HLA-G1 and soluble HLA-G5 isoforms (Fig. 2B). In addition, the level of HLA-G was directly associated with the rate of inhibition of proliferation of Phytohemagglutinin (PHA)-activated peripheral blood lymphocytes [62]. Data from other works and our own clearly show that MSCs$^{HLA\text{-}G+}$ have better immunosuppressive functions than do MSCs$^{HLA\text{-}G+/-}$, as evidenced by enhanced inhibition of allogeneic T-cell proliferation in vitro (Fig. 2C) [63]. Although further studies are essential to appreciate the strict mechanisms that control the MSC immunomodulatory functions via HLA-G, HLA-G can be used as a marker to recognize MSCs with potent immunomodulatory functions. The use of MSCs$^{HLA\text{-}G+}$ instead of non-selected MSCs for clinical applications in allogeneic transplantation or in regenerative medicine would likely enhance the efficiency of the treatment to prevent immune rejection.

MSCs$^{HLA\text{-}G+}$ As Immunosuppressant Cells in Allogeneic Transplantation

Graft-versus-host disease (GvHD) is a severe and frequent complication in allogeneic HSC transplantation [64]. The coinfusion of MSCs and HSCs improves engraftment and reduces

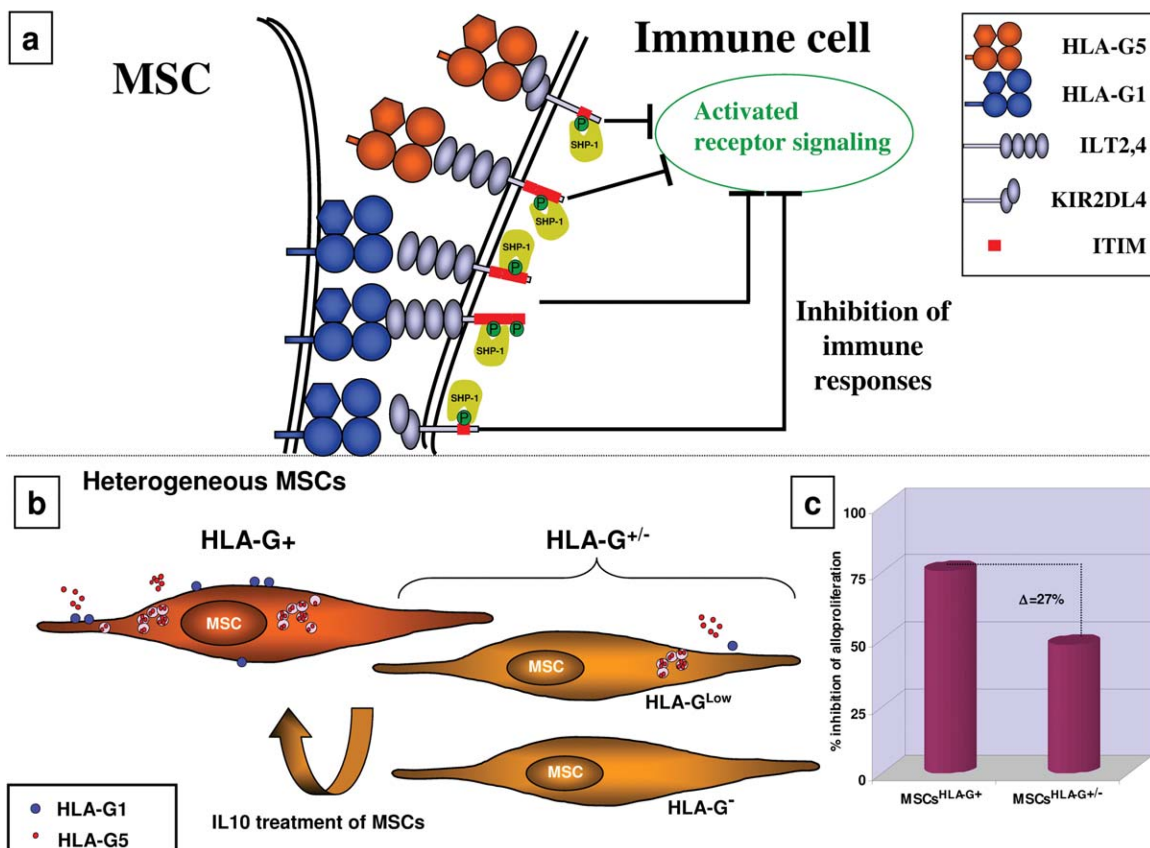

Figure 2. Heterogeneity of MSCs according to HLA-G expression and immunosuppression. **(A):** Mechanisms of HLA-G immunosuppression: membrane (HLA-G1) or soluble (HLA-G5) bind ILT2 or ILT4 (ILT2,4) receptors found on lymphocytes or monocytes as well as KIR2DL4 expressed by NK cells. This induced activation by phosphorylation of immunoreceptor tyrosine-based inhibition motif in the cytoplasmic tails of HLA-G receptors recruits the SHP-1, which results in inhibition of immune responses. **(B):** Different populations of MSCs with HLA-G expression: $MSCs^{HLA\text{-}G+}$, $MSCs^{HLA\text{-}Glow}$, and $MSCs^{HLA\text{-}G-}$. To prevent the decrease of HLA-G expression in the course of in vitro expansion of MSCs, cells can be treated with IL-10. **(C):** $MSCs^{HLA\text{-}G+}$ exhibit better immunosuppressive functions than do a heterogeneous population of MSCs ($MSCs^{HLA\text{-}G+/-}$). Abbreviations: HLA-G, human leukocyte antigen G; ILT-2, Ig-like transcript 2; KIR, killer Ig-like inhibitory receptor; MSC, mesenchymal stem cell; SHP-2, Src homology 2 domain-containing phosphatase 1.

GvHD, as demonstrated in a number of clinical trials worldwide [36, 65, 66]. Thus, MSCs might provide an interesting tool for improving allogeneic organ transplantation, in general, and for solid organs in particular, without the use of elevated concentration of immunosuppressive drugs. The first clinical data describing MSC infusion in allogeneic solid-organ transplant recipients are now available and seem to confirm that use of MSCs is a promising cell therapy [21, 67]. Three phase I and II trials are registered to assess the safety and efficiency of MSCs in reducing immune rejection episodes or inducing tolerance in allogeneic kidney and liver transplantation (Tables 1, 2). However, further clinical research is needed to refine and develop effective MSCs as treatment to prevent both acute and chronic rejection. Fortunately, the considerable amount of clinical data regarding HSC transplantation and the use of MSCs to prevent GvHD should facilitate the use of MSCs in allogeneic solid-organ tranplantation. Nevertheless, the efficacy of such treatment remains variable in some clinical trials [68], which mitigates the enthusiasm regarding the use of MSCs as immunosuppressant cell biotherapy. Such variations in efficacy might be due to the heterogeneity of MSCs and the different culture conditions [24–26]. To better adapt protocols to isolate potent immunosuppressive MSCs aimed at reducing allograft rejection, we suggest the selective use of $MSCs^{HLA\text{-}G+}$.

HLA-G has been broadly investigated for allogeneic solid-organ transplantation and has been well associated with reduced number of immune rejection episodes in kidney and liver allogeneic transplantation [69]. Indeed, data from more than 1,000 patients show that HLA-G is associated with better graft acceptance, and a high level of HLA-G in plasma is associated with less acute and chronic rejection episodes after heart and liver/kidney transplantation [70–73]. Interestingly, the protein level of soluble HLA-G is greatly increased 2 hours after injection of immunosuppressive drugs (cyclosporine) in heart transplant patients, which suggests that HLA-G expression is related to the immunosuppression pathway. In addition to these clinical findings, some experiments showed the direct effects of HLA-G on graft acceptance. For instance, when peripheral blood mononuclear cells were treated with a solution containing HLA-G before their infusion in patients, they did not elicit responses to allogeneic stimuli. Peripheral blood from liver and liver/kidney transplanted patients who exhibit >80% graft acceptance contained significantly higher HLA-G and IL-10 plasma levels and over-representation of $CD3+CD4^{low}$ or $CD3+CD8^{low}$ suppressor T cells when compared with kidney transplanted patients with 53% graft acceptance or with healthy patients. This finding was confirmed by in vitro experiments showing that HLA-G

might induce functional $CD3+CD4^{low}$ and $CD3+CD8^{low}$ suppressive T cells [69]. Finally, injection of HLA-G tetramer-coated beads into recipient mice before allogeneic skin transplantation induced suppressive T cells with prolonged graft survival or acceptance [74]. Therefore, coupling two entities with strong immunosuppression activities (i.e., MSCs overexpressing HLA-G molecules) should be of benefit in preventing immune rejection.

$MSCs^{HLA-G+}$ As Immunosuppressant Cells in Regenerative Medicine

MSCs are also of particular interest in regenerative medicine because of their multipotential capabilities, and testing $MSCs^{HLA-G+}$ could be of primary interest. Because BM-MSCs can consistently differentiate into bona fide chondrocytes and osteoblasts, a number of research projects have focused on designing tools to restore bone in certain diseases, even in allogeneic contexts [75, 76]. In human, BM-MSCs were used in clinical trials to treat several types of bone diseases (bone defect repair, osteonecrosis, osteogenesis imperfecta). Despite the fact that the trials have to be improved, MSCs represent promising tools for regeneration of bone in skeletal diseases [77, 78]. Beyond their differentiation properties, MSCs have trophic abilities that improve healing and regeneration of multiple tissues not directly derived from MSCs [7, 8, 79]. By infusing MSCs for tissue injury, the extent of damage and cell death can be controlled and allow for subsequent regeneration of various tissues [79]. Several clinical trials are using allogeneic MSCs for regeneration of the heart after myocardial infarction and for meniscal restoration following meniscal injury in part because of the trophic capabilities of MSCs [80–83]. Moreover, the European Regenerating Bone Defects using New biomedical Engineering approaches (REBORNE) project recently launched international clinical trials to evaluate bone repair in nonunion bone fractures with MSCs (from BM or adipose tissue) combined with biomaterials (www.reborne.org). A particular focus is on whether MSCs are beneficial in bone repair. In addition, since HLA-G is expressed by osteoblastic cells induced during skeletogenesis or during bone healing after fracture, HLA-G will be assessed as a bone-healing biomarker [52]. Therefore, MSC^{HLA-G+} and osteoblastic cells positive for HLA-G might be valuable in regenerative medicine to enhance the MSC reparative/regenerative abilities or to be used as biomarker of tissue regeneration.

$MSCs^{HLA-G+}$ Derived from Pluripotent Stem Cells

Most clinical trials use MSCs derived from BM, umbilical cord blood, or adipose tissue [66]. However, the most common source of MSCs for clinical trials is still BM (Tables 1, 2). The doses currently used in clinical trials have been $1–2 \times 10^6$ cells per kg. Hence, we need to produce MSCs ex vivo extensively [83]. For instance, 10,000 doses of fully characterized MSCs can be produced from one BM donor for therapeutic injection. However, HLA-G expression tends to diminish in long-term cultures as described above. Otherwise, lesser doses can be produced for decreasing the time of culture but this necessitates more donors. Nevertheless, this is less consistent and consequently more random in terms of effectiveness due to intrinsic donor-dependent variabilities in the immunomodulatory efficiency of MSCs [83]. In addition, the discrepancies between results of the effectiveness of the treatment might be explained by the facts that MSCs derived from different sources [83]. One solution to these problems is to have an infinite number of $MSCs^{HLA-G+}$ which should be derived from a single source, that is, embryonic stem (ES) cells or induced-pluripotent stem (iPS) cells. MSCs from human ES cells could be produced under conditions relevant for therapeutic uses. These types of MSCs do not form teratomas in vivo despite their typical MSC phenotype and function [84, 85]. Recently, a study provided evidence of the expression of HLA-G by ES- and iPS-cell-derived MSCs [86]. These data show the practicability of obtaining an infinite number $MSCs^{HLA-G+}$ with potent immunosuppressive functions (i.e., inhibition of NK activation). However, pluripotent stem-cell-derived MSCs must be compared to BM-MSCs in terms of immunomodulation capacities. Results to date show that human ES-cell-derived MSCs are indeed immunosuppressive, but more clarification on the mechanisms and comparison with BM-MSCs are needed notably in the in vivo context. Interestingly, several research groups reported that human ES-cell-derived MSCs are more resistant to NK-mediated lysis probably because of membrane HLA-G [87]. Therefore, further enriching MSCs expressing HLA-G is needed to ensure and enhance the MSC immunosuppressive functions.

Conclusions

To improve current protocols of MSCs used as immunosuppressant cell biotherapy in allogeneic transplantation and regenerative medicine, recent research suggests the following: (a) increase the number of MSCs expressing HLA-G with IL-10 stimulation, and ultimately (b) isolate and use MSCs expressing a high level of HLA-G. This latter point could be achieved through the use of iPS-derived MSCs. These recommendations would certainly enhance the immunomodulation properties of MSCs and ensure consistency in their beneficial effects in preventing immune rejection in allogeneic transplantation.

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Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

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