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Comprehensive Review

Immunogenicity of Adult Mesenchymal Stem Cells: Lessons from the Fetal Allograft

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ABSTRACT

Herein we review recent data that support host tolerance of allogeneic adult mesenchymal stem cells (MSC). Evidence is emerging that donor MSC deploy a very powerful array of mechanisms that allow escape from host allogeneic responses. These mechanisms include limited expression of alloantigen by the stem cell and cell contact-dependent and -independent mechanisms. MSC modulate host dendritic cell and T cell function, promoting induction of suppressor or regulatory T cells. These effects are complemented by the induction of divisional arrest anergy in T cells and by stem cell production of soluble immunomodulatory factors, including interleukin-10, transforming growth factor–β, prostaglandin E2, and hepatocyte growth factor. In addition, MSC express the enzyme indoleamine 2,3-dioxygenase, which creates a tryptophan-depleted milieu that promotes immunosuppression. We propose that these observations show striking similarity to emerging data on the maternal acceptance of the fetal allograft. This comparison suggests new approaches to determine the contribution of different mechanisms to the successful use of MSC in regenerative medicine.

INTRODUCTION

STEM CELLS HAVE A CAPACITY for self-renewal and a potential to differentiate into multiple lineages. This has made them an attractive target for the development of novel cell-based therapies that are usually referred to as regenerative medicine. The immunogenicity and capabilities of embryonic stem and hematopoietic stem cells are well known and have been thoroughly reviewed elsewhere (1–3). However, a number of recent studies have thrown new light on the possible use of allogeneic adult stem cells. Adult human stem cells can be isolated from a range of tissues, however most attention has focused on a stem cell population resident at low density in the bone marrow stroma termed mesenchymal stem cells (MSC), first described by Friedenstein and Petrokova (4). Recent advances in the isolation, culture, and differentiation of these cells has sparked intense interest in their therapeutic use in tissue engineering and regenerative therapies (5–8).

The challenge facing researchers is to exploit MSC in the clinical setting. This immediately poses questions of the immunogenicity of MSC. There may well be cases where autologous graft of MSC may be beneficial (9), but the ability to engraft allogenic MSC is likely to be much more attractive therapeutically. Indeed, the commercially viable exploitation of regenerative medicine may well rest on the capacity to use allogenic MSC on a large scale.

Although stem cell biologists may be aware of the varied literature supporting the possibility of host tolerance

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of allogeneic MSC, the broader immunology community is often less convinced. Nevertheless, if progress is to be made in the use of MSC in an allogeneic environment, then it is useful to consider the state of the art in allotransplantation and to draw from parallel disciplines those salient features which inform our own studies.

One difficulty that confronts attempts to provide a comprehensive review of the field is the bewildering array of cells being studied by different groups. There is not universal agreement of what is an MSC, and there is certainly a need for standardization. The biological characterization of different MSC populations has been covered in another recent review and will not be the main focus of this study (5,10); neither will we consider the multipotent adult progenitor cells of different varieties that seem to have broader differentiation potential and quite different surface marker expression (11,12). Accounts of the transplant potential of these multipotent cells have been published (11), but full descriptions of their immunogenicity are eagerly awaited. The goal of this article is to review the numerous lines of investigation that convincingly indicate the mechanisms by which adult MSC escape the normal processes of allogeneic rejection. We propose a conceptual framework in which MSC phenotype mirrors aspects of the most successful form of immune tolerance–the fetal allograft.

MSC: DO THEY AVOID ALLOGENEIC REJECTION MECHANISMS?

For Immunologists, the capacity for autologous or syngeneic MSC to be tolerated by a host poses few conceptual problems (9). However, the potential use of allogeneic cells is often viewed with scepticism. This scepticism is unjustified; a large body of data from in vitro human, as well as large and small animal in vivo studies, support an immunosuppressive or at least hypoimmunogenic character to allogeneic MSC (5,12–16). The in vivo data from humans is at present preliminary; however, a small number of studies suggest that MSC play a role in enabling alloantigen tolerance. For example, Horwitz described how transplantation of sibling bone marrow in children with osteogenesis imperfecta resulted in engraftment of donor-derived MSC (17). Furthermore Koc et al. concluded from a study of metachromatic leukodystrophy and Hurler syndrome that allogeneic MSC were safe and associated with a reversal of pathology in some tissues (18); this study also showed no evidence of alloreactive T cells or graft-versus-host disease (GVHD) in recipients (18). This work supported earlier findings by this group in breast cancer patients, suggesting that autologous MSC were safe and promoted hematopoiesis after myeloablative therapy (19).

Studies in animal models of allogeneic transfer are also impressive (13,20). Saito et al. demonstrated that MSC were tolerated in a xenogeneic environment while retaining trafficking capacity to an injured pericardium (21). MSC present at low levels in hematopoietic stem cell transfers are considered to be immunosuppressive, and human MSCs can promote unrelated haematopoitic stem cell survival and suppress T cell activation in a nonobese diabetic/severe combined immunodeficiency (NOD-SCID) model (22). Indeed, a range of therapeutic possibilities based on this immunosuppressive effect have been proposed (10). Using bone marrow derived allogeneic Flk-1^+, Sca-1^- MSC in a mouse model, Deng et al. demonstrated not only long-term tolerance of allogeneic skin grafts, but also provided data suggesting the induction of haematopoietic chimerism (23). Perhaps the key recent paper has come from Aggarwal and Pittenger, which clearly demonstrates that human MSC modulate allogenic immune responses (14) and supports the tolerance of allogeneic MSC.

A large body of work based on allogeneic co-cultures or mixed lymphocyte reactions (MLR) have generated convincing evidence supporting an immunosuppressive role for MSC (13,23–27). Many of these data will be discussed in more detail below, but at the fundamental level they all share a similar observation: that the use of mismatched MSC does not provoke a proliferative T cell response in an allogeneic MLR. These findings have been seen with human cells, with rat or mice cells and even in xenogenic MLR (13,24,26–28). It is clear that a broad body of data using different cells, different species, and diverse readouts all support the description of MSC as being exceptions to the regular allogeneic rejection mechanisms. Two questions arise out of these observations. First, how do MSC mediate this effect? This gap in our understanding has prompted the focus of research to the discovery of mechanisms by which hypoimmunogenicity is maintained. Two mechanisms have been evoked, one involving direct cell–cell interaction (29), the other requiring the secretion of immunomodulatory factors. The potential mechanisms will be outlined below, but (like the provision of T cell help to B cells for production of antibody) it is most likely that both processes are involved (30). The second question arising from hypoimmunogenicity of MSC is more difficult to address. The mammalian immune system plays a fundamental role in the organism's interaction with the environment; in effect it is a major mechanism by which the body "sees" the biochemical world. In which case, why has evolution maintained a hypoimmunogenic phenotype for MSC? And why do these cells escape the usual laws of allogeneic rejection? The answer, or at least an indication, may come from the only routine form of allogeneic interaction in the mammal, the carriage of the allogeneic fetus.

MATERNO–FETAL INTERACTION, PREVENTING REJECTION OF THE FETAL ALLOGRAFT

In successful mammalian pregnancy, the mother accepts a fetal allograft. This occurs in the presence of a maternal T cell and antibody response to a paternal antigen. Therefore, the mother is not immunologically ignorant of the paternal antigen and that the fetus is not "walled off" from maternal immune recognition. Indeed, the maternal immune system can delete circulating allogeneic fetal cells without rejecting the fetus (31,32). Researchers since Medawar have attempted to comprehend how the fetus is tolerated in the presence of immune recognition. It is of direct interest to stem cell researchers, because it is possible that similar tolerogenic mechanisms operate during pregnancy as during allogeneic MSC engraftment (33). An obvious mechanism by which MSC could bypass allogeneic rejection mechanisms would be to migrate and differentiate into thymic epithelium and thereby promote central tolerance. However, three lines of evidence suggest that this is unlikely to be a major mechanism operating here. The rapid timing of regular allogeneic rejection of non-MSC, the results from in vitro studies (where such a mechanism is precluded), and the constrained differentiation potential of MSC as opposed to multipotent adult progenitor cells (11). These data would tend to argue against central tolerance to chimeric or alloantigen-bearing cells as being the primary mechanism of allograft survival. Although this mechanism cannot be excluded in vivo, central tolerance cannot be operating in the various in vitro systems.

A full understanding of materno–fetal tolerance is not yet available, but major advances have been made recently that show profound alteration in maternal immune responses during gestation (31,34–38). These immunomodulatory changes are linked with suppression of inflammatory or Th1-like cytokines and the induction of T cells with regulatory or suppressive phenotypes (34,35,38,39). Foremost among the mediators of such effects are interleukin-10 (IL-10) and transforming growth factor-β (TGF-β). IL-10 is expressed by the placenta and endometrium and has a broad, complex role at the materno–fetal interface (40–42). Interestingly, IL-10 abnormalities are associated with pre-eclampsia (40–42) whereas reduced IL-10 is associated with recurrent spontaneous abortion (43). Given that IL-10 exerts a broad immunosuppressive influence (34,35,38,39), the evidence suggests that this soluble factor plays an important role in tolerance to the fetal allograft.

One consequence of IL-10 production in the human placenta is that the nonclassical major histocompatibility complex class I (MHC-I)-like protein HLA-G is synthesized (44) and MHC class I antigen production is downregulated (44). Indeed, the human trophoblast is MHC class II negative and the expression of MHC class I is limited (45,46). HLA-G expression is significant because this surface marker can bind to inhibitory receptors on natural killer (NK) cells and thereby protect against rejection (47). Thus, soluble and cell contact-dependent mechanisms cooperate at the materno–fetal interface to prevent fetal loss.

Other soluble factors with immunomodulatory potential have a role in the materno–fetal interaction. TGF-β isoforms and closely related molecules are elevated in the circulation of human and rodent fetal and maternal circulations during gestation (48–50). Because TGF-β has well-documented immunosuppressive and regulatory influences (51–53), it has been proposed that this factor contributes to survival of the fetal allograft (49). Like TGF-β, hepatocyte growth factor (HGF) plays a complex role in early fetal–maternal interactions. HGF is involved in the cross talk between thecal and granulosa cells (54) and has a role in regulating folliculogenesis (55). This physiological function continues in the developing organism (56,57). Recent data suggest that HGF has a second important function. In addition to developmental roles, HGF promotes allograft survival (58–60); thus, this factor may contribute to fetal tolerance. In contrast to HGF and TGF, prostaglandin E-2 (PGE-2) synthesis by the conceptus shows marked differences between species; however, in most mammals PGE-2 plays a role in luteal maintenance and parturition (61). It is also an immune mediator with broad suppressive activity (62–65), although in the human materno–fetal context, this role may be subservient to an immune function within the uterus at term (66). The salient point is that each of these factors, TGF-β, HGF, and PGE-2, appear to supplement a physiological or developmental function during pregnancy with an immunomodulatory role that serves to protect the fetal allograft.

It is now known that the enzyme indoamine 2,3-dioxygenase (IDO) is expressed and active in the human placenta and suggests a role for this enzyme in allograft tolerance (67) as well as a direct physiological function (31). IDO catabolizes L-tryptophan, depleting the local environment of an essential amino acid. Studies from infectious models have shown that tryptophan depletion can limit microbial proliferation (68,69), and recent evidence has confirmed that depletion of this essential amino acid by IDO + plasmacytoid dendritic cells (DC) induces regulatory/suppressor type T cells (70). In human studies of the materno–fetal interaction, IDO expression is moderate in the syncytial trophoblast and endothelium but high in the fetal-derived invasive extravillous trophoblast (67). Although murine placental organization differs from that of humans, mouse studies show similar findings with IDO expression by trophoblast giant cells under the control of (maternally derived) fetal cells (71). Consistent with these studies are observations that tryptophan con-

centration in maternal circulation falls steadily over pregnancy (72,73). The emerging paradigm is that fetus-driven IDO expression creates a "tryptophan desert" in the trophoblast, an environment that is conducive to the induction of regulatory DC and T cell subsets that contribute to fetal allograft survival (70). The mechanisms that support tolerance of the fetal allograft therefore include a hypoimmunogenic phenotype (e.g., low MHC class I and no MHC II expression) by fetal cells at the materno–fetal interface combined with specific cognate and noncognate mechanisms operating locally. The parallels to the tolerance of allogeneic MSC described below are striking.

MHC EXPRESSION BY MSC: A HYPOIMMUNOGENIC PHENOTYPE

The highly regulated control of MHC expression at the materno–fetal interface is an important but not the sole mechanism of fetal allograft tolerance. Knowledge of the expression of MHC class I and class II in both human and animal MSC is critical to research attempts to understand the immunology of these cells. However, the difficulties arising from the use of different models, the multitude of MSC-like populations used, the different isolation and culture protocols, and the variable timings for measurement have led to considerable differences in the reported expression of these molecules by MSC. Although there is a need for increased standardization of approach, some consensus can be discerned. MSC from both human and rodents appear to be MHC class I positive (15,29,74). This is an important finding because there are powerful immune rejection mechanisms operating against cells that are class I negative occupying sites that lack immunological privilege (75). Indeed, a major function of NK and NK-like cells is to kill tumor cells that have down-regulated class I (75). It will be interesting to see if multipotent adult-derived progenitor cells that are class I negative actually survive in vivo in an inflamed allogeneic environment. It will also be important to measure expression of nonclassical MHC molecules such as HLA-E, -F, or -G to assess their contribution to MSC survival in the presence of NK activity. Regardless of these future findings, the main challenge for workers using MHC I^+ MSC will be to define the mechanisms by which these cells escape the allogeneic rejection process

Although there is some consensus regarding MHC I expression on MSC, attempts to define MHC II expression have been more controversial. However, the findings are of utmost importance because this is likely to be a key molecular target in allogeneic responses. Most studies have found adult and fetal MSC to be class II negative or low (76) and this has become incorporated into definitions of MSC summarized in Table 1. Nevertheless, a number of reports suggest some degree of class II expression. Although it is possible to ascribe some data to contaminating hematopoietic precursors, this is clearly not always the case. For example, Potian et al. show quite broad expression of HLA-DR by population and single-cell analyses, although interestingly expression was absent on cell projections of MSC and was punctate in areas close to the nuclei (24). Whereas many workers use MSC that are MHC II negative (74,77), the definitive description of class II transactivator (CIITA) expression, the master controller of class II expression, remains to be published.

Table 1. Surface Marker Expression by Adult Human MSC[a]

Human MSC typically express[b]	*Human MSC typically do not express*
MHC class I^{lo} (HLA-A)	MHC class II (HLA-DR)
CD29	CD11b
CD44	CD14
CD54	CD22
CD90	CD31
CD105 (Endoglin both SH1 and SH2)	CD34
CD106	CD40
CD120a	CD40L
	CD80
	CD86
	CD95L
	CD117

[a]Data compiled from sources cited in text, and data not shown.

[b]Considerable variation from this pattern of expression may be seen due to difference of cell source and culture history.

Recently, another possible mechanism by which class II could appear on a cell's surface has emerged that may resolve these discrepancies. This is not through direct expression of MHC proteins by the cell itself, but through a transfer mechanism in which target cells can acquire surface MHC molecules (and certain other proteins) by intermembrane transfer following a cognate interaction (78,79), or via nanotubule "highways" (80,81). The extent of this phenomenon in vivo has yet to be determined, but it may well explain some of the diverse results obtained by different laboratories. For example, if MSC have been cultured at sufficient density with MHC class II-positive cells, then it is possible that such transfer mechanisms explain the observed differences between reports. More intriguingly, if such processes operate in vivo, then MSC may acquire host MHC molecules at the cell surface in the absence of direct expression (78,79). In effect, the donor MSC might mimic the host, a process that would not be detected by gene expression anal-

ysis, but should be amenable to protein analyses. To date, intercellular protein transfer mechanisms have had a modest impact on existing paradigms; however, if the process is shown to be widespread in vivo, then a number of cherished immunological theories, including allogeneic rejection, will need to be reevaluated.

Whereas there are certainly MHC II-positive MSC in use, most data suggest that commonly used MSC express and display little or no surface class MHC II proteins (or indeed exogenous antigen processing machinery) (74). Because MHC class II proteins are potentially powerful alloantigens, this widespread observation supports a role for MSC as having reduced immunogenicity. It is important to note that all MSC described to date lack surface expression of the T cell co-stimulatory molecules CD80 and CD86 (29,74). These are important observations, because in the absence of costimulation, T cell engagement can result in anergy that would contribute to tolerance. These observations need to be extended to support therapeutic utility, for example, many applications envisage delivery of MSC to inflamed sites, will MSC remain MHC-II negative under those conditions? and will CD80 and CD86 be negative also? MSC expression of MHC I and II appears to be up-regulated by interferon-γ (IFN-γ) (29,82), and studies with fetal MSC showed that prolonged exposure to IFN-γ was required to induce surface expression of HLA II, but, even under those conditions, these cells suppressed alloreactive lymphocytes (76) and showed attenuated costimulation (29). Of course, while MHC class I and II proteins are powerful alloantigens in other contexts, it does not follow that MHC expression is central to the mechanism of MSC-mediated suppression. In fact, the published data suggest that suppression is MHC independent (15). Taken together, the published work described above gives cause for optimism with regard to the therapeutic use of MSC. Most studies suggest that the limited surface protein expression by these cells indicates that they will avoid NK activation (MHC I positive), but lack the antigen processing, presentation, and costimulatory machinery to drive a profound activatory T helper cell response.

MSC MODULATION OF DC, T CELL, AND NK CELL FUNCTION

The regular process of antigen-specific $CD4^+$ T cell induction requires antigen capture and processing by DC (or other sentinel cells), accompanied by a process of maturation and trafficking to local lymph nodes. It has long been realized that this process contributes to the graft rejection process, and the absence of such a process is thought to be a reason behind the ease of transplantation for tissues that lack lymphatic drainage (83). However, it is now recognized that in some situations the process of presentation results in T cells with a suppressive or regulatory phenotype (84). A number of recent studies from infectious models show that pathogens are adept at exploiting these mechanisms. Clearly, MSC could be preventing normal allogeneic responses either through modulation of DC function or by direct effects on T cells. Different lines of evidence support this contention. Zhang et al. showed that MSC inhibit upregulation of CD1a, CD40, CD80 CD86, and HLA-DR during DC maturation (25). Similarly, Jiang et al. reported that MSC maintain DC in an immature state (27). This group also shows that IL-12 p70 secretion is down-regulated by MSC (25). Recent data have built on these findings. Beyth et al. showed reduced IFN-γ, IL-12, and tumor necrosis factor-α (TNF-α) in human MSC/monocyte co-culture (85). In their hands, DC cultured with allogeneic MSC resulted in aberrant DC maturation (85). Taken together, these results suggest that a key mechanism of allogeneic tolerance of MSC is mediated by MSC directing maturing antigen-presenting cells (APC) toward a suppressor or inhibitory phenotype that results in an attenuated or regulatory T cell response.

An indirect effect on T cell induction through modulation of DC function does not preclude other mechanisms. Indeed, there is also evidence that MSC interact directly with T cells. The induction of split tolerance will be discussed below; however, Krampera et al. showed that MSC can inhibit T cell proliferation by mechanisms that do not require APC, or indeed $CD4^+$ $CD25^+$ regulatory T cells (Treg) (26). However, because the phenotypes of regulatory T cells are still contentious issues, it may be that suppression is mediated by other less-characterized $CD4^+$ cell subsets (84,86). Certainly the induction of T cell regulation or suppression is likely to be involved in the process of allograft acceptance, as suggested by reports that MSC modulate T cell production of IL-2 (87) and down-regulate the expression of the IL-2 receptor on activated T cells (82). The observation that MSC suppression can be reduced in the presence of IL-2 (13) strongly supports a role for either a direct (T cell phenotype) or indirect (DC phenotype) mechanism of immune modulation directed by MSC.

The influence of MSC on cytotoxic T cells ($CD8^+$ CTL) and NK cells has also been addressed. Rasmusson showed that MSC escape recognition by alloreactive CTL and NK cells; MSC were not lysed by these cells in coculture (87). This effect appears to be mediated by soluble factors (87), a finding supported by data showing that MSC-secreted factors also inhibit differentiation of CTL from precursors (88). These findings are all the more surprising given the known NK cell ability to lyse allogeneic MHC class I expressing cells when there is a KIR ligand mismatch (75), again a role for nonclassical MHC expression may prove a fruitful approach to explaining these findings (47).

Whereas MSC limit MHC expression, escape from allorecognition appears to be MHC independent (15). Therefore, the influence of MSC on apoptosis and control of T cell division has become a subject for investigation. It is difficult to find a biological process in which apoptosis or other forms of physiological cell death are not involved; however, the hypoimmunogenicity of MSC does appear to be an exception. There seems to be no major endogenous caspase activity in cultured MSC, consistent with their long-lived phenotype, although active caspases can be induced in MSC differentiating along the osteoblast pathway by sclerostin an antagonist of bone morphogenetic protein (BMP) (89). However, recent work by Bu et al. suggests that even these cells are protected from apoptosis by multiple nonsignaling TNF receptors (90). This is consistent with the stem cell's longevity and escape from allogeneic deletional mechanisms. In terms of avoiding allogeneic responses, it is more interesting to discover possible veto effects on cells that could interact with MSC. Di Nicola found no evidence to suggest that MSC induce apoptosis in T cells (91), and careful reading of the broader literature supports this observation. There is some evidence from immortalized mini-pig-derived MSC to indicate a role for FasL (CD95L) in suppression (92). This is in line with findings that showed an essential role for FasL in transplantation tolerance induced by donor bone marrow (93), and studies suggesting that allogeneic bone marrow cells modulate T cell receptor signal transduction (94). Nevertheless, in terms of MSC interaction with DC and T cells, it seems that direct induction of apoptotic deletion is not a factor involved.

Direct apoptotic deletion of alloreactive T cells may not be mediated by MSC, but there are observations that MSC influence control over cell division cycle pathways in cells of immunological relevance. The key work in this field comes from Glennie et al., who used the murine HY model to show that T cells stimulated in the presence of MSC display a profound inhibition of cyclin D2 and upregulation of the cyclin dependent kinase inhibitor $p27^{kip1}$ (30). In their system, IL-2 could not reverse T cell inhibition and so these cells were not rendered anergic in the classical sense. As those authors suggest, it is more likely that MSC are inducing the alternative condition of divisional arrest anergy in interacting T cells, a phenomenon usually associated with CTLA-4 signaling (95). Intriguingly, Glennie et al. show that on removal of MSC from the system, only IFN-γ production but not T cell proliferation could be restored (30), suggesting that MSC induce a condition resembling split anergy (96) or split tolerance (97,98). This work clearly shows that MSC can exert veto effects on T cells and is important in understanding the full range of mechanisms that contribute to the hypoimmunogenic role for MSC. The question that leads from these observations is subtle. To what degree are veto effects integral to the process of maintaining the condition of tolerance? Is this phenomenon subservient to other effector mechanisms such as those mediated by soluble factors? How much true redundancy exists? Or is there an orchestration of soluble and contact-dependent intercell communication. There is as yet no definitive answer to these questions. Nevertheless, the work reviewed above provides ample evidence to demonstrate convincingly that MSC modulate the immune function of the major cell populations involved in alloantigen recognition and elimination, including DC, $CD4^+$ T cells, $CD8^+$ T cells, and NK cells. When the observational evidence is also considered, the picture that emerges is of a MSC population with a very powerful array of mechanisms that allow escape from allogeneic responses.

SOLUBLE FACTORS CONTRIBUTING TO MSC SUPPRESSION OF ALLOGENEIC RESPONSES

The possibility that immunological suppression may also be mediated by soluble factors as opposed to direct cell–cell interaction has already been alluded to (15). Obviously these factors will be underestimated in MLR models that involve MSC irradiation or attenuation, but the importance of soluble mediators may also be overlooked in systems involving very nutrient-rich culture media. Again, the lack of standardization in isolation and culture conditions has given rise to multiplicity of findings and interpretations. These are not assisted by descriptions of cytokine production based solely on mRNA expression profiles from populations with potential hematopoietic contamination. Despite these caveats, a number of groups have shown that MSC modulation of alloresponsiveness is in part mediated through soluble factors (14,85,91).

The characterisation of cytokines produced by MSC is still rudimentary and is again hampered by the diversity of cells and culture systems studied. It is clear that MSC do not constitutively express IL-2, IL-3, IL-4, and IL-5 (99,100). However, there are reports of MSC secreting IL-1, IL-6, IL-7, IL-8, IL-11, IL-12, IL-14, IL-15, IL-27, leukemia inhibitory factor (LIF), stem cell factor (SCF), granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte (G)-CSF, and macrophage (M)-CSF (101,102). Recent papers have tended to be more conservative in the descriptions of MSC-derived cytokines and growth factors, and the number of cytokines constitutively produced by MSC and consistently detected at both the mRNA and protein level is surprisingly small. Perhaps the most intriguing factor with immunomodulatory effects produced by MSC is HGF.

Although some groups do not detect HGF in MSC cocultures (15), more reports suggest that HGF is constitu-

tively produced by MSC (82,91,103). Our own studies support these and show that concentrations of constitutive HGF production can be high (Fig. 1A). HGF is a small-secreted factor that has pleiotrophic immunological influences and can be produced by a range of cell types. In addition to the role in placental organization described above, HGF has been described as exerting morphogenic, angiogenic, antiapoptotic, and tumorigenic effects in different systems (104–106). HGF has a well-characterized role in wound repair (106–108) and, like many repair mechanisms, this has an immunological component. HGF preserves graft versus leukemia (GVL) but ameliorates GVHD effects (104,109). More interestingly, studies using rat dermal-derived "multipotent" cells that expressed HGF demonstrate that these cells promote wound healing (108). Also relevant are data showing that pretransplant islet adenoviral gene therapy with HGF markedly improves islet transplant outcomes in allogeneic rodent diabetes models (60,110). HGF treatment also prevents chronic allograft nephropathy in rats (58), and suppresses acute and chronic rejection in murine cardiac transplant models (59). Demonstrations that MSC produce HGF (82,91,103) supports a role for these cells in tissue repair (103). Furthermore, the data from Di Nicola et al. show that HGF in combination with TGF-β promotes the allo-escaping phenotype (91).

Although these studies suggest that HGF plays a role in the acceptance of allogeneic grafts, some caution is needed before extrapolating these results to humans. Le Blanc, for example, did not find evidence to support a role for HGF in mediating the suppression of alloresponses in vitro (82). More importantly from the clinical perspective, HGF has a well-characterized mitogenic (105) and differentiating (56,57) influence that could act counter to the desired properties required of a stem cell to be used therapeutically. Again this should not be surprising given the pleiotrophic nature of this factor. It is very likely that HGF contributes in diverse ways to both immunological and other functions of MSC. If this includes the escape of allogeneic rejection by MSC, then it is another interesting parallel between MSC and the survival of the fetal allograft.

To the immunologist exploring allogeneic responses perhaps the key cytokines of interest are IL-10 and TGF-β, which have well documented roles in T cell regulation and in the promotion of a "regulatory" or suppressor phenotype. However, it should be remembered that these cytokines influence cell lineages broader than lymphocytes (111) and that the bone marrow is rich in endogenous TGF (16). Human MSC production of TGF-β1 has not been seen constitutively in our system to date although other related cytokines and growth factors can be detected (data not shown). This is in line with LeBlanc, who found no difference in TGF-β1 concentration in co-cultures containing or lacking MSC (82). In contrast, Beyth et al. detected TGF-β1 in supernatants of human MSC and immune cells, but again co-culturing did not increase TGF-β1 concentration (85). In contrast to the results of DiNicola (91), a number of studies using neutralizing monoclonal antibodies could not demonstrate a role for TGF-β1 in evasion of allogeneic responsiveness (82,85,112), although TGF did influence MSC differentiation (8,16). The discrepancies here are compounded by the paucity of understanding of TGF-β isoforms and the notorious difficulties inherent in TGF-β measurement (113). Therefore, it is still premature to dismiss a role for alternative TGF isoforms in MSC allo-escape mechanisms.

In contrast to TGF-β1, constitutive IL-10 production is a notable feature of human MSC in our hands (Fig. 1B), whereas Beyth et al. and Rasmusson et al. only observe IL-10 in co-culture (85,112). IL-10 can be produced by many cell types and has a key role in promoting regulatory or suppressor T cell populations and can act antagonistically to IL-12 during induction of inflammatory immune responses (111,114–118). IL-10 is a known

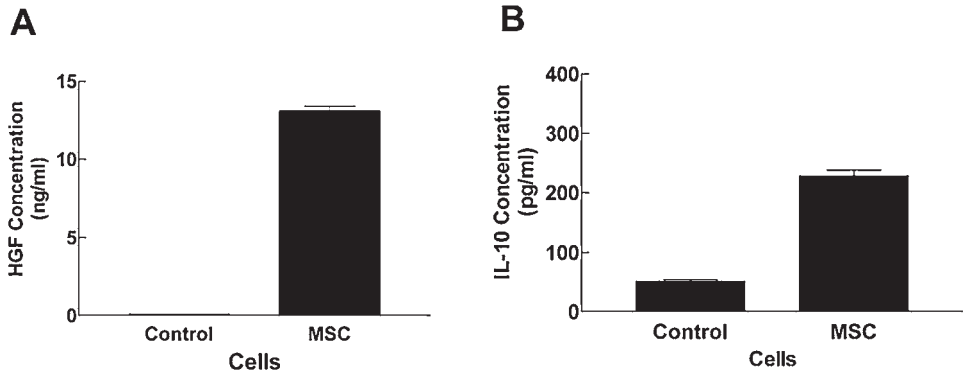

FIG. 1. Human MSC but not fibroblasts (control) cultured as previously described (5,6,8), constitutively secrete HGF (**A**) and IL-10 (**B**) into culture supernatant as detected by enzyme-linked immunosorbent assay (ELISA).

growth factor for regulatory T cells (119) and is a candidate factor for inhibition of allo-responsiveness. This is supported by studies showing that the inhibitory effect of MSCs was partially reverted by blocking IL-10 activity in MLR cultures (85,112). Taken together, the observation that these immunomodulatory cytokines are produced by at least some MSC populations could explain mechanisms that promote or maintain the survival of the allografted populations. Once again there is a strong parallel to survival of the fetal allograft where IL-10 and TGF-β play a well-characterized role in preventing allorejection.

Another parallel between the tolerance of the fetal allograft and MSC can be seen in the production of immunomodulatory eicosanoids. MSC constitutively express both COX-1 and COX-2 isomers of cyclooxygenase, which results in the constitutive expression of the eicosanoid PGE-2 (120). This may be down-regulated on differentiation (120) or up-regulated in co-culture (14,29). In addition to a role in materno–fetal interaction (61,66,121), PGE-2 influences numerous immune functions, including suppression of B cell activation (63) and induction of regulatory T cells (62). It is also a major mechanism by which tumors avoid immune surveillance (122). Although the observation of PGE-2 production by MSC provides a potential mediator for suppression of alloresponses in MLR cultures, the data conflicts with regard to the actual contribution of this mechanism. Studies from Tse, suggested that PGE-2 was a nonessential component to suppression (29). This is similar to the findings of Rasmusson et al., who show that blocking PGE-2 production did not restore allogeneic MLR responses but did influence mitogen-driven proliferation (112). Very recently, Aggarwal has shown that PGE-2 production in co-cultures follows a time-dependent, bell-shaped pattern, and that inhibitors of PGE-2 production mitigated hMSC-mediated immune modulation (14). Although conflicting reports are present in the current literature, it would be foolish to dismiss the potential influence of eicosanoids in suppressing allogeneic responses. It should be remembered that both COX-1 and in particular COX-2 expression appear to be constitutive in MSC rather than inducible (120), which may mean that COX-2 inhibitor protocols need to be reassessed in MSC experiments. Furthermore, the emphasis in this area has focused on PGE-2, but there are a range of other prostaglandins and eicosanoids with immunomodulatory function. A full lipidomic analysis of human MSC would be a major benefit to the understanding of MSC function and the potential eicosanoids that could be influencing alloresponses.

The present discussion has focused on soluble factors produced by MSC, but it is also possible that MSC withdraw essential factors and nutrients from the microenvironment and thereby create a tolerogenic environment. One obvious candidate for such a function is the induction of IDO and subsequent depletion of tryptophan. The expression of IDO has two well-characterized functions that are relevant to the present enquiry—an antimicrobial role and the observation IDO expression serves to maintain the fetal allograft (123–126). It is intriguing to notice the parallels with MSC. Human MSC do not constitutively express IDO, but they can express this enzyme on exposure to IFN-γ (127) and inhibit allogeneic T cell responses by tryptophan degredation (127). This is consistent with findings from other systems reporting that IDO-mediated tryptophan depletion inhibits allogeneic T cell responses, and this involves more than one T cell inhibitory mechanism (128). It is now clear that IDO is a central mechanism in allograft survival; be that in the materno–fetal or other contexts (71–73,129,130).

The fascinating recent finding is that MSC utilize the same process. The key observation is that this process is

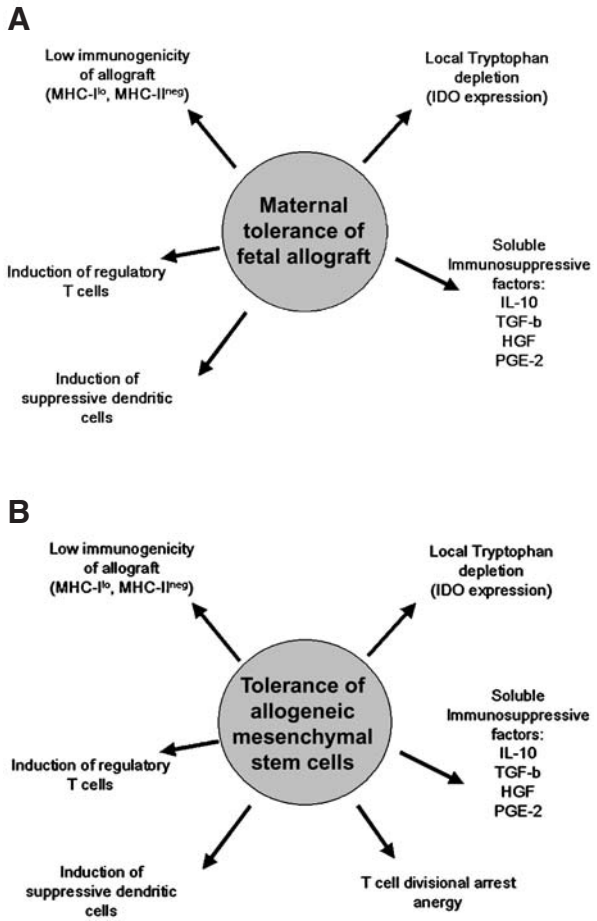

FIG. 2. Comparison of putative mechanisms involved in preventing rejection of the fetal allograft by the maternal immune system (**A**) and allogeneic adult mesenchymal stem cells by the mismatched host (**B**).

dependent upon IFN-γ stimulation (127). The significance is that this is precisely the scenario in which mismatched MSC are likely to face the most powerful allogeneic rejection mechanisms, as well as the most important setting for future therapeutic use of MSC in regenerative medicine. The detection of this mechanism, which conceivably parallels the creation of the tryptophan desert, is a very positive indicator for MSC use. Nevertheless, there are still questions to be resolved concerning the necessity of this pathway for preserving the hypoimmunogenicity of MSC. For example Tse et al. showed that peripheral blood mononuclear cell (PBMC) proliferation was not substantially reversed by either the addition of supplementary tryptophan or the addition of a specific IDO inhibitor (29).

One problem of in vitro culture attempts to address the importance of these mechanisms is that few in vitro systems replicate even simple aspects of the tissue microenvironment in which these interactions occur. For example, transwell studies may be elaborated to mimic some aspects of tissue architecture, but tryptophan depletion is likely to be a highly localized and temporally regulated event in vivo. This will be difficult to replicate or control in experimental systems involving culture media with other nutrients in excess that could interfere with indirect effects of tryptophan metabolites. However, support for a more cautious interpretation comes from recent work from Aggarwal and Pittenger (29), who suggest that the absence of T cell apoptosis and the kinetics of suppression are not consistent with IDO mediated alloescape mechanisms (14). Nevertheless, it is again intriguing to note that MSC exploit a mechanism operating at the materno–fetal interface, which is thought to play a role in tolerance of the fetal allograft (123–126).

SUMMARY

The last 48 months have seen a large amount of new data supporting the potential for allogeneic MSC to avoid provocation of a functional allogeneic response in the host. This has been supported by in vitro data proposing diverse mechanisms through which this occurs. The challenge now is to determine the extent and hierarchy of such mechanisms and to integrate these into a conceptual framework that supports the hypoimmunogenicity of MSC. A comparison of the mechanisms operating at the materno–fetal interface with the evidence from MSC shows remarkable similarities (Fig. 2). Our contention is that the mechanisms that promote survival of the fetal allograft may well be retained by MSC and explain the surprising ability of these cells to avoid allogeneic effector mechanisms, thus opening the possible use of allogeneic MSC in regenerative medicine.

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