Stem Cell Rev and Rep (2011) 7:342–363
DOI 10.1007/s12015-010-9196-4

Wharton's Jelly Mesenchymal Stem Cells as Candidates for Beta Cells Regeneration: Extending the Differentiative and Immunomodulatory Benefits of Adult Mesenchymal Stem Cells for the Treatment of Type 1 Diabetes

Rita Anzalone · Melania Lo Iacono · Tiziana Loria · Antonino Di Stefano · Pantaleo Giannuzzi · Felicia Farina · Giampiero La Rocca

Published online: 23 October 2010

Abstract Mesenchymal stem cells (MSC) are uniquely capable of crossing germinative layers borders (i.e. are able to differentiate towards ectoderm-, mesoderm- and endoderm-derived cytotypes) and are viewed as promising cells for regenerative medicine approaches in several diseases. Type I diabetes therapy should potentially benefit from such differentiated cells: the search for alternatives to organ/islet transplantation strategies via stem cells differentiation is an ongoing task, significant goals having been achieved in most experimental settings (e.g. insulin production and euglycaemia restoration), though caution is still needed to ensure safe and durable effects in vivo. MSC are obtainable in high numbers via ex vivo culture and can be differentiated towards insulin-producing cells (IPC). Moreover, recent reports evidenced that MSC possess immunomodulatory activities (acting on both innate and acquired immunity effectors) which should result in a reduction of the immunogenicity of transplanted cells, thus limiting rejection. Moreover it has been proposed that MSC administration should be used to attenuate the autoimmune processes which lead to the destruction of beta cells. This review illustrates the recent advances made in differentiating human MSC to IPC. In particular, we compare the effectiveness of the differentiation protocols applied, the markers and functional assays used to characterize differentiated progeny, and the in vivo controls. We further speculate on how MSC derived from Wharton's jelly of human umbilical cord may represent a more promising regenerative medicine tool, as recently demonstrated for endoderm-derived organs (as liver) in human subjects, also considering their peculiar immunomodulatory features compared to other MSC populations.

Keywords Mesenchymal stem cells · Umbilical cord · Wharton's jelly · Type 1 diabetes · Beta cells · Differentiation markers · Pancreas development · Inflammation · Immune modulation · Hypoimmunogenicity

Rita Anzalone and Melania Lo Iacono contributed equally to this paper.

R. Anzalone · M. Lo Iacono · T. Loria · F. Farina · G. La Rocca (✉)
Sezione di Anatomia Umana,
Dipartimento di Biomedicina Sperimentale e Neuroscienze Cliniche,
Università degli Studi di Palermo,
Via del Vespro 129,
Palermo, PA 90127, Italy
e-mail: giampylr@hotmail.com

A. Di Stefano · P. Giannuzzi
Laboratorio di Citoimmunopatologia apparato cardio Respiratorio e Divisione di Cardiologia, Fondazione S. Maugeri, IRCCS,
Istituto Scientifico di Veruno (NO),
Veruno, Italy

Main Features of Mesenchymal Stem Cells

Friedenstein and colleagues were the first to isolate, from bone marrow (BM), a population of cells forming clonal fibroblast-like colonies, capable of differentiating into multiple mesenchymal lineages [1]. According to their features, Caplan called these cells "mesenchymal stem cells" (MSC) [2], although another term currently used is "multipotent mesenchymal stromal cells" [3]. BM-MSC are a non-hematopoietic stromal marrow population, able to adhere to plastic culture surfaces and characterized by long-term self-renewal and the ability to differentiate into connective tissues cells such as osteoblasts, chondrocytes

and adipocytes [4]. More recent studies elucidated that these cells can differentiate into other mesoderm-derived cytotypes, nervous cells, and endoderm-derived cells (such as hepatocytes and pancreatic islet-cells), therefore expanding their potential clinical uses [5].

BM-MSC express a typical set of markers including CD29, CD44, CD73, CD90, CD105, CD166 CD49e, CD51, CD54, CD59, CD71, [6] and recently Delorme and colleagues also suggested the expression of CD200 [7]. Unlike endothelial and hematopoietic cells, BM-MSC do not express CD14, CD31, CD34, CD45, CD79, CD86, CD117 and glycophorin A [8, 9]. In addition, these cells express molecules of class I major histocompatibility complex (MHC), but not class II (such as HLA-DR) [9, 10].

Even if bone marrow represents the most common source of adult MSC, the yield of MSC is substantially low and decreases progressively with the advancing age of the donor [11]. Many researchers have therefore searched for alternative sources of MSC, such as adipose tissue, umbilical cord blood, amniotic fluid, placenta and Wharton' s Jelly [12, 13].

All of these populations share a high similarity with the BM-MSC phenotype (with some key exceptions), and are capable of undergoing differentiation towards mesenchymal and non-mesenchymal cell types.

Recent data suggest that in particular Wharton's Jelly (WJ) contains mesenchymal stem cells that can differentiate into ectodermal, mesodermal and endodermal cellular lineages and successfully be expanded ex vivo and cryopreserved [5, 14]. WJ-derived MSC (WJ-MSC) have a gene expression profile similar to BM-MSC [15], even if expressing also additional markers (e.g. for CD117) [13]. Their basic immune features resemble those of BM-MSC, since these cells express type I MHC molecules (such as HLA-A, -B, -C), but do not express type II MHC (HLA-DR). Moreover, as we [13, 16] and others [17] demonstrated, these cells are further characterized by the expression of key molecules which may exert immunomodulatory activities. It is clear, as we show in the following sections of this paper, that a correct understanding of the molecular mechanisms governing MSC-mediated immune regulation in vitro and in vivo is a fundamental prerequisite for clinical approaches using either autologous or allogeneic MSC.

MSC Immnomodulatory Properties In Vitro and In Vivo

Multiple Evidence of Interactions Between the Immune System and MSC

Immunomodulation is increasingly viewed as a key property of mesenchymal stem cells, isolated from multiple organ sources, which can extend (or even supercede) their importance as versatile multipotent cells. Multiple reports have evidenced, first in vitro and then in vivo, the ability of MSC to express molecules that can interact with both innate and adaptive immunity processes, both in a cell contact-mediated fashion and in experimental settings where cell-cell interaction is precluded, thus suggesting the importance of soluble factors in mediating these processes [18, 19]. Table 1 lists the molecules which have been shown to be expressed in two different MSC populations: BM-MSC, the prototype of this class of stem cells, and WJ-MSC, which are the focus of this review.

One important immunological feature of MSC is the inhibition of T-cell proliferation and dendritic cell (DC) differentiation [18]. It has been reported that MSC can modulate T-cell proliferation by their low expression of costimulatory molecules and the absence of class II HLA [13, 19]. In addition, the immunosuppressive capacity of MSC may also be mediated by the secretion of soluble factors, and by the induction of T-cell anergy and regulatory T-cells (Tregs), with important consequences for treatment, e.g. improvement of current tolerization strategies using anti-CD3 antibodies [20, 21]. Recent studies demonstrated that the secretion of key soluble factors is not a constitutive process: secretion is often a consequence of cross-talk between MSC and T-lymphocytes, the latter being able to trigger this de novo expression [22].

Non-classical type I HLA molecules are an interesting as yet only partly explored field in MSC immune function. Several reports of our and other groups reproducibly showed that BM-MSC and WJ-MSC express the HLA-G molecule, both as mRNA and protein, and its soluble form HLA-G5 [13, 17, 23]. However, little attention has been devoted to other members of this family of molecules, e.g. HLA-E and HLA-F, by researchers. As shown in Table 1, only HLA-E expression has been investigated in BM-MSC, while data are lacking regarding the expression of these molecules by WJ-MSC. Nevertheless, these molecules can be expressed in vivo in close proximity (and in some cases by the same cell types) to HLA-G ones. Moreover, different reports have highlighted the presence of a functional autocrine loop in placental trophoblast cells. Indeed, HLA-G expression is indirectly fundamental for modulating NK cells activity by direct stimulation of HLA-E expression [24].

Di Nicola and colleagues suggested that transforming growth factor-β (TGF-β) and hepatocyte growth factor (HGF) are two possible mediators for suppression of T-cell proliferation in mixed lymphocyte reaction. In addition, the authors demonstrated that T-lymphocytes inhibited by BM-MSC do not enter apoptosis, since they actively proliferate on re-stimulation with cellular and humoral activators [19]. In a very recent report, Ren and co-workers showed that the adhesion molecules ICAM-1 (inflammatory cytokine-

Table 1 Comparison of immunoregulatory molecules expressed by BM-MSC and WJ-MSC; n.a.: not applicable

Markers	BM-MSC	WJ-MSC	References
HLA-A	+	+	[9, 13, 38]
HLA-B	+	+	[9, 39]
HLA-C	+	+	[9, 39]
HLA-DR	−	−	[10, 13, 39]
HLA-E	+	n.a.	[40]
HLA-F	n.a.	n.a.	
HLA-G	+	+	[13, 17, 41]
HLA-G5	+	n.a.	[41]
B7-1 CD80	−	+	[13, 42]
B7-2 CD86	−	−	[13, 38, 42, 43]
B7-H1 (CD274)	+	n.a.	[44]
B7-H3 (CD276)	n.a.	n.a.	
B7-H4	+	−	[45]
CD54 ICAM-1	+	n.a.	[25, 40]
CD58 LFA3	+	+	[40, 46]
CD106 VCAM-1	+	+	[13, 25, 40]
CD166 ALCAM	+	+	[13, 40]
IL-1a	+	+	[46, 47]
IL-6	+	+	[46, 47]
IL-10	−	n.a.	[40]
HGF	+	+	[46, 48]
HO-1	+	n.a.	[32]
NO	+ (mice[a])	n.a.	[35, 36]
IDO-1	+[b]	+[b]	[37]
PGE2	+	+	[30, 49–51]
Factor H	+	n.a.	[34]
TGFbeta1	+	+	[46, 49]
Galectin-1	+	n.a.	[52]
Galectin-3	+	n.a.	[54]
TSG-6	+	n.a.	[53]

[a] The role of NO in modulating T cell response by MSC has been reproducibly demonstrated for mice MSC, while its role in human MSC is still under investigation

[b] The authors reported a low level of expression (both protein and RNA) for IDO in unstimulated cells, with marked increase after IFNγ and TNFα exposure

induced intercellular adhesion molecule-1) and VCAM-1 (vascular cell adhesione molecule-1) are critical for the MSC immunosuppression on T cells, and are inducible by the parallel presence of IFN-γ and inflammatory cytokines (as IL-1 and TNF-α) [25]. Another mechanism underlying BM-MSC mediated T-cell suppression is anergy. It was suggested that BM-MSC determine T-cell arrest in G0-G1 phase by inhibition of cyclin D2 expression [26]. MSC can also induce immune suppression, stimulating the production of CD8+ regulatory T-cells that inhibit allogeneic lymphocyte proliferation [27]. Other studies reported that BM-MSC interfere with dendritic cell (DC) differentiation maturation and activation [28]. In addition, MSC inhibit B cell proliferation and activation in a dose-dependent manner and modulate their differentiation, antibody production and chemotactic abilities [29]. In a very recent report, Németh and co-workers demonstrated that BM-MSC can actively reprogram macrophages in a murine sepsis model, through prostaglandin E2 (PGE2) stimulation of the EP2 and EP4 receptors. Reprogrammed macrophages produced more IL-10, thus reducing neutrophil infiltration in tissues (which is a component of multi-organ dysfunction) and increasing neutrophil numbers in the circulation leading to bacterial clearance [30]. These data received further confirmation from the very recent work of Mei and collaborators, who demonstrated that MSC improve survival in sepsis by reducing inflammation, while enhancing bacterial clearance [31].

Table 1 lists further immunomodulatory molecules which have been recently described for BM-MSC: heme oxigenase-1, for which a protective role has been demonstrated in neural differentiation experiments [32, 33], and factor H, which should make these cells capable of regulating complement-mediated innate immunity processes [34]. Moreover, MSC express also IDO (indoleamine deoxygenase) and NO (nitric oxide), for which species-specific differences have been proposed in their ability to favor regulation of immune responses by MSC [35–37].

Tolerance Induction as a Key Immunoregulatory Feature of MSC

Tolerance to self antigens is a process of fundamental importance for the correct development of the human immune system. Peripheral tolerance mechanisms act together with central tolerance, based on the role of a novel class of lymphocytes known as Treg (regulatory T) cells [55] which exert active suppression by cytokine expression or by promoting the so-called infectious tolerance [55]. A key molecule in which a tolerance-induction role has been demonstrated is HLA-G. This non-classical type Ib HLA molecule was first characterized in trophoblast cells, where it mediates tolerance towards the semi-allogeneic embryo, together with other factors such as EPF (early pregnancy factor), HLA-E, etc. [56, 57]. HLA-G has been found to be expressed in different MSC populations, such as BM-MSC [41] and WJ-MSC [13]. This molecule has been directly linked to the tolerogenic ability of MSC, e.g. inducing the expansion of CD4+CD25+FoxP3+ Tregs which would contribute to the suppression of effector responses to alloantigens [41, 55]. HLA-G would act both in its membrane-bound isoform, implicating direct cell-cell contact, or by its shedding from the cellular surface (the isoform defined as HLA-G5). Furthermore, a cooperative

interplay between different immunomodulatory molecules, co-expressed by MSC, is hypothesizable on the basis of recent advances. In fact, post-translational modifications, such as nitration, are important biochemical reactions that play a fundamental role in cellular redox mechanisms, in both physiological and stress conditions, in different cell types [58–61]. Diaz-Lagares and co-workers recently demonstrated the existence of a functional interplay between NO and HLA-G in favoring the establishment of immune tolerance. In fact, HLA-G is a target of protein nitration, a reaction which is favored by increased NO in the extracellular space. Nitration of HLA-G renders it sensitive to metalloprotease-dependent shedding. Therefore, HLA-G should exert a tolerogenic action in a paracrine fashion [62].

In a recent paper, Gonzalez et al demonstrated that the beneficial action of adipose tissue (AT)-derived MSC on experimental rheumatoid arthritis was due to the generation and activation of Treg cells [63]. Moreover, in an experimental model of diabetes, Madec and co-workers recently demonstrated that MSC exerted a protective function on NOD mice by inducing regulatory T cells. In fact, MSC reduced the ability of diabetogenic T cells to infiltrate islets. Moreover, MSC suppressed both allogeneic and insulin-specific proliferative responses in vitro, an effect linked to IL-10 production by Treg cells [64]. In a parallel report, Zhao and co-workers demonstrated that in NOD mice autologous Tregs (which are functionally impaired by the underlying disease) can be modulated by co-culture with cord blood stem cells, reverting the autoimmunity via systemic and local immune modulation and promoting beta cells regeneration [65].

Taken together, these data indicate that one of the great expectations linked to the use of MSC may reside in their ability to successfully engraft, evading an immune response and inducing peripheral host tolerance.

Contrasting Evidence on the MSC Immune Privilege In Vivo

While numerous data in the literature have highlighted the ability of MSC to modulate immune cells proliferation and activation in in vitro settings, contrasting reports exist regarding the potential generation of immune and memory responses by MSC when administered in vivo. This constitutes a serious issue in cellular therapy, since xenogenic and allogeneic MSC should be eliminated by the host immune system previous to exert any beneficial action. Actually in our opinion MSC are paying the price for their popularity: data in the literature show that they are being used in almost every disease setting where autoimmunity or tissue regeneration have to be targeted. Therefore, in reality one should not be surprised that in some model organisms (above all for xenogenic approaches) MSC fail to deliver the expected outcome when applied in vivo. Moreover, in this "race for the cure" researchers sometimes lose of sight the categorical need for a clear and complete immune characterization of MSC before their implant, and as a consequence generate frustrating confusion in the scientific community. However, the general feeling and our personal opinion is that negative results in MSC administration in vivo ought to provide instead key information on the molecular mechanisms of MSC-mediated immune modulation, above all in allogeneic settings, for which most hopes are placed on MSC as a possible "off the shelf" therapy for a number of diseases.

One key question which remains still to be solved despite the intense work of researchers is to define actual levels of immune privileges of MSC when transplanted in vivo. The response to this question will in our opinion have a dramatic effect in boosting the use of MSC in cellular therapy. WJ-MSC should be recovered in high numbers and should auspicably be banked in parallel to cord blood units. This should lead to the increase of cell numbers available for both autologous and HLA-matched heterologous administration. On the other hand, once the immune mechanisms of the diffferent MSC populations are fully characterized and validated in most in vivo settings, then the path to use of HLA-mismatched heterologous cells will be opened.

In a recent review, Griffin and co-workers reviewed the multiple aspects of allogeneic mesenchymal stem cell therapy. While examining the evidence for and against the use of MSC as immunoprivileged cells in vivo, the authors stressed the concept that a better understanding of the mechanisms of MSC-immune cell interactions in vivo is tantamount for the success of allogeneic therapy [55].

Here we provide a brief analysis of the contrasting reports existing in the literature on the maintenance of the immune privileges of MSC in in vivo settings. In a seminal work, with in vivo approaches in human subjects, Sundin and co-workers demonstrated that allogeneic MSC, transplanted in patients undergoing HSCT (hematopoietic stem cells transplantation), did not cause production of alloantibodies in the host, while anti FCS (fetal calf serum) antibodies were detectable. While these antibodies seemed however clinically insignificant, the important datum is that in vivo, in human subjects, no humoral response was detected against MSC [66]. In a parallel report, Rasmusson and colleagues demonstrated that human BM-MSC were resistant to CTL (cytotoxic T lymphocytes), failing to induce IFN-γ or TNF-α. Therefore the authors stated that only an "abortive" activation program should be induced in fully differentiated CTL [67]. Subsequently Morandi and co-workers demonstrated that human MSC can process and present viral or tumor antigens to specific CTL with only

limited efficiency. This is due to defects in the antigen-processing machinery, some of whose components are not expressed in MSC [68]. Data supporting the use of allogeneic and xenogenic MSC as effective in muscular regeneration come from the study of Shabbir and collaborators, who demonstrated that dystrophic hamsters treated with porcine or human MSC exhibited 1 month after infusion both muscle regeneration and attenuated oxidative stress [69]. Moreover, in a recent report, Quevedo and co-workers demonstrated the restoration of cardiac function in chronic ischemic cardiomiopathy by long-term engraftment and the differentiative ability of allogeneic porcine MSC [70]. In addition, Chen and co-workers have recently demonstrated, in in vivo experiments of excisional wound healing, that allogeneic and syngeneic BM-MSC had similar engraftment ability and resulted in enhanced wound healing, without effects on the numbers of CD45+ leukocytes and lymphocytes [71].

In contrast to these and other studies pointing to the in vivo hypoimmunogenicity as a key property of MSC, other reports have raised doubts about the general validity of this theory. Eliopoulos and co-workers claimed that allogeneic BM stromal cells were rejected by MHC class I and class II mismatched recipient mice [72]. In a subsequent work, Nauta and co-workers further extended this concept suggesting that allogeneic MSC are immunogenic and stimulate donor graft rejection [73]. These data are obviously in contrast with those reported above, but our initial point about the need to accurately characterize MSC before in vivo assays maintains all of its validity when accurately analyzing these data. In fact, for both papers, MSC characterization was not optimal, with the cells used by Eliopoulos and colleagues being negative for CD90 expression and positive for both CD80 and class II MHC [72], therefore bearing key differences to standard bone marrow stromal cells immunophenotype. Data in the paper from Nauta and co-workers further show that, apart from tri-lineage differentiation (although proved exclusively by histochemical methods), MSC characterization was limited to CD106, CD45, CD14 and CD31 [73]. In a more recent report, Prigozhina and colleagues further suggested that allotransplantation of MSC (again from mice) leads to loss of their in vitro immunosuppressive potential, failing to reduce GVHD (graft versus host disease) [74]. However, again some remarks should be made on the characterization process (based on positivity to four MSC markers alone) and on the isolation protocol from BM, placenta and WJ. In fact, initial passages in culture showed massive contamination with CD45+ cells (up to 93% in BM preparations, and 64% in placenta preparations) [74]. Moreover, no positive selection was performed prior to implanting cells in vivo. Regarding the application of human MSC in an animal model of disease, Chiavegato and co-workers recently showed that human amniotic fluid-derived stem cells were rejected after transplantation in the myocardium of normal, ischemic, immuno-suppressed or immuno-deficient rat. A more accurate analysis of the results reported allowed to establish that AF-derived cells were positive for both CD80 and CD86 B7 co-stimulators, leading the authors to suppose that these cells should act as donor APC (antigen presenting cells) [75]. Again, the lack of an extended characterization in vitro of cells prior to their use in vivo led to disappointing in vivo results.

The data obtained so far claim the need for caution in the administration of MSC for pre-clinical or clinical trials. The characterization of cells before implantation is a sine qua non which must be observed in all cases, since otherwise it would lead to contrasting data on the therapeutic efficacy of these cells. Moreover, we must consider that animal models (and in particular mouse) are not perfectly equal to the human counterpart. As we stated previously, there are differences between the immunomodulatory molecules expressed in vitro by human and mouse MSC (see also Table 1, for the expression of NO or IDO). Indeed, Ren and co-workers have recently highlighted that while immuno-suppression in murine MSC is driven by iNOS-derived NO secretion, human and primate MSC use IDO as an immunosuppressant molecule [76]. This is a first proof that animal models do not always mirror exactly the conditions of the human disease or the behaviour of human cells. When examining the contrasting reports on the beneficial effects of MSC in GVHD, some reports clearly evidenced that in BM-transplant receiving mice, allogeneic BM-MSC failed to prevent GVHD in mice [77, 78]. On the contrary, Ringden and colleagues demonstrated that allogeneic human BM-MSC contributed to alleviate GVHD in BM-transplant recipient patients [79]. Again the species-specific differences between intrinsic immunomodulatory potency of MSC may explain the striking differences between animal models and actual clinical effects in patients [78]. Moreover, it is expected that when human cells are being used, the extended characterization of markers expressed should be viewed as an additional "safety" feature to prevent immune reactions in the recipient host.

MSC and Immunosuppressants in Prevention of Allograft Rejection: Sometimes Two is Better Than One

Immunosuppressive therapy associated with organ or islet transplant is accompained and limited by a number of heavy side effects. Therefore MSC are ideally viewed as cellular therapy devices which should completely abrogate the need for immunosuppressants [80]. This is particularly needed in islets transplantation, which is a therapeutic approach that is limited by the recurrent immune response against transplanted beta cells. Recent reports evidenced

that MSC immune modulation should exert a beneficial effect on allogeneic islets transplanted in diabetic subjects. In a very recent report, Ito and co-workers demonstrated that co-transplantation of islets and BM-MSC improved islets graft morphology and function. The authors hypothesized that this improvement was due to the promotion of graft revascularization by MSC [81]. In a parallel report, Li and co-workers co-transplanted allogeneic BM-MSC and allogeneic islets under the kidney capsule of diabetic mice. They found a reduction of rejection by MSC, which exerted suppressive effects acting on T lymphocyte subsets (both naïve and memory cells) and dendritic cells [82]. In another recent report, Longoni and colleagues employed allogeneic and syngeneic rat MSC to determine the effects on rejection of islets administered through the portal vein. The authors showed normalization of glucose levels and low-grade rejections for up to 15 days, together with reduction of pro-inflammatory cytokines. Moreover, the authors demonstrated that the same effect was obtained with both syngeneic and allogeneic MSC, and at levels comparable to those achieved with standard immunosuppressive therapy [83].

Indeed, as MSC properties are being determined by researchers, it is clear that in some cases MSC and immunosuppressant drugs may have the same target (i.e. lymphocyte proliferation) [84]. In a recent report, Popp and co-workers reported that MSC should act synergistically with mycophenolate mofetil (MMF) to induce long term acceptance of solid organ allograft in a rat heart transplantation model [85]. In another report, Ge and colleagues demonstrated that MSC also synergize with rapamycin to attenuate autoimmune responses and promote cardiac allograft tolerance [86].

All of these experiments showed that allo-MSC should be useful in preventing rejection of allogeneic islets as well as of solid organs, the latter in synergism with low doses of immunosuppressants. Experiments with islet allografts are limited by the short observation time, so that longer monitoring of the recipient is required to understand if the effect of MSC is transient and if the addition of a synergistic immunosuppressant would potentiate it in order to completely abrogate even the low-grade rejections observed in some reports.

Maintenance of the Immune Privileges of MSC by MSC-Derived Differentiated Cells: In Vitro and In Vivo Evidence

The hypoimmunogenicity of undifferentiated MSC should be a key factor in the application of these cells as cellular therapy vectors. But regenerative medicine, in the presence of limited host reparative processes, should also provide cells able to differentiate to mature cytotypes and replacing existing malfunctioned cells. Therefore the question whether differentiated MSC should lose their immunomodulatory features is of striking importance to decide whether to infuse differentiated cells alone or mixed with undifferentiated cells (which should protect them from immune system attacks). The research on this intriguing issue stemmed from a seminal work of Le Blanc and colleagues, who first defined the HLA expression and the immunological properties of differentiated MSC compared to their undifferentiated counterpart [87]. The researchers performed differentiation of MSC towards bone, cartilage or adipose, and the differentiated cells upregulated expression of HLA class I, but not class II. Moreover, with respect to control cells, IFN-γ-driven expression of HLA class II at the surface of cells was clearly diminished. Moreover, in vitro alloreactivity was not seen for all of the differentiated cells, even after IFN-γ pretreatment (besides, IFN-γ increased MSC suppression of mixed lymphocyte cultures) [87]. In a more recent report, Liu and co-workers, using a novel in vivo model of osteogenesis, highlighted that differentiated allogeneic MSC retained their immunomodulatory potential and were detectable 4 weeks post-implant in the regenerated tissue, where they secreted bone matrix proteins, without evidence of cell clearance. No evidence of the induction of a frank memory response was obtained by the authors. Indeed, MHC II expression was detectable after in vivo implantation, even if IFN-γ was not the critical factor for this expression [88]. In a more recent report, Zheng and co-workers demonstrated that in rheumatoid arthritis (RA) both allogeneic MSC and MSC-derived chondrocytes suppressed the response of type II collagen-reactive T cells isolated from RA patients, and the suppressive effects were mediated by TGFβ1 [89]. Another in vitro set of experiments published by Chen and co-workers, examined the xenogenic immunogenicity of rat MSC, differentiated into bone, adipose and cartilage. The experiments demonstrated that xenogenic MSC-derived chondrocytes were chemotactic to human dendritic cells (DC), and upon differentiation upregulated the two costimulatory B7 molecules (CD80 and CD86, which were absent in untreated cells), leading to DC maturation [90]. Therefore, rat MSC differentiated towards a chondrogenic fate do not show the same immunomodulatory features as their human counterpart, while osteogenic and adipogenic cells maintained their immune privilege. Again, contrasting reports can be seen regarding the maintenance of immune privileges of MSC both in vitro and in vivo. The confusion may derive first from the attempt to compare homologous cellular populations from different species (which should not have the same immunomodulatory mechanisms) and to compare different experimental settings (i.e. xenogenic versus allogeneic transplantation). A higher number of studies, which still lack for the most complex differentiation protocols (e.g. hepatocytes or beta cells), are required to

definitively shed light on the immune properties of differentiated MSC. This valuable information can then be fruitfully used to design better transplantation strategies which should take into account the expected reactions that the recipient could manifest against donor cells.

Into the Fire: MSC Targeting of Inflammation Sites as a Key Pre-requisite to Dampen Inflammatory Reactions

Several reports show that MSC after systemic infusion have the tendency to migrate to sites of tissue injury and inflammation, and initially accumulate there [91]. This may seem not a sound strategy, since inflammatory sites should also be the sites where MSC are scavenged more easily by resident and migrating phagocytes. Our growing understanding about the interaction between MSC and the actors and effectors of inflammation is revealing new roles for these cells in the regulation of inflammation. There is growing evidence that MSC plays a role in reducing inflammation in vitro and in vivo. Neurological disorders seem to benefit from MSC treatments based on the anti-inflammatory and oxidative stress-dampening properties of MSC. In particular, Kemp and co-workers recently described the secretion of SOD3 (superoxide dismutase 3) by MSC. SOD3 exerts a direct antioxidant activity, attenuating tissue damage and reducing inflammation, and thus has a neuroprotective role [92]. Interestingly, the authors showed that SOD3 secretion by BM-MSC was upregulated synergistically by inflammatory stimuli (e.g. TNFα and IFNγ), rather than by the exposure of cells to elevated ROS (reactive oxygen species) concentrations. Moreover, in a recent report, Liao and colleagues demonstrated that human umbilical cord-derived MSC exerted anti-inflammatory and pro-angiogenic effects in a rat model of intracerebral haemorrhage [93].

Further reports of MSC action in inflammatory in vivo settings indicated that MSC may reduce inflammation in obstructive sleep apnea [94], ischemia/reperfusion induced acute renal failure [95], liver fibrosis [96], experimental colitis [97], asthma [98], acute myocarditis [99], and myocardial infarction [100]. Moreover, one of the first reports of use of umbilical cord blood-derived mononuclear cells in diabetes pointed out that NOD mice treated with UCB mononuclear cells normalized blood glucose levels and increased their lifespan. In treated mice a reduction of insulitis was also detected [101].

Another key question is the role of inflammatory cytokines in the regulation of the differentiation potential of MSC, and the in vivo effects of such interactions. One example comes from the work of Wehling and colleagues, who recently demonstrated that chondrogenesis in human MSC was inhibited by both IL-1β and TNF-α [102]. This study pointed out that in inflamed joints the cellular reparative mechanisms may fail if not supported by the contemporary administration of specific antagonists of these inflammatory cytokines. These issues arose from immature cells implantation in inflamed cartilage, and would be avoided if the differentiation process was carried out pre-implantation.

The accumulated evidence strongly suggests not only that MSC preferentially home in inflamed tissues, but that they also can attenuate inflammation by the secretion of a number of mediators. Their usefulness has been demonstrated in several in vivo models of acute and chronic inflammatory diseases.

Using Stem Cells for Beta Cells Repopulation Strategies: Morphofunctional Basis and Therapeutic Needs

In the light of the multipotency of MSC, different groups have shown that these cells can give rise to IPC.

The pancreas is composed of an endocrine and exocrine compartment. The vast majority of the organ is formed by exocrine acinar cells, while the endocrine compartment, accounting for less than 2% of the organ volume, consists of multiple insular endocrine glands known as islets of Langerhans. Inside these islets, four cellular types are able to synthesize peptide hormones: α-cells, β-cells, PP-cells and δ-cells. β-cells secrete insulin, a hypoglycemizing hormone which regulates blood glucose concentration.

Pancreas regeneration in vivo physiologically occurs only in limited cases. For example, exocrine acinar cells can easily regenerate after severe toxin-induced pancreatitis, therefore re-establishing exocrine function. On the contrary, while beta cells can undergo intense replication in vivo in some physiological conditions (e.g. during pregnancy), they are not able to overcome the injuries which lead to their destruction [103, 104].

Diabetes is a metabolic disease, constituting a leading cause of death in many countries. This disease is characterized by absolute or relative insulin deficiency. In type 1 diabetes, there is an absolute insulin decrease caused by β-cells destruction by T-cells. In type 2 diabetes, there is a relative insulin deficiency due to decreased peripheral insulin sensitivity [105]. Pathological conditions such as atherosclerosis, kidney failure, blindness and coronary artery disease are favoured by the presence of diabetes. In particular, heart failure commonly coexists with diabetes, making the treatment of each condition more problematic due to the presence of the other condition [106–108].

Type 1 diabetic patients need insulin injections every day, but this external administration only grossly mimics the physiological insulin secretion process. Therefore, whole pancreas or islet transplantation studies have been

developed in a number of clinical settings. Different reports show that human islet transplantation in type 1 diabetic patients achieves insulin independence during the first year, with a subsequent decline [109]. Due to the need for a concurrent immune suppression protocol and the paucity of human donor pancreases, clinical interest has switched towards cellular therapy applications, and much research has been focused on trying to promote differentiation of insulin-producing cells (IPC) from embryonic stem cells (ESC), foetal-associated stem cell (e.g. umbilical cord blood cells), adult stem cells (e.g. BM-MSC, adipose tissue cells and Wharton's Jelly cells) and even hepatic progenitors [110–112].

The relative abundance of sources for stem cells derivation raises the question of how MSC should be used to treat diabetic patients. On the one hand, autologous cells (e.g. from BM or adipose tissue of the same patient) should pose no limitations due to host immune reactions. On the other hand, subjective and disease-linked factors limit the broad use of autologous stem cells. In fact, as stated previously, the number of MSC in BM declines with age [11]; moreover, functional impairment of MSC, due to the underlying disease, has been demonstrated in diabetic subjects [113, 114]. Therefore, while the use of donor-derived MSC was initially thought to be useful following HLA-matching, in order to avoid undesired reactions two alternative routes are now being explored for the use of these cells. Firstly, if umbilical cord matrix banking is promoted parallel to cord blood banking, the number of MSC for autologous therapy will increase dramatically. Secondly, it is expected that developments in the field of immune regulation by MSC will lead to the definition of optimal conditions, drugs and starting cellular population, to obtain beta cell regeneration and/or immunosuppression without HLA matching between donor and recipient.

The field in which most progress has been made, even if not all of the desired mature functions of beta cells seem attainable at the moment, is the differentiation of MSC towards endocrine-committed cells. The molecular bases for these different attempts in differentiating phylogenetically-unrelated cellular populations, may be researched in the developmental history of the pancreas, and in particular of islet cells, as well as in the expression of key transcription factors in differentiating cells.

Molecular Regulation of Endocrine Pancreas Development

Endocrine pancreas development is a multistep process with recapitulation as the main object of current differentiation strategies of various undifferentiated populations. This process is tightly regulated during organogenesis and a precise sequence of transcription factors is followed in the subsequent differentiation steps from definitive endoderm specification to mature beta cell generation. Several recent comprehensive reviews have described the processes of molecular regulation of pancreas development [115–118], and a detailed analysis of these developmental processes is beyond the scope of the present review. However, since the attempt to recapitulate initial and late stages of islet cells differentiation directs the attention of scientists towards the analysis of expression of key markers in stem cells after application of differentiative protocols, some basic notions are helpful to better understand the current results achieved worldwide. As reviewed recently, most of our knowledge of pancreas development derives from animal (mainly rodent) models [115]. Therefore, even if differentiation strategies are based on developmental biology steps which are well defined in non human species, the same events are not always exactly recapitulated in human pancreas development, suggesting caution in the interpretation of the results and in the definition of better differentiation strategies.

The human pancreas originates from the fusion of two distinct outgrowths of the foregut endoderm, one dorsal, the other ventral. Cells expressing insulin, glucagon and somatostatin, even if rare, have been observed between 7.5 and 8.5 weeks of development [reviewed in 118].

One of the most important factors in the endocrine pancreas developmental process is PDX1 (pancreatic and duodenal homeobox 1) [reviewed in 116]. During development, its expression has been reported in both endocrine and exocrine progenitors, while in the mature organ it is mainly expressed by beta cells [119]. Following the first wave of expression of PDX1, cells which will give rise to both acinar and endocrine cells start co-expressing the bHLH (basic Helix-Loop-Helix) transcription factor Ptf1a (pancreas specific transcription factor 1a) [117, 120, 121]; the absence of Ptf1a results in almost apancreatic animals [122]. The endocrine program is initiated by Ngn-3 (Neurogenin 3), a bHLH transcription factor. Ngn3-null mice do not have islets in their pancreases and die within a short time due to hyperglycaemia [123]. Ngn-3+ cells are the progenitors of each endocrine cell type in mature islets [117]. Then, specification of single hormone positive cells occurs via other transcription factors, acting either as activators or repressors, e.g. Pax4 (paired box 4), Arx (aristaless elated homeobox), Pax6 (paired box 6), and NeuroD1. Among the latter factors involved in endocrine cells differentiation, a key regulator of beta cells differentiation is Nkx2.2 (NK2 homeobox 2). Its inactivation gives rise to Isl1 (Islet1)+/Ins (Insulin)- cells, thus suggesting its role in blocking the definitive differentiation of such endocrine elements [117, 124, 125].

Endocrine Pancreas-Directed Differentiative Capacity of Different Stem Cell Types

Embryonic Stem Cells

ESC are historically viewed as promising cells for recapitulating the developmental pathways leading to beta cells differentiation [reviewed in 126]. Soria et al. were the first to differentiate mouse ESC in insulin producing cells and to note that the differentiated cells, upon transplantation in diabetic mice, induced a reversal of hyperglycaemia [127]. Lumelsky and co-workers described a protocol to obtain IPCs by mouse ESC via a nestin expression step [128]. In all experiments, the differentiated ES cells were able to synthesize insulin, cleaving pro-insulin to C-peptide and insulin, and expressed voltage-activated calcium channels. Despite the fact that these ESC-derived cells showed features resembling β-cells, they were unable to secrete insulin exclusively in response to high glucose levels, and did not show the presence of insulin-containing secretory granules, results confirmed also by other groups [129]. In addition, independently of the disease treated, the use of ESC involved per se a higher risk of teratoma formation. This, together with ethical issues related to ESC sourcing, still constitutes a strong limitation concerning use of these cells, as well as of induced pluripotent stem cells (iPS), for the regeneration of beta cells [130]. Moreover, the inability to secrete insulin in response to glucose stimulation, which is the key function of beta cells, remains an issue to be resolved by future research [131].

Adult Mesenchymal Stem Cells: Bone Marrow Mesenchymal Stromal Cells

More recently, adult stem cells and in particular BM-MSC, have been reported as a promising source to obtain insulin-producing cells. As stated earlier, during pancreas development, the differentiation of the endocrine compartment is controlled by the activity of key transcription factors such as Pdx-1, Ngn-3, NeuroD1, Pax4, and Pax6 [117, 132]. Experiments aimed at inducing BM-MSC to differentiate into IPCs were conceived to correctly reprogram cells in order to activate the pathways driven by such factors. As detailed in Table 2, three parallel reports in 2004 opened up this field showing the results obtained by different groups using rat BM-MSC.

Chen an co-workers reported that rat BM-MSC, cultured in serum-free medium in the presence of high glucose concentration, nicotinamide and β-mercaptoethanol, were able to differentiate towards IPC. These cells were tested in vitro only for the expression of insulin and nestin. In vivo experiments with the transplantation of differentiated cells in diabetic rats showed a reduction of glucose levels which however failed to reach statistical significance [133]. In this first report, the authors made use of stimulating agents which are rather unspecific for the desired phenotype. Neuron-like cells were present amongst rounded beta-like ones, thus indicating incomplete and even unwanted differentiation. This would further justify the limited results obtained in vivo.

Wu and co-workers demonstrated that rat BM-MSC cultured with a two-step differentiation protocol (high glucose concentration for 14 days with the subsequent addition of nicotinamide and exendin-4) gave rise to beta-like cells. Exendin-4 probably stimulated β-cell replication and conversely inhibited β-cell apoptosis. These IPC expressed insulin, IAPP (islet amyloid polypeptide), Pax-6, Ngn-3, NeuroD1 and Glut-2 (Glucose transporter type 2) genes, and low levels of insulin and IAPP proteins, showing low ability to secrete insulin and a weak glucose response. When transplanted in streptozotocin (STZ)-induced diabetic rats, differentiated cells reduced hyperglycaemia for the first 2 weeks only, after which the trend to increase resumed [134].

Oh and co-workers suggested that rat BM-derived cells, cultured in a medium supplemented with DMSO (dimethylsulfoxide) and high glucose concentration, transdifferentiated into IPC. In particular, these researchers highlighted the formation of aggregates by differentiated cells, which, upon transplantation into hyperglycaemic mice, acquired a three-dimensional architecture resembling islets of Langerhans. These cells expressed typical pancreatic genes as insulin, glucagon and somatostatin, therefore giving rise to a mixed population of islet-like cells. These cells possessed granules with relatively low insulin content and, when transplanted into diabetic mice, favoured the normalization of blood glucose levels over almost 3 months [135]. In a very recent report, Paz and co-workers demonstrated that rat MSC overexpressing β-cellulin were able to produce insulin in vitro and to revert hyperglycaemia in STZ-induced diabetic rats [136].

While the studies listed so far were based on animal MSC sources and models, in the last few years several researchers have focused on the differentiation of human BM-MSC (hBM-MSC) towards IPC.

Moriscot and co-workers infected hBM-MSC with adenoviral vectors coding for mouse Pdx-1, HLXB9 (homeobox protein HB9) and FOXA2 (forkhead box A2) transcription factors. While HLXB9 and FOXA2 are involved in the hepatic and pancreatic developmental pathways, Pdx-1 is a factor expressed exclusively during pancreatic development. The transfected cells were cultured alone or in the presence of pancreatic islets or islet-conditioned medium: in all of these settings, hBM-MSC generated cells expressing insulin, as well as NeuroD1 and Islet1 [137].

Table 2 Summary of literature reports indicating the differentiative ability of several MSC populations towards islet-like cells

MSC population	Pancreatic differentiation protocol	Analysis of markers expression	Functional assays	References
Rat bone marrow mesenchymal stem cells	Two step,. 34 h protocol Pre-induction: L-DMEM with nicotinamide and β-mercaptoethanol for 24 h Re-induction: serum-free H-DMEM with nicotinamide and mercapoethanol for 10 h	I. Expression of insulin by RT-PCR and RIA; II. Expression of insulin and nestin by immunohistochemistry	*In vitro*: cell morphology changes by inverted microscope and expression of insulin by RT-PCR, IHC, RIA *In vivo*: differentiated MSC transplanted in STZ-induced diabetic rats. Evaluation of blood glucose levels in the rats by Roche ACCU-CHEK	[133]
Rat bone marrow mesenchymal stem cells	Three steps: 28 days protocol DMEM with FBS and high glucose (FBS HG-DMEM)for 14 days. (FBS HG-DMEM) with nicotinamide for 7 days (FBS HG-DMEM) with exendin- for 7 days	I. Expression of CD29, CD45, CD90, insulin by flow cytometric analysis; II. Expression of insulin I, Insulin II, Gcg, SS, IAPP, GLUT-2, GK, GLP-1R, PDX-1, Ngn-3, NeuroD1, PAX-6, Nkx2.2, GAPDH by RT-PCR; III. Expression of insulin, C-pepide, Gcg, SS, IAPP by IF; IV. expression of insulin by ELISA	*In vitro*: cell morphology changes by electron and converted microscope Expression of insulin by flow cytometry and ELISA *In vivo:* differentiated MSC transplanted in STZ-induced diabetic rats. Evaluation of blood glucose levels in the rats by Roche ACCU-CHEK glucose meter. Expression of insulin, Gcg, SS and IAPP by IHC	[134]
Rat bone marrow mesenchymal stem cells	Three steps protocol: 10 days Serum-free DMEM with 1% DMSO for 3 days, DMSO-free DMEM with FBS with either high or low glucose concentration for 7 days. Cells were plated on six well plates or on slide coverslips coated with type I collagen	I. Expression of PDX-1, NKX2.2 NKX6.1, Glut-2, GAPDH, Gcg, Insulin I, insulin II, SS, PP; II. Expression of insulin by Northern Blotting and ISH; III. expression of insulin PP, SS, C-peptide by ICC	*In vitro:* measurement of insulin content an secretion by immunoprecipitation, western blotting and ELISA *In vivo:* differentiated cells transplanted in NOD-Scid STZ-induced diabetic mice. Evaluation glucose level by a standard glucose meter Expression of insulin by immunogold	[135]
Human bone marrow mesenchymal cells (hBM-MSC)	hBM-MSC infected with adenoviruses coding for mouse IPF1, HLXB9, FOXA2 using various MOI ratios	I. Expression of CD11b, CD31, CD34, CD45, CD49b, CD117, CD44, CD73, CD90, CD105, CD147, CD10 by FACS; II. Expression Hlxb9, FoxA2, PDX-1, Ngn-3 NeuroD1, Nkx2.2, Pax-4, Nkx6.1, Isl-1, Pax-6, PC1/3, Glut-2, GK, Insulin, CK18, CK19, CD90, Oct4 by RT-PCR	*In vitro*: Expression Hlxb9, FoxA2, PDX-1, Ngn-3 NeuroD1, Nkx2.2, Pax-4, Nkx6.1, Isl-1, Pax-6, PC1/3, glut-2, GK, Insulin, CK18, CK19, CD90, Oct4 by RT-PCR after that hBM-MSC infected with adenovirus	[137]
Human bone marrow mesenchymal cells (hBM-MSC)	hBM-MSC infected with PDX-1 adenovirus in serum-free DMEM with B27 and GLP-1	I Expression of h actin, h-PDX-1, mPDX-1, h-Ngn-3, h-brain-4, h Gcg, h-insulin, h-GLP-1R, Glut-2, GK by RT-PCR; II. Expression of h-insulin and h-C-peptide by IF	*In vitro:* expression of insulin and C-peptide by ELISA kit *In vivo:* differentiated PDX-1+ h-BM-MSC into STZ-induced diabetic mice. IPGTT to measure blood glucose levels Expression of C-peptide and insulin by ELISA kit	[138]
Human bone marrow mesenchymal cells (hBM-MSC)	None	None	*In vivo*: hBM-MSC transplanted in NOD/SCID STZ-induced mice. Evaluation of glucose levels by glucometer Expression of insulin by ELISA Detection of human Alu sequences by RT-PCR Expression of human/mouse insulin, human nuclei antigen, mouse and human PDX-1, mouse/human fibronectin, mouse/human podocalyxin Urine assay	[139]

Table 2 (continued)

MSC population	Pancreatic differentiation protocol	Analysis of markers expression	Functional assays	References
Human bone marrow mesenchymal cells (hBM-MSC)	Three steps protocol: 18 days Step 1: serum-free high glucose DMEM, β-mercaptoethanol for 2 days Step 2: medium with NEAA, EGF, β-FGF, EGFB27, glutamine for 8 days Step 3: new medium with β-cellulin, activin-A, B27, nicotinamide for 8 days.	I. Expression of CD34, CD45, CD14, CD29, CD44, CD106 by flow cytometry; II. Expression of Nestin PDX-1, Ngn-3, Pax-4, Insulin, Gcg by RT-PCR; III. Expression of insulin and Gcg by ICC.	*In vitro*: cell morphology changes by inverted microscope, DTZ staining insulin assay by ELISA	[140]
Human bone marrow mesenchymal cells (hBM-MSC)	h BM-MSC infected with a retrovirus expressing rat PDX-1 cDNA under control of the viral long terminal repeat.	I. Expression of CD34, CD45, CD105, CD29, CD90, CD14, CD44 by FACS; II. expression of rPDX-1, hPDX-1, Insulin, Gcg, SS, Pp, Glut-2, GK, NeuroD1, NGN3, PAX4, PAX6, NKX2.5, NKX6.1, HLXB9, HNF6, ISL1, PC1/3, PC2, SUR1, KIR6.2 by qRT-PCR; III. expression of insulin, Gcg, HSP27, PDX1, PP, C-peptide by IF	*In vitro*: expression of rPDX-1, hPDX-1, Insulin, Gcg, SS, Pp, Glut-2, GK, NeuroD1, NGN3, PAX4, PAX6, NKX2.5, NKX6.1, HLXB9, HNF6, ISL1, PC1/3, PC2, SUR1, KIR6.2, syntaxin3, synaptobrevin1, synaptotagmin 1 by microarray analysis; Insulin and C-peptide assay by ELISA *In vivo*: infected cells transplanted in STZ-diabetic SCID mice. Insulin and C-peptide assay Expression of SS, PP, PDX1, NeuroD1, SUR1, KIR6.2, GLUT2 by qRT-PCR.. Expression of insulin, Gcg, hC-peptide by IF	[141]
Human bone marrow mesenchymal cells (hBM-MSC)	Three steps protocol: 15 days Step 1: H-DMEM with bFGF, DMSO, FBS, glucose for 3 days Step 2. serum-free DMEM/F12 with glucose, nicotinamide, EGF, bFGF, exendin-5, B27 and N for 7 days Step 3: RMPI 1640 with glucose, nicotinamide, Hepes, activin-A, exendin-4, for 5 days	I. Expression of CD29,CD44, CD105, CD90, CD73, CD45, CD34, CD14 by flow cytometry; II. Expression of Oct-4, ISL-1, Nkx6.1, Beta-2, NeuroD, Glut2, CK18, CK19, Insulin PDX-1, Nestin, Pax-6, Ngn-3, Gcg by RT-PCR; III. Expression of CK-18, CK19, Glut-2, ISL-1, GAPDH by western blotting; IV. Expression of Oct3/4, insulin Gcg, SS and C-peptide by IF	*In vitro:* DTZ staining to stain insulin granules in β-cells, insulin secretion assay and glucose challenge test *In vivo:* Differentiated BM-MSC transplanted in STZ-induced diabetic nude mice. HE staining and glucose challenge test	[142]
Human adipose tissue derived mesenchymal stem cells	One step protocol Serum-free DMEM/F12 with glucose, nicotinamide, activin-a, exendin-4, HGF, pentagastrin B27 for 3 days in ultra-low attachment plates.	I. Expression of HPRT, insulin, Ipf-1, ABCG2, Pax-6, ISL-1, Nestin, Ngn-3, SS, Gcg, Thy-1, SCF, c-kit by qRT-PCR; II. Expression of SS by RIA; III. Expression of c-peptide, ISL-1, Ipf-1 by ICC	*In vitro:* Expression of SS by RIA	[144]
Human adipose tissue derived mesenchymal stem cells (AD-MSC)	One step protocol DMEM with glucose, nicotinamide, activin-a, exendin-4, pentagastrin, HGF, B-27, for 3 days	I. Expression of CD45, CD90, CD73 by FACS; II. Expression of Pax-6, ISL-1, PDX-1, by IF	*In vivo*: insulin-producing AD-MSC transplanted with hematopoietic stem cells in insulinopenic diabetic patients. Expression of serum C-peptide and insulin by chemiluminescence. Expression of glycosylated haemoglobin (HB A1c)	[145]
Mononuclear umbilical cord blood cells (UCB cells)	UCB cells cultured in IMDM with FBS and glutamine. After 48–72 h the cells adhered to the substrate and after 7–8 days they have fibroblast-like morphology.	I. Expression of nestin, Ngn-3, CK-8/18, CK-19 by ICC and Western blotting; II. Expression of Nestin, PDX-1, ISL-1, Ngn-3, CK-8, CK18, CK-19, PAX-4, GAPDH, actin by RT-PCR	None	[146]

Table 2 (continued)

MSC population	Pancreatic differentiation protocol	Analysis of markers expression	Functional assays	References
Umbilical cord blood cells (UBC cells)	None	None	*In vivo:* UCB cells transplanted in NOD mice with autoimmune type I diabetes. Evaluation of glucose levels, survival and insulitis. Expression of HGH and HPRT by RT-PCR	[101]
Umbilical cord blood cells (UCB cells)	None	None	*In vivo:* UCB cells transplanted in type 2 diabetic mice. Evaluation of glucose level, survival and renal pathology by statistical analysis.	[148]
Mononuclear umbilical cord blood cells (UCB cells)	UCB-derived T- cell depleted mononuclear cells transplanted in newborn Nod/SCID/β_2m^{null} mice	I. Expression of CD33, CD19, CD3 and CD45 by flow cytometry; II. Expression of insulin and CD45 by FISH; III. Expression of insulin, C-peptide and CD45 by IF; IV. Expression of h-insulin, m-insulin and GAPDH by RT-PCR analysis	*In vivo:* expression of h-insulin and m-insulin by FISH	[149]
Umbilical cord blood cells (UCB cells)	Two steps, 7 days protocol: UCB cells plated into poly-L-ornithine/laminin coated tissue culture dishes. Step 1:DMEM/F12 witsh progesterone, putrescine, laminin, insli, sodium selenite, nicotinamide transferring fibronectin, B27 and FCS for 24 h Step 2: stage1 medium without DMEM/F12 but with high glucose DMEM for 5–7 days	I Expression of CD4, CD20, CD29, CD34, CD44, CD45, CD90, CD105 and CD133 by FACS. II Expression of OCT-4, SSEA-4, h-insulin, h-C-peptide by IHC III Expression of C-peptide and insulin by western blotting	*In vitro*: expression of C-peptide and OCT-4 by western blotting. Expression of h-insulin and h-C-peptide by IHC	[106]
Umbilical cord blood cells (UCB cells)	Two types of differentiation protocols Protocol 1: four steps,22 days Step1: high glucose-DMEM with MTC, FBS, glutamine, NNEA for 4d Step 2: serum free insulin, transferring, selenium(ITS) in basal medium-A for 6 d Step 3: serum-free pancreatic proliferation medium containing N2-supplement-A and B27 supplements and bFGF in basal medium-A for 6d Step 4: pancreatic proliferation medium containing N2-supplement-A and B27 supplements and nicotinamide in basal medium-A with serum for 6d Protocol 2: Three steps, for 2 weeks Step1: After 2 h in chamber slide and addition of activin-a for 24 h, the cells cultured in DMEM with FBS, all-trans retinoic acid for 24 h Step2: DMEM with FBS, bFGF, for 3-5d Step3: DMEM/F12 with N2 supplement, B27 supplement, laminin, bFGF and nicotinamide for 3–5d	I. Expression of CD33, CD45, Cd7, Cd4, CD133 by MACs; II. Expression of h-C-peptide, h-nestin, vimentin, insulin SSEA-4 by IHC; III. Expression of CD34, CD38, CD133by flow cytometry	None	[150]
Umbilical cord blood-derived mesenchymal stem cells (UCB-MSC)	Three steps protocol: about 2 weeks Step1: H-DMEM with FBS, retinoic acid for 24 h after in H-DMEM with only FBS for 2 days. Step 2: cells coated in	I. Expression of h-NSE, h-C-peptide, h-insulin, h-Gcg by IF; II. Expression of h-C-peptide, h-insulin by Western blotting; III. Expression of CD14, CD19,	*In vitro*: evaluation of insulin secretion by BCA protein assay kit	[151]

Table 2 (continued)

MSC population	Pancreatic differentiation protocol	Analysis of markers expression	Functional assays	References
	plates with ECM gel. L-DMEM with FBS, nicotinamide and EGF for 6d Step3: low-glucose medium with FBS, exendin-4 for 6d	CD29, CD34, CD44, CD45, CD90, CD105 by flow cytometry; IV. Expression of Gcg, insulin, Ngn-3, Pax-4, PDX-1, GLUT-2,		
Umbilical cord blood-derived mononuclear cells(UCB-MNC)	UCB-MNC transplanted in FVB/NJ or NOD/SCID mice	I. expression of CD34, CD44, CD45, CD90 by flow cytometry; II. Expression of insulin, SMA, Gcg, vWF, GLP1R, PDX1 by immunostaing; III. expression of ngn3, glp1r,PAX-6, NKX6.1, brn4,gck, GATA4, GATA6, Hes1, NEUROD, SMA, vimentin, pdx1, gcg, isl-1, ki67 by qRT-PCR	*In vivo:* expression of insulin by ELISA kit	[152]
Placenta-derived adherent cells	One step, 14 days protocol: DMEM-H, with 10%FBS, 1% NEAA, 0,1 mmol/l β-mercaptoethanol, 1 mmol/l l-glutamine, 5 μg/l bFGF	I. expression of CD29, CD44, CD105; II. PDX-1, insulin 1 and Insulin 2 expression by RT-PCR.	In vitro: DTZ staining, Expression of insulin 1 and 2, Pdx-1.	[153]
Wharton's jelly derived mesenchymal stem cells (WJC)	Four steps protocol: 28 days Step 1: DMEM with FBS for 3 to 6 d. Step.2: neuronal conditioned medium for 7 d. Step.3: DMEM7F12 with FBS, glucose, nicotinamide, B27 for 7 d. Step.4: stage 3 medium with SCM	I. Expression of insulin, PDX-1, HLXB9, NKX2.2, NKX6.1, GLUT-2, r-insulin by qRT-PCR; II. Detection of human nuclei and human insulin by IF	*In vitro:* expression of insulin and C-peptide by ELISA *In vivo:* islet-like cells transplanted in STZ-induced diabetic rats. Expression of insulin and C-peptide by ELISA IPGT test	[155]
Wharton's jelly derived mesenchymal stem cells (WJC) Bone marrow mesenchymal stem cells	One step protocol: The cells were seeded in an ultra-low attachment culture plates in DMEM-F12, with 17.5 mM glucose, 10 mM nicotinamide, 2nM activin-A 10 nM exendin-4 100pM HGF and 10 nM pentagastrin.	I. Expression of CD29, CD44, CD59, CD34, by flow cytometry analysis; II. Expression of hC-peptide, h Gcg, h PDX-1 by ICC; III. expression of insulin by RIA	*In vitro:* expression of insulin by RIA expression of c-peptide by flow cytometry analysis Analysis of viability by cell counting Kit8 and apoptosis by Annexin V-FITC apoptosis detection kit.	[157]
Wharton's jelly derived mesenchymal stem cells (WJC)	Three steps protocol, 34 days: Cells were cultured for 7 days in CMRL1066 medium containing 10% FBS, 1% Penicillin/streptomycin/ amphotericin B, 100 ng/ml of β-nerve growth factor, 4 nM activin-A, 10 mM nicotinamide and 25 ng/ml of epidermal growth factor. DMEM/F12 for 7–10 days. Finally, 10 mM nicotinamide, insulin/transferrin/selenium and 10 ng/ml of basic fibroblastic growth factor were added and incubation was continued for 17 days.	I. Expression of C-peptide by ICC and ELISA; II. Expression Insulin, Glut2, MafA, Pax4, NeuroD, Isl1, Nkx2.2 by RT-PCR; III. immunolocalization of insulin and c-peptide by immunogold	*In vitro:* C-peptide secretion in response to different glucose concentrations *In vivo*: transplantation if insulin producing cells in NOD mice; Detection of C-peptide and human nuclei in liver; improvement of glucose tolerance.	[158]

Abbreviations: *Gcg* glucagon, *SS* somatostatin, *IAPP* Islet amyloid polypeptide, *GK* glucokinase, *GLP-1R* glucagon-like peptide 1-receptor, *PDX-1* pancreatic duodenal homeobox-1, *Ngn-3* neurogenin-3, *PAX-6* gene paired box-6, *NKx2.2* homeobox protein NKX2.2, *GAPDH* Glyceraldehyde_3-phosphate_dehydrogenase, *PP* polypeptide pancreatic, *ISL-1* Insulin gene enhancer protein ISL-1, *CK 18* citokeratin-18, *CK-19* citokeratin-19, *Pax-4* Paired gene box-4, *IPF-1* insulin promoter factor-1, *HLXB9* homeobox protein HB9, *FOXA2* forkhead box A2, *PC 1/3.* pro-convertase, *SUR1* sulfonylurea receptor 1, *KIR6.1* subunit of the ATP-sensitive K^+ channel, *HSP27* heat shock protein 27, *HPRT* Hypoxanthine-guanine phosphoribosyltransferase, *OSM* oncostatin M, *ABCG2* ATP-binding cassette, sub-family G member 2. Thy-1: or CD90, *SCF* stem cell factor, *HGH* human growth hormone, *SSEA-4* stage-specific embryonic antigen-4, *SMA* smooth muscle actin, *vWF* Von Willembrand factor, *brn4* brain-4, *Hes1* Hairy and enhancer of split 1

Li and colleagues suggested that hBM-MSC transfected with recombinant adenovirus carrying Pdx-1 gene differentiated in IPC. The transfected Pdx-1+ cells expressed multiple islet cell-specific genes including neurogenin3 (Ngn3), insulin, GK (glycerol kinase), Glut2, and glucagon, and produced and released insulin and C-peptide in a weak glucose-regulated manner. Moreover, if transplanted in diabetic mice, restoration of euglycaemia was achieved and maintained for least 42 days [138]. Even if few markers were analyzed in this work, it is hypothesizable that the transfer in vivo would have ensured the favourable conditions that boosted the terminal differentiation of such cells.

Lee and co-workers reported that undifferentiated hBM-MSC infused in STZ-induced diabetic NOD/Scid mice normalized hyperglycaemia over 1 month by increasing blood insulin levels. In addition, when these cells were engrafted into kidneys, the researchers observed an improved glomerular morphology and decreased mesangial thickening and macrophage infiltration. This was the first documented evidence that hBM-MSC could be useful per se not only to enhance insulin secretion but also to improve the outcome of the renal lesions that develop in diabetic patients [139].

Sun and co-workers were the first to report that hBM-MSC isolated from type I diabetic patients, cultured in high glucose medium supplemented with nicotinamide, activin A and β-cellulin, could differentiate into IPC. Activin A (a TGFbeta family member) regulates neogenesis of β-cells in vivo, while β-cellulin regulates growth and differentiation of pancreatic endocrine precursor cells. The combined action of these factors with nicotinamide effectively induced IPC differentiation. Moreover, this report suggested that differentiated cells produced insulin in response to different glucose concentrations [140].

Karnieli and co-workers induced β-cells differentiation from hBM-MSC using a Pdx-1 gene-transfer approach. Differentiated cells expressed insulin, Pax4, Pax6, Nkx2.2, Nkx6.1 (NK6 homeobox 1), HNF-6 (hepatocyte nuclear factor 6), Isl-1 HLXB9 but surprisingly did not express Ngn3 or NeuroD1. The authors hypothesized that in vitro Pdx-1 probably upregulated transcripts for insulinoma-associated antigen 1, histone deacetylase 1 and 3, and cyclin D, all factors acting as repressors of NeuroD1. Interestingly, in an in vivo setting these IPC, transplanted in diabetic mice, decreased hyperglycaemia and restored the expression of some of the genes not expressed in vitro, e.g. NeuroD1, SUR1 (sulfonylurea receptor 1) and KIR6 (Inward rectifier potassium channel Kir6.1) [141]. This work therefore pointed out the importance of local microenvironmental signals, as well as cell-cell contacts with host cells, in complementing and completing the incomplete differentiation achieved in vitro [104, 141]. However, on the basis of the current notions of pancreas development, it should be noted that HNF6 is a transcription factor which is expressed early in the pancreatic epithelium (where it acts as an activator of both Pdx-1 and Ngn-3 expression) but its down-regulation is essential later for the definitive maturation of beta cells [117]. Therefore, these data suggest that gene-transfer techniques should lead to a mixed phenotype population, which maintains characters of pluripotency and undifferentiation, even if the local microenvironment exerts a key role in directing the final differentiation of cells.

More recently, Xie and co-workers demonstrated that hBM-MSC were able to give rise to IPC by a three-step differentiation protocol, with the final addition of Activin A as the differentiating agent. The acquisition of a beta cell-like phenotype by these cells was attested by morphological analyses and the expression of typical pancreatic genes such as Nkx6.1, Isl-1, Beta2/NeuroD, Glut2, Pax6, nestin, Pdx-1, ngn3, insulin, glucagon and C-peptide. More importantly, differentiated cells released insulin in a glucose-dependent manner and improved hyperglycaemia for over 1 month in STZ-induced diabetic rats [142].

Adult Mesenchymal Stem Cells: Adipose-Derived MSC as a Source of IPC

Adipose tissue (AT) has emerged recently as another reliable source of MSC populations. The stromal-vascular cell fraction of AT is characterized by the presence of multipotent stem cells known as adipose tissue-derived stromal cells (ADSC). Zuk and co-workers observed that these cells are similar to BM-MSC in both gene expression and differentiation capacities [143].

Timper and colleagues were the first to suggest that ADSC can adopt a pancreatic endocrine phenotype. As detailed in Table 2, a multi-step differentiation protocol favoured the generation of IPC, which were characterized for the expression of molecules as Ipf-1, Isl1, Ngn-3, insulin, glucagon and Pax-6 [144].

More recently, Trivedi and co-workers differentiated ADSC in vitro towards IPC. Using a xenogenic-free culture medium, the authors transplanted induced cells together with hematopoietic stem cells (HSC) into several insulinopenic diabetic patients without the use of immunosuppressants. Pancreatic function was then evaluated by expression of C-peptide and insulin [145], and strongly confirmed the effectiveness of delivery and function of such cells, which did not exert an immune response in the host. In particular, the insulin requirement for each patient was, if not abolished, notably reduced. The implications of this study are that, even if total remission and exogenous insulin-independence are still unattained goals, the safety and effectiveness of MSC have been consistently demonstrated.

With the start of the first clinical trials on BM-MSC as a therapeutic agent to treat diabetes, the data gained from in vitro and in vivo transdifferentiation of cells was of vital importance to evaluate the subsequent clinical strategies. All of the attempted differentiation protocols listed so far tended to confirm the feasibility of a cellular therapy approach aimed at generating new beta cells by different stimuli applied to MSC. While euglycaemia restoration seems an achieved goal for some studies, even with a long-lasting effect, the issue of incomplete or unwanted differentiation is still present, therefore raising questions about the real efficacy of the implanted cell numbers. Moreover, no information is available about the fate of these partly differentiated cells. Another related question is the engraftment success of these cells. The difficulties in evaluating the actual numbers of engrafted cells with current imaging and molecular techniques still prevent our full understanding of the real potential of these cells. Two key factors do influence engraftment success: the maintenance or not of immunoprivileged status by MSC-derived beta cells, and the potential impact of the inflamed microenvironment due to insulitis. Research efforts will hopefully be directed to determine if immune privileges and anti-inflammatory activities are retained by differentiated BM-MSC or AT-MSC.

MSC from Umbilical Cord Blood

Another source of MSC is umbilical cord blood (UCB). From this tissue, MSC can be routinely harvested without any risk to the donor. Moreover, these UCB-MSC have low immunogenic properties. Recently different groups successfully induced the differentiation of UCB-MSC into IPC.

Pessina and co-workers were the first to highlight that naïve UBC-MSC, cultured in a medium with serum but without specific cytokines or growth factors, expressed a panel of typical markers in the pancreatic differentiation pathway (Ngn3, Nestin, Cytokeratin-18 Cytokeratin-19, Isl-1 and Pax-4). These cells did not express Pdx-1 [146]. Taking into account the observations made by Soria [147], the UCB cells isolated by these researchers would represent a cellular population with a phenotype partially overlapping that of the endocrine cell precursors in transition to beta cells [146].

Ende and colleagues showed that the transplantation of UCB mononuclear cells in Nod mice with autoimmune type 1 diabetes induced a decrease of blood glucose levels, probably due to improvement of insulitis in these mice [101]. In a correlated report, the transplantation of UCB mononuclear cells in type 2 diabetic mice improved glucose levels, attenuating also glomerular hypertrophy and tubular dilatation. Interestingly, the improvement of clinical signs was obtained without any prior or concomitant immune-suppression strategy [148].

Yoshida and co-workers investigated in vivo the capacity of human UCB mononuclear cells to give rise to IPC when transplanted in newborn NOD/SCID/β2-m ^{null} mice. These cells, 1-2 months after transplantation were able to generate IPC, as testified by by the presence of human insulin at the RNA level and human chromosome-containing insulin positive cells in situ. In addition, this study clarified that UCB cells could generate IPC by both fusion-dependent and -independent mechanisms in vivo [149].

Sun and co-workers used SSEA-4 (stage-specific embryonic antigen-4)+/Oct4 (octamer binding protein 4)+ UCB culture in a pancreatic differentiation medium. After 5–7 days islet–like structures expressing insulin and C-peptide were observed [106].

Denner and co-workers applied a pancreatic differentiation protocol to three different UCB-derived cell populations. All of the tested cells expressed insulin and C-peptide [150].

More recently, Gao and colleagues induced MSC derived from umbilical cord blood to generate IPC through a 3 step-protocol. The induced cells formed islet-like clusters, expressed pancreatic β-cell markers such as Ngn-3, Pdx-1, Pax-4, Glut-2 and secreted glucagon and C-peptide, but did not express insulin in response to glucose challenge. This report also suggested the important role of the extracellular matrix to induce the formation of three-dimensional structures in pancreatic endocrine cell maturation [151].

According to the last reports, the UCB should contain pancreatic-committed cells capable of differentiating in vivo into IPC. Parekh and co-workers, in fact, confirmed that UCB-derived mononuclear cells can express some pancreatic genes and, when transplanted in NOD/SCID (nonobese diabetic/severe combined immunodeficiency) FBV/NJ (Friend leukaemia virus B) mice, can differentiate into insulin-producing cells. Certainly, further studies are necessary to understand the underlying pancreatic differentiation of these UBC derived cells, which could provide a source of “lineage-committed” progenitors for diabetes therapy [152].

The key feature which has been highlighted by multiple reports on UCB cells is the possibility that these cells could exert both immunomodulatory and anti-inflammatory actions, therefore possessing a higher engraftment potential in the context of diseased human pancreas. Therefore, more work needs to be done to uncover the masked diffferentiative ability of naïve UCB-MSC in the pancreatic microenvironment, while in parallel we need to know to what extent UCB-derived beta cells retain immune privileges and anti-inflamatory activity.

Placenta Derived Stem Cells

A very recent report [153] has suggested the differentiability of placenta-derived adherent cells into insulin-producing cells. The authors demonstrated for the first time that differentiated placenta-derived cells expressed Pdx-1, Insulin and Glut 2 genes. This pilot study could be of major importance in extending our knowledge about the differentiability of placenta-derived cells, which may constitute another important therapeutic option for regenerative medicine [154].

Wharton's Jelly Mesenchymal Stem Cells (WJ-MSC)

Wharton's jelly-derived MSC recently gained much attention due to their easy sourcing, culture and differentiability into several tissues. A few important pilot reports indicate that these cells can be successfully differentiated into IPC.

Chao and co-workers differentiated WJ-MSC into IPC through a stepwise culture protocol using neuron-conditioned medium (see Table 2). To assess in differentiated IPC the presence of typical β-cell functions in vivo, the cell clusters obtained were transplanted into liver of diabetic mice. The authors demonstrated the expression of insulin in response to physiological glucose levels, as well as secretion of C-peptide and expression of pancreas-specific genes Pdx-1, Nkx2.2, HLXB-9 and Glut-2 [155, 156].

Subsequently, a comparative study carried out by Wu and colleagues compared the differentiative ability of WJ-MSC and BM-MSC in order to acquire a IPC phenotype. Both cellular types were able to form islet-like clusters on the first day of culture in a medium containing nicotinamide, activin, HGF, exendin-4 and pentagastrin. The researchers found a higher expression of Pdx-1 in differentiated WJ-MSC than in differentiated BM-MSC. Secretion of insulin and mRNA expression of insulin and C-peptide was comparably higher in the differentiated WJ-MSC [157]. In a very recent report, Wang and co-workers further corroborated these data with in vitro and in vivo experiments using differentiated human WJ cells to treat diabetes in NOD mice. After transplantation, IPC were located in the liver, and were able to normalize glycaemia [158]. Taken collectively, these promising data suggest that WJ-MSC possess the ability, both in vitro and in vivo, to differentiate into insulin-secreting cells.

The cumulative data on extraembryonic mesenchymal stem cells highlight the extreme cellular plasticity and transdifferentiation ability of these cells. The frank immune privilege of undifferentiated WJ-MSC has not been investigated in the differentiated beta cells, nor the anti-inflammatory activity. Nonetheless, recent results highlight that differentiated beta cells may engraft and survive in organs other than pancreas (e.g. in liver) and survive maintaining their differentiated state, thus contributing to glycaemia normalization. Moreover, comparative studies have demonstrated that WJ-MSC can be differentiated better than BM-MSC towards a mature beta cell phenotype, therefore increasing their usefulness in beta cell replacement therapy. The possibility of umbilical cord cells to be banked in parallel to cord blood units, which is desirable for the future, should render these cells available in high numbers for multiple patients. Indeed much work remains to be performed to better characterize the immune features of these cells and their differentiated progeny, and to increase their engraftment potential and survival in vivo.

Achieved Goals, Unsolved Challenges and Promising Features: Why Use MSC for Type I Diabetes Therapy?

Due to the critical shortage of supply of donor pancreases for organ or islet transplantation strategies, therapy for type 1 diabetes should certainly benefit from strategies aiming to repopulate the diseased organ with properly differentiated and hypoimmunogenic IPC. Mesenchymal stem cells recently came to light as reliable candidate cells to perform this task, due to their key properties of multipotency and immunomodulatory activities. Several reports evidenced that MSC from different sources can express naïvely some markers expressed by pancreatic progenitors and mature endocrine cells. The same cells can be successfully differentiated either with use of transfection approaches aimed to overexpress key transcription factors of the beta cells developmental program, or by culturing MSC in the presence of growth factors mimicking the signals leading to the development of the endocrine pancreas. While the results obtained using different MSC populations are in large part encouraging, and often adequately confirmed by the amelioration of clinical signs in animal models, several points need to be addressed in order to obtain a reliable cellular therapy approach which can constitute a valid clinical alternative to the standard therapeutic options currently available.

In particular, in our opinion, two key points need to be accurately addressed:

1- There is still a great variability in the differentiation protocols used, in terms of both the stimuli applied to cells and the length of the differentiation protocol. While serum-free and xenogenic-free culture conditions would be preferable for subsequent application in vivo in human subjects, this is still a difficult task which requires more research. Reports that ECM or a reconstituted three-dimensional microenvironment can boost differentiation should be taken into account to improve future protocols for IPC derivation.

2- The assessment of complete beta cells differentiation is currently performed using just a few markers, and a limited number of in vitro and in vivo functional assays. Insulin expression and secretion has been achieved in vitro by almost all of the investigated MSC populations, differentiated using variable and unrelated stimuli, from simple serum deprivation to exendin/activin stimulation. However, despite these promising results, more clinically-effective features of beta cells, such as the glucose-mediated regulation of insulin production, the formation of insulin-containing granules, and the ability to maintain the phenotypycal changes acquired in vitro after transplantation, are goals currently far from achieved. The complexity of the insulin promoter and the regulatory mechanisms which can determine its activation and proper glucose-dependent regulation in undifferentiated cells need to be further investigated [119, 159].

Nevertheless, the use of MSC to derive IPC could have positive side effects besides restoration of euglycaemia in patients. Type 1 diabetes is a T-cell driven disease, in which the role of CD8+ cells is assuming a growing importance [160]. The promising immunomodulatory features of MSC could therefore be of key importance for the resolution of the disease. In a recent review, Abdi and colleagues suggested that MSC could be used "per se", even as undifferentiated cells, in the therapy of type 1 diabetes, for their intrinsic ability to negatively modulate the immune effectors by acting on dendritic cells, T-cells and NK-cells [161]. Furthermore, as recently shown by Madec and co-workers, mesenchymal stem cells can reduce in vivo the ability of diabetogenic T cells to penetrate islets and can further induce regulatory T cells [64].

Interestingly, as stated previously, some recent pilot studies on MSC biology suggested also that the expression of immune-related molecules by MSC is unaffected by the application of a differentiative protocol [87–89], with the key exception of chondrogenic differentiation [90]. While such data are currently available only for HLA (both type I and II molecules) expression in MSC, and in one case for the expression of B7 co-stimulators by differentiated cells, researchers should be encouraged to evaluate, when performing a IPC differentiation protocol, the presence of immunomodulatory molecules in the differentiated progeny both in vitro and in vivo. In this perspective, the use of WJ-MSC would be favoured on account of their expression in the naïve state of a number of molecules which can promote T-cell tolerance and anergy, as demonstrated by different groups for the expression of HLA-G. The use of a "natural" source of immunomodulatory molecules acting at the very early stages of the pathogenetic process could overcome the heavy side effects of current immunosuppressant therapeutic strategies, perhaps favouring the physiological repopulation of the organ. Since inflammation and innate immunity are viewed now as potential initiators of the immune attack against beta cells following the developmet of insulitis [163], the production of anti-inflammatory molecules by transplanted MSC could attenuate local inflammation and contribute positively to the restoration of a physiological microenvironment. Current views offer a contrasting panorama, with some authors hypothesizing the possible differentiation of beta cells starting from resident or migrating progenitors, and other reports highlighting beta cell replication as the sole mechanism of islet repopulation and maintenance by means of the slow differentiation of resident progenitors [163, 164]. In each case, a differentiated IPC, which should also positively interact with the host immune system, could provide an immediate advantage with the restoration of euglycaemia and the presence of a higher number of functioning beta cells, which should start a virtuous circuit resulting in beta cell repopulation.

Recent data from multiple groups has shown that MSC administration should not necessarily be considered an alternative to immunosuppressant therapy, since very low doses of drug, while not altering the differentiative potential of co-administered MSC, could provide key protection to the limited generation of an allogeneic response by the recipient immune system [84]. This synergistic action would, hopefully, not generate the same heavy side effect as standard immunosuppressive therapy alone. In our opinion, this prospect constitutes further motivation for researchers to investigate the therapeutic options, which may provide a key benefit for the presumptive longer lasting of engrafted MSC which should potentiate their action in vivo.

Conclusions and Future Perspectives

The data illustrated here, obtained from different research groups, consistently point out the benefits of differentiated MSC for therapeutic applications in noninsulin therapies for type I diabetic patients. The promising features of these cells, i.e. their multipotency which makes them able to easily cross lineage borders, and a strong ability to interact with the immune system cells and processes, can be effectively used, either singly or as a powerful combination. This would allow to achieve an immediate result (e.g. restoration of euglycaemia in patients) as well as favour the blockade of inflammatory and immune processes acting in the islets microenvironment, thus indirectly promoting the slow and physiological replacement of beta cells starting from the proliferation of local precursors. With these objectives in mind, researchers should pursue with determination the road of characterization of the immune

features of both undifferentiated and differentiated MSC and the ways they interact with immune cells. Perhaps in the run to obtain "perfectly differentiated" beta cells we will achieve another, more interesting goal for the patient: even if differentiated cells secrete little insulin, no matter; terminal differentiation will result from local signals derived from the in vivo transfer in the organ while, on the other hand, immune-modulating MSC can exert indirect actions which should render them able, in the long term, to restore islet functions by favouring the existing reparative mechanisms. The latter will be restored if MSC maintain their promise to effectively dampen inflammatory and autoimmune processes acting in the diseased organ.

Acknowledgements We thank Rosemary Allpress for her revision of the English language. This work was partly supported by University of Palermo grants (ex 60% 2007) to RA, FF, GLR. The authors acknowledge the support of the Foundation S. Maugeri (ricerca corrente).

Disclosure The authors declare no potential conflicts of interest.

References

1. Friedenstein, A. J., Gorskaya, J. F., & Kulagina, N. N. (1976). Fibroblast precursors in normal and irradiated mouse hematopoietic organs. *Experimental Hematology, 4*, 267–274.
2. Caplan, A. I. (1991). Mesenchymal stem cells. *Journal of Orthopaedic Research, 9*, 641–650.
3. Horwitz, E. M., Le Blanc, K., Dominici, M., et al. (2005). Clarification of the nomenclature for MSC: the international society for cellular therapy position statement. *Cytotherapy, 7*, 393–395.
4. Secco, M., Moreira, Y. B., Zucconi, E., et al. (2009). Gene expression profile of mesenchymal stem cells from paired umbilical cord units: cord is different from blood. *Stem Cell Reviews and Reports, 5*, 387–401.
5. Anzalone, R., Lo Iacono, M., Corrao, S., et al. (2010). New emerging potentials for human Wharton's jelly mesenchymal stem cells: immunological features and hepatocyte-like differentiative capacity. *Stem Cells and Development, 19*, 423–438.
6. Hung, S. C., Chen, N. J., Hsieh, S. L., Ma, H. L., & Lo, W. H. (2002). Isolation and characterization of size-sieved stem cells from human bone marrow. *Stem Cells, 20*, 249–258.
7. Delorme, B., Ringe, J., Gallay, N., et al. (2008). Specific plasma membrane protein phenotype of cultureamplified and native human bone marrow mesenchymal stem cells. *Blood, 111*, 2631–2635.
8. Reger, R. L., Tucker, A. H., & Wolfe, M. R. (2008). Differentiation and characterization of human MSCs. *Methods in Molecular Biology, 449*, 93–107.
9. Turnovcova, K., Ruzickova, K., Vanecek, V., Sykova, E., & Jendelova, P. (2009). Properties and growth of human bone marrow mesenchymal stromal cells cultivated in different media. *Cytotherapy, 11*, 874–885.
10. Weiss, M. L., Medicetty, S., Bledsoe, A. R., et al. (2006). Human umbilical cord matrix stem cells: preliminary characterization and effect of transplantation in a rodent model of Parkinson's disease. *Stem Cells, 24*, 781–792.
11. Rao, M. S., & Mattson, M. P. (2001). Stem cells and aging: expanding the possibilities. *Mechanisms of Ageing and Development, 122*, 713–734.
12. Romanov, Y. A., Svintsitskaya, V. A., & Smirnov, V. N. (2003). Searching for alternative sources of postnatal human mesenchymal stem cells: candidate MSC-like from umbilical cord. *Stem Cells, 21*, 105–110.
13. La Rocca, G., Anzalone, R., Corrao, S., et al. (2009). Isolation and characterization of Oct-4+/HLA-G+ mesenchymal stem cells from human umbilical cord matrix: differentiation potential and detection of new markers. *Histochemistry and Cell Biology, 131*, 267–282.
14. Majore, I., Moretti, P., Stahl, F., Hass, R., & Kasper, C. (2010). Growth and differentiation properties of mesenchymal stromal cell populations derived from whole human umbilical cord. *Stem Cell Reviews and Reports*. doi:10.1007/s12015-010-9165-y.
15. Fong, C. Y., Chak, L. L., Biswas, A., et al. (2010). Human Wharton's jelly stem cells have unique transcriptome profiles compared to human embryonic stem cells and other mesenchymal stem cells. *Stem Cell Reviews and Reports*. doi:10.1007/s12015-010-9166-x.
16. La Rocca, G., Anzalone, R., & Farina, F. (2009). The expression of CD68 in human umbilical cord mesenchymal stem cells: new evidences of presence in non-myeloid cell types. *Scandinavian Journal of Immunology, 70*, 161–162.
17. Weiss, M. L., Anderson, C., Medicetty, S., et al. (2008). Immune properties of human umbilical cord Wharton's jelly-derived cells. *Stem Cells, 26*, 2865–2874.
18. Vija, L., Fargec, D., Gautier, J. F., et al. (2009). Mesenchymal stem cells: stem cell therapy perspectives for type 1 diabetes. *Diabetes & Metabolism, 35*, 85–93.
19. Di Nicola, M., Carlo-Stella, C., Magni, M., et al. (2002). Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. *Blood, 99*, 3838–3843.
20. Li, D. S., Warnock, G. L., Tu, H. J., et al. (2009). Do immunotherapy and β cell replacement play a sinergistic role in the treatment of type 1 diabetes? *Life Sciences, 85*, 549–556.
21. Nichols, J., & Cooke, A. (2009). Overcoming self-destruction in pancreas. *Current Opinion in Biotechnology, 20*, 511–515.
22. Alma, J., Nauta, W., & Fibbe, E. (2007). Immunomodulatory properties of mesenchymal stromal cells. *Blood, 110*, 3499–3506.
23. Selmani, Z., Naji, A., Gaiffe, E., et al. (2009). HLA-G is a crucial immunosuppressive molecule secreted by adult human mesenchymal stem cells. *Transplantation, 87*(9 Suppl.), S62–S66.
24. Le Bouteiller, P. (2000). HLA-G in the human placenta: expression and potential functions. *Biochemical Society Transactions, 28*, 208–212.
25. Ren, G., Zhao, X., Zhang, L., et al. (2010). Inflammatory cytokine-induced intercellular adhesion molecule-1 and vascular cella adhesion molecule-1 in mesenchymal stem cells are critical for immunosuppression. *Journal of Immunology, 184*, 2321–2328.
26. Krampera, M., Glennie, S., Dyson, J., et al. (2003). Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. *Blood, 101*, 3722–3729.
27. Djouad, F., Plence, P., Bony, C., et al. (2003). Immunosuppressive effect of mesenchymal stem cells favors tumour growth in allogeneic animals. *Blood, 102*, 3837–3844.
28. Aggarwal, S., & Pittenger, M. F. (2005). Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood, 105*, 1815–1818.
29. Corcione, A., Benvenuto, F., Ferretti, E., et al. (2006). Human mesenchymal stem cells modulate B-cell functions. *Blood, 107*, 367–372.
30. Németh, K., Leelahavanichkul, A., Yuen, P. S. T., et al. (2009). Bone marroew stromal cells attenuate sepsis via prostaglandin

E2-dependent reprogramming of host macrophages to increase their interleukin-10 production. *Nature Medicine, 15*, 42–49.
31. Mei, S. H., Haitsma, J. J., Dos Santos, C. C., et al. (2010). Mesenchymal stem cells reduce inflammation while enhancing bacterial clearance and improving survival in sepsis. *American Journal of Respiratory and Critical Care Medicine*. doi:10.1164/rccm.201001-0010OC.
32. Barbagallo, I., Tibullo, D., Di Rosa, M., et al. (2008). A cytoprotective role for the heme oxygenase-1/CO pathway during neural differentiation of human mesenchymal stem cells. *Journal of Neuroscience Research, 86*, 1927–1935.
33. Jiang, Y., Chen, L., Tang, Y., et al. (2010). HO-1 gene overexpression enhances the beneficial effects of superparamagnetic iron oxide labeled bone marrow stromal cells transplantation in swine hearts underwent ischemia/reperfusion: a MRI study. *Basic Research in Cardiology, 105*, 431–442.
34. Tu, Z., Li, Q., Bu, H., & Lin, F. (2010). Mesenchymal stem cells inhibit complement activation by secreting factor H. *Stem Cells and Development*. doi:10.1089/scd.2009.0418.
35. Sato, K., Ozaki, K., Oh, I., et al. (2007). Nitric oxide plays a crucial role in suppression of T-cell proliferation by mesenchymal stem cells. *Blood, 109*, 228–234.
36. Ren, G., Zhang, L., Zhao, X., et al. (2008). Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. *Cell Stem Cell, 2*, 141–150.
37. Yoo, K. H., Jang, I. K., Lee, M. W., et al. (2009). Comparison of immunomodulatory properties of mesenchymal stem cells derived from adult human tissues. *Cellular Immunology, 259*, 150–156.
38. Troyer, D. L., & Weiss, M. L. (2007). Concise review: Wharton's jelly-derived cells are a primitive stromal cell population. *Stem Cells, 26*, 591–599.
39. Wang, H. S., Hung, S. C., Peng, S. T., et al. (2004). Mesenchymal stem cells in the Wharton's jelly of the human umbilical cord. *Stem Cells, 22*, 1330–1337.
40. Götherstrom, C., West, A., Liden, J., Uzunel, M., Lahesmaa, R., & Le Blanc, K. (2005). Difference in gene expression between human fetal liver and adult bone marrow mesenchymal stem cells. *Haematologica, 90*, 1017–1026.
41. Selmani, Z., Naji, A., Zidi, I., Favier, B., et al. (2008). Human leukocyte antigen-G5 secretion by human mesenchymal stem cells is required to suppress T lymphocyte and natural killer function and to induce CD4+CD25highFOXP3+ regulatory T cells. *Stem Cells, 26*, 212–222.
42. Deans, R. J., & Moseley, A. B. (2000). Mesenchymal stem cells: biology and potential clinical uses. *Experimental Hematology, 28*, 875–884.
43. Xu, W., Zhang, X., Qian, H., et al. (2004). Mesenchymal stem cells from adult human bone marrow differentiate into a cardiomyocyte phenotype in vitro. *Experimental Biology and Medicine, 229*, 623–631.
44. Sheng, H., Wang, Y., Jin, Y., et al. (2008). A critical role of IFNγ in priming MSC-mediated suppression of T cell proliferation through up-regulation of B7-H1. *Cell Research, 18*, 846–857.
45. Xue, Q., Luan, X. Y., Gu, Y. Z., et al. (2010). The negative co-signaling molecule b7-h4 is expressed by human bone marrow-derived mesenchymal stem cells and mediates its T-cell modulatory activity. *Stem Cells and Development, 19*, 27–38.
46. Friedman, R., Betancur, M., Boissel, L., Tuncer, H., Cetrulo, C., & Klingemann, H. (2007). Umbilical cord mesenchymal stem cells: adjuvants for human cell transplantation. *Biology of Blood and Marrow Transplantation, 13*, 1477–1486.
47. Guo, Z., Zheng, C., Chen, Z., et al. (2009). Fetal BM-derived mesenchymal stem cells promote the expansion of human Th17 cells, but inhibit the production of Th1 cells. *European Journal of Immunology, 39*, 2840–2849.
48. Prasanna, S. J., Gopalakrishnan, D., Shankar, S. R., & Vasandan, A. B. (2010). Pro-inflammatory cytokines, IFNgamma and TNFalpha, influence immune properties of human bone marrow and Wharton jelly mesenchymal stem cells differentially. *PLoS One, 5*, e9016.
49. Sotiropoulou, P. A., Perez, S. A., Gritzapis, A. D., Baxevaxis, C. N., & Papamichail, M. (2006). Interactions between human mesenchymal stem cells and natural killer cells. *Stem Cells, 24*, 74–85.
50. Zanone, M. M., Favaro, E., Miceli, I., et al. (2010). Human mesenchymal stem cells modulate cellular immune responses to islet antigen glutamic acid decarboxylase in type 1 diabetes. *Journal of Clinical Endocrinology and Metabolism, 95*, 3788–3797.
51. Chen, K., Wang, D., Du, W. T., et al. (2010). Human umbilical cord mesenchymal stem cells hUC-MSCs exert immunosuppressive activities through a PGE2-dependent mechanism. *Clinical Immunology, 135*, 448–456.
52. Lee, R. H., Pulin, A. A., Seo, M. J., et al. (2009). Intravenous hMSCs improve myocardial infarction in mice because cells embolie in lung are activated to secrete the anti-inflammatory protein TSG-6. *Cell Stem Cell, 5*, 54–63.
53. Kadri, T., Lataillade, J. J., Doucet, C., et al. (2005). Proteomic study of galectin-1 expression in human mesenchymal stem cells. *Stem Cells and Development, 14*, 204–212.
54. Sioud, M., Mobergslien, A., Boudabous, A., & Fløisand, Y. (2010). Evidence for the involvement of galectin-3 in esenchymal stem cell suppression of allogeneic T-cell proliferation. *Scandinavian Journal of Immunology, 71*, 267–274.
55. Griffin, M. D., Ritter, T., & Mahon, B. P. (2010). Immunological aspects of allogeneic mesenchymal stem cell therapies. *Human Gene Therapy*. doi:10.1089/hum.2010.156.
56. Hunt, J. S., Petroff, M. G., McIntire, R. H., & Ober, C. (2005). HLA-G and immune tolerance in pregnancy. *The FASEB Journal, 19*, 681–693.
57. Corrao, S., Campanella, C., Anzalone, R., et al. (2010). Human Hsp10 and Early Pregnancy Factor (EPF) and their relationship and involvement in cancer and immunity: current knowledge and perspectives. *Life Sciences, 86*, 145–152.
58. Anzalone, R., La Rocca, G., Di Stefano, A., et al. (2009). Role of endothelial cell stress in the pathogenesis of chronic heart failure. *Frontiers in Bioscience, 14*, 2238–2247.
59. La Rocca, G., Di Stefano, A., Eleuteri, E., et al. (2009). Oxidative stress induces myeloperoxidase expression in endocardial endothelial cells from patients with chronic heart failure. *Basic Research in Cardiology, 104*, 307–320.
60. Eleuteri, E., Magno, F., Gnemmi, I., et al. (2009). Role of oxidative and nitro sative stress biomarkers in chronic heart failure. *Frontiers in Biosciences, 14*, 2230–2237.
61. Eleuteri, E., Di Stefano, A., Ricciardolo, F. L., et al. (2009). Increased nitrotyrosine plasma levels in relation to systemic markers of inflammation and myeloperoxidase in chronic heart failure. *International Journal of Cardiology, 135*, 386–390.
62. Dìaz-Lagares, A., Alegre, E., LeMaoult, J., Carosella, E. D., & Gonzalez, A. (2009). Nitric oxide produces HLA-G nitration and induces metalloprotease-dependent shedding creating a tolerogenic milieu. *Immunology, 126*, 436–445.
63. Gonzalez, M. A., Gonzalez-Rey, E., Rico, L., Büscher, D., & Delgado, M. (2009). Treatment of experimental arthritis by inducing immune tolerance with human adipose-derived mesenchymal stem cells. *Arthritis & Rheumatism, 60*, 1006–1019.
64. Madec, A. M., Mallone, G., Alfonso, E., et al. (2009). Mesenchymal stem cells protect NOD mice from diabetes by inducing regulatory T cells. *Diabetologia, 52*, 1391–1399.
65. Zhao, Y., Lin, B., Darflinger, R., Zhang, Y., Holterman, M. J., & Skidgel, R. A. (2009). Human cord blood stem cell-

modulated regulatory T lymphocytes reverse the autoimmune-caused type 1 diabetes in nonobese diabetic (NOD) mice. *PloS ONE, 4*, e4226.
66. Sundin, M., Ringdén, O., Sundberg, B., Nava, S., Götherström, C., & Le Blanc, K. (2007). No alloantibodies against mesenchymal stem cells, but presence of anti-fetal calf serum antibodies, after transplantation in allogeneic hematopoietic stem cell recipients. *Haematologica, 92*, 1208–1215.
67. Rasmusson, I., Uhlin, M., Le Blanc, K., & Levitski, V. (2007). Mesenchymal stem cells fail to trigger effector functions of cytotoxic T lymphocytes. *Journal of Leukocyte Biology, 82*, 887–893.
68. Morandi, F., Raffaghello, L., Bianchi, G., et al. (2008). Immunogenicity of human mesenchymal stem cells in HLA-class I-restricted T-cell responses against viral or tumor-associated antigens. *Stem Cells, 26*, 1275–1287.
69. Shabbir, A., Zisa, D., Leiker, M., Johnston, C., Lin, H., & Lee, T. (2009). Muscular dystrophy therapy by non-autologous mesenchymal stem cells: muscle regeneration without immunosuppression and inflammation. *Transplantation, 87*, 1275–1282.
70. Quevedo, H. C., Hatzistergos, K., Oskouei, B. N., et al. (2009). Allogeneic mesenchymal stem cells restore cardiac function in chronic ischemic cardiomiopathy via trilineage differentiating capacity. *PNAS USA, 106*, 14022–14027.
71. Chen, L., Tredget, E. E., Liu, C., & Wu, Y. (2009). Analysis of allogenicity of mesenchymal stem cells in engraftment and wound healing in mice. *PloS ONE, 4*, e7119.
72. Eliopoulos, N., Stagg, J., Lejeune, L., Pommey, S., & Galipeau, J. (2005). Allogeneic marrow stromal cells are immune rejected by MHC class I- and class II-mismatched recipient mice. *Blood, 106*, 4057–4065.
73. Nauta, A. J., Westerhuis, G., Kruisselbrink, A. B., Lurvink, E. G. A., Willemze, R., & Fibbe, W. E. (2006). Donor-derived mesenchymal stem cells are immunogenic in an allogeneic host and stimulate donor graft rejection in a nonmyeloablative setting. *Blood, 108*, 2114–2120.
74. Prigozhina, T. B., Khitrin, S., Elkin, G., Eizik, O., Morecki, S., & Slavin, S. (2008). Mesenchymal stromal cells lose their immunosuppressive potential after allotransplantation. *Experimental Hematology, 36*, 1370–1376.
75. Chiavegato, A., Bollini, S., Pozzobon, M., et al. (2007). Human amniotic Xuid-derived stem cells are rejected after transplantation in the myocardium of normal, ischemic, immunosuppressed or immuno-deficient rat. *Journal of Molecular and Cellular Cardiology, 42*, 746–759.
76. Ren, G., Su, J., Zhang, L., et al. (2009). Species variation in the mechanisms of mesenchymal stem cell-mediated immunosuppression. *Stem Cells, 27*, 1954–1962.
77. Sudres, M., Norol, F., Trenado, A., et al. (2006). Bone marrow mesenchymal stem cells suppress lymphocyte proliferation in vitro but fail to prevent graft-versus-host disease in mice. *Journal of Immunology, 176*, 7761–7767.
78. Iyer, S. S., & Rojas, M. (2008). Anti-inflammatory effects of mesenchymal stem cells: novel concept for future therapies. *Expert Opinion in Biological Therapy, 8*, 569–581.
79. Ringdén, O., Uzunel, M., Rasmusson, I., et al. (2006). Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease. *Transplantation, 81*, 1390–1397.
80. Zhang, X., Jiao, C., & Zhao, S. (2009). Role of mesenchymal stem cells in immunological rejection of organ transplantation. *Stem Cell Reviews and Reports, 5*, 402–409.
81. Ito, T., Itakura, S., Rawson, J., et al. (2010). Mesenchymal stem cell and islet co-transplantation promotes graft revascularizatioon and function. *Transplantation, 89*, 1438–1445.
82. Li, F. R., Wang, X. G., Deng, C. Y., Qi, H., Reb, L. L., & Zhou, H. X. (2010). Immune modulation of co-transplantation mesenchymal stem cells with islet on T and dendritic cells. *Clinical and Experimental Immunology, 161*, 357–363.
83. Longoni, B., Szilagyi, E., Quaranta, P., et al. (2010). Mesenchymal stem cells prevent acute rejection and prolong graft function in pancreatic islet transplantation. *Diabetes Technology and Therapy, 12*, 435–446.
84. Hoogduijn, M. J., Popp, F., Verbeek, R., et al. (2010). The immunotherapy properties of mesenchymal stem cells and their use for immunotherapy. *International Immunopharmacology*. doi:10.1016/j.intimp.2010.06.019.
85. Popp, F. C., Eggenhofer, E., Renner, P., et al. (2008). Mesenchymal stem cells can induce long-term acceptance of solid organ allografts in synergy with low-dose mycophenolate. *Transplant Immunology, 20*, 55–60.
86. Ge, W., Jiang, J., Baroja, M. L., et al. (2009). Infusion of mesenchymal stem cells and rapamycin synergize to attenuate alloimmune responses and promote cardiac allograft tolerance. *American Journal of Transplantation, 9*, 1760–1772.
87. Le Blanc, K., Tammik, C., Rosendahl, K., Zettenberg, E., & Ringdén, O. (2003). HLA expression and immunogenic properties of differentiated and undifferentiated mesenchymal stem cells. *Experimental Hematology, 31*, 890–896.
88. Liu, H., Kemeny, D. M., Heng, B. C., Ouyang, H. W., Melendez, A. J., & Cao, T. (2006). The immunogenic and immunomodulatory function of osteogenic cells differentiated from mesenchymal stem cells. *The Journal of Immunology, 176*, 2864–2871.
89. Zheng, Z. H., Li, X. Y., Ding, J., Jia, J. F., & Zhu, P. (2008). Allogeneic mesenchymal stem cell and mesenchymal stem cell-differentiated chondrocyte suppress the responses of type II collagen-reactive T cells in rheumatoid arthritis. *Rheumatology, 47*, 22–30.
90. Chen, X., McClurg, A., Zhou, G. Q., McCaigue, M., Armstrong, M. A., & Li, G. (2007). Chondrogenic differentiation alters the immunosuppressive property of bone marrow-derived mesenchymal stem cells, and the effect is partially due to the upregulated expression of B7 molecules. *Stem Cells, 25*, 364–370.
91. Pittenger, M. (2009). Sleuthing the source of regeneration by MSCs. *Cell Stem Cell, 5*, 8–10.
92. Kemp, K., Gray, E., Mallam, E., Scolding, N., & Wilkins, A. (2010). Inflammatory cytokine induced regulation of superoxide dismutase 3 expression by human mesenchymal stem cells. *Stem Cell Reviews and Reports*. doi:10.1007/s12015-010-9178-6.
93. Liao, W., Zhong, J., Yu, J., et al. (2009). Therapeutic benefit of human umbilical cord derived mesenchymal stromal cells in intracerebral hemorrage rat: implication for anti-inflammation and angiogenesis. *Cellular Physiology and Biochemistry., 24*, 307–316.
94. Carreras, A., Almendros, I., Montserrat, J. M., Navajas, D., & Farré, R. (2010). Mesenchymal stem cells reduce inflammation in a rat model of obstructive sleep apnea. *Respiratory Physiology & Neurobiology, 172*, 210–212.
95. Cao, H., Quian, H., Xu, W., et al. (2010). Mesenchymal stem cells derived from human umbilical cord ameliorate ischemia/reperfusion-induced acute renal failure in rats. *Biotechnology Letters, 32*, 725–732.
96. Tsai, P. C., Fu, T. W., Chen, Y. M. A., et al. (2009). The therapeutic potential of human umbilical mesenchymal stem cells from Wharton's jelly in the treatment of rat liver fibrosis. *Liver Transplantation, 15*, 484–495.
97. Gonzalez, M. A., Gonzalez-Rey, E., Rico, L., Büscher, D., & Delgado, M. (2009). Adipose-derived mesenchymal stem cells alleviate experimental colitis by inhibiting inflammatory and autoimmune responses. *Gastroenterology, 136*, 978–989.
98. Bonfield, T. L., Koloze, M. F., Lennon, D. P., Zuchowski, B., Yang, S. E., & Caplan, A. L. (2010). Human mesenchymal stem

cells suppress chronic airway inflammation in the murine ovalbumin asthma model. *American Journal of Physiology Lung Cellular and Molecular Physiology.* doi:10.1152/ajplung.00182.2009.
99. Ishikane, S., Yamahara, K., Sada, M., et al. (2010). Allogeneic administration of fetal membrane-derived mesenchymal stem cells attenuates acute myocarditis in rats. *Journal of Molecular and Cellular Cardiology.* doi:10.1016/j.yjmcc.2010.07.019.
100. Kim, Y. S., Park, H. J., Hong, M. H., et al. (2009). TNF-alpha enhances engraftment of mesenchymal stem cells into infarcted myocardium. *Frontiers in Bioscience, 14*, 2845–2856.
101. Ende, N., Chen, R., & Reddi, A. S. (2004). Effect of human umbilical cord blood cells on glycemia and insulitis in type 1 diabetic mice. *Biochemical and Biophysical Research Communications, 325*, 665–669.
102. Wehling, N., Palmer, G. D., Pilapil, C., et al. (2009). Interleukin-1β and tumor necrosis factor α inhibit chondrogenesis by human mesenchymal stem cells through NF-κB-dependent pathways. *Arthritis & Rheumatism, 60*, 801–812.
103. Weir, G. C., & Bonner-Weir, S. (2010). Dreams for type 1 diabetes: shutting off autoimmunity and stimulating beta-cell regeneration. *Endocrinology, 151*, 2971–2973.
104. Dor, Y., & Stanger, B. Z. (2007). Regeneration in liver and pancreas: time to cut the umbilical cord? *Science STKE, 414*, pe66.
105. Eberhard, D., Lammert, E. (2009). The pancreatic β-cell in the islet and organ community. *Current Opinion in Genetics & Development, 19*, 469–475.
106. Sun, B., Roh, K. H., Lee, S. R., Lee, Y. S., Kang, K. S. (2007). Induction of human umbilical cord blood-derived stem cells with embryonic stem cell phenotypes into insulin producing islet- like structure. *Biochemical and Biophysical Research Communications, 354*, 919–923.
107. Wu, L. F., Wang, N. N., Liu, Y. S., Wei, X. (2009). Differentiation of Wharton's Jelly primitive stromal cells into insulin-producing cells in comparison with bone marrow mesenchymal stem cells. *Tissue Engineering Part A, 15*, 2865–2873.
108. MacDonald, M. R., Petrie, M. C., Hawkins, N. M., et al. (2008). Diabetes, left ventricular systolic dysfunction, and chronic heart failure. *European Heart Journal, 29*, 1224–1240.
109. Scrutinio, D., Giannuzzi, P. (2008). Comorbidity in patients undergoing coronary artery bypass graft surgery: impact on outcome and implications for cardiac rehabilitation. *European Journal of Cardiovascular Prevention and Rehabilitation, 15*, 379–385.
110. Palma, C. A., Lindeman, R., Tuch, B. E. (2008). Blood into β-cells: can adult stem cells be used as a therapy for type 1 diabetes? *Regenerative Medicine, 3*, 1–15.
111. Sahu, S., Tosh, D., Hardikar, A. A. (2009). New sources of β-cells for treating diabetes. *Journal of Endocrinology, 202*, 13–16.
112. Aguayo-Mazzuccato, C., & Bonner-Weir, S. (2010). Stem cell therapy for type 1 diabetes mellitus. *Nature Reviews Endocrinology, 6*, 139–148.
113. Fadini, G. P., Sartore, S., Schiavon, M., et al. (2006). Diabetes impairs progenitor cell mobilization after hindlimb ischaemia-reperfusion injury in rats. *Diabetologia, 49*, 3075–3084.
114. Gallagher, K. A., Liu, Z. J., Xiao, M., et al. (2007). Diabetic impaiorments in NO-mediated endothelial progenitor cell mobilization and homing are reversed by hyperoxia and SDF-1 alpha. *Journal of Clinical Investigation, 117*, 1249–1259.
115. McKnight, K. D., & Kim, S. K. (2010). Deconstructing pancreas development to reconstruct human islets from pluripotent stem cells. *Cell Stem Cell, 6*, 300–308.
116. Puri, S., & Hebrok, M. (2010). Cellular plasticity within the pancreas—lessons learned from development. *Developmental Cell, 18*, 342–356.
117. Guney, M. A., & Gannon, M. (2009). Pancreas cell fate. *Birth Defects Research (Part C), 87*, 232–248.
118. McDonald, E., Krishnamurthy, M., Goodyer, C. G., & Wang, R. (2009). The emerging roles of SOX transcription factors in pancreatic endocrine cell development and function. *Stem Cells and Development, 18*, 1379–1387.
119. Shao, S., Fang, Z., Yu, X., & Zhang, M. (2009). Transcription factors involved in glucose-stimulated insulin secretion of pancreatic beta cells. *Biochemical and Biophysical Research Communications, 384*, 401–404.
120. Burlison, J. S., Long, Q., Fujitani, Y., et al. (2008). Pdx-1 and Ptf1a concurrently determine the fate specification of pancreatic multipotent progenitor cells. *Developmental Biology, 316*, 74–86.
121. Kawaguchi, Y., Cooper, B., Gannon, M., et al. (2002). The role of the transcriptional regulator Ptf1a in converting intestinal to pancreatic progenitors. *Nature Genetics, 32*, 128–134.
122. Sellick, G. S., Barker, K. T., Stolte-Dijkstra, I., et al. (2004). Mutations in Ptf1a cause pancreatic and cerebellar agenesis. *Nature Genetics, 36*, 1301–1305.
123. Gradwohl, G., Dierich, A., LeMeur, M., & Guillemot, F. (2000). Neurogenin3 is required for the development of the four endocrine cell lineages of the pancreas. *Proceedings of the National Academy of Sciences USA, 97*, 1607–1611.
124. Sussel, L., Kalamaras, J., Hartingan-O'Connor, D. J., et al. (1998). Mice lacking the homeodomain transcription factor Nkx2.2 have diabetes due to arrested differentiation of pancreatic beta cells. *Development, 125*, 2213–2221.
125. Servitja, J. M., & Ferrer, J. (2004). Transcriptional networks controlling pancreatic development and beta cell function. *Diabetologia, 47*, 597–613.
126. Sulzbacher, S., Schroeder, I. S., Truong, T. T., & Wobus, A. M. (2009). Activin A-induced differentiation of embryonic stem cells into endoderm and pancreatic progenitors-the influence of differentiation factors and culture conditions. *Stem Cell Reviews, 5*, 159–173.
127. Soria, B., Roche, E., Berna, G., et al. (2000). Insulin secreting cells derived from embryonic stem cells normalize glycemia in streptozotocin-induced diabetic mice. *Diabetes, 49*, 157–162.
128. Lumelsky, N., Blondel, O., Laeng, P., et al. (2001). Differentiation of embryonic stem cells to insulin secreting structures similar to pancreatic islets. *Science, 292*, 1389–1394.
129. Sipione, S., Eshpeter, A., Lyon, J. G., Korbutt, G. S., & Bleackley, R. C. (2004). Insulin expressing cells from differentiated embryonic stem cells are not beta cells. *Diabetologia, 47*, 499–508.
130. Furth, M. E., & Atala, A. (2009). Stem cell sources to treat diabetes. *Journal of Cellular Biochemistry, 106*, 507–511.
131. Rolletscheck, A., Kania, G., & Wobus, A. M. (2006). Generation of pancreatic insulin-producing cells from embryonic stem cells—"proof of principle", but questions still unanswered. *Diabetologia, 49*, 2541–2545.
132. Chakrabarti, S. K., & Mirminra, R. G. (2003). Transcription factors direct the development and function of pancreatic β-cells. *Trends in Endocrinology and Metabolism, 14*, 78–84.
133. Chen, L. B., Jiang, X. B., & Yang, L. (2004). Differentiation of rat marrow mesenchymal stem cells into pancreatic islet beta-cells. *World Journal of Gastroenterology, 10*, 3016–3020.
134. Wu, X. H., Liu, C. P., Xu, K. F., et al. (2007). Reversal of hyperglycemia in diabetic rats by portal vein transplantation of islet-like cells generated from bone marrow mesenchymal stem cells. *World Journal of Gastroenterology, 13*, 3342–3349.
135. Oh, S. H., Muzzonigro, T. M., Bae, S. H., LaPlante, J. M., Hatch, H. M., & Petersen, B. E. (2004). Adult bone marrow-derived cells trans-differentiating into insulin-producing cells for treatment of type I diabetes. *Laboratory Investigation, 84*, 607–617.
136. Paz, A. H., Salton, G. D., Ayala-Lugo, A., et al. (2010). Betacellulin overexpression in mesenchymal stem cells induces

insulin secretion in vitro and ameliorates streptozocin-induced hyperglycemia in rats. *Stem Cells and Development*. doi:10.1089/scd.2009.0490.
137. Moriscot, C., De Fraipont, F., Richard, M. J., et al. (2005). Human bone marrow mesenchymal stem cells can express insulin and key transcription factors of the endocrine pancreas developmental pathway upon genetic and/or microenvironmental manipulation in vitro. *Stem Cells, 23*, 594–604.
138. Li, Y., Zhang, R., Qiao, H., et al. (2007). Generation of insulin-producing cells from PDX-1 gene-modified human mesenchymal stem cells. *Journal of Cellular Physiology, 211*, 36–44.
139. Lee, R. H., Seo, M. J., Reger, R. L., et al. (2006). Multipotent stromal from human marrow home to and promote repair of pancreatic islets and renal glomeruli in diabetic NOD/scid mice. *Proceedings of the National Academy of Sciences USA, 103*, 17438–17443.
140. Sun, Y., Chen, L., Hou, X. G., et al. (2007). Differentiation of bone marrow-derived mesenchymal stem cells from diabetic patients into insulin-producing cells in vitro. *Chinese Medical Journal, 120*, 771–776.
141. Karnieli, O., Izhar-Prato, Y., Bulvik, S., & Efrat, S. (2007). Generation of insulin-producing cells from human bone marrow mesenchymal stem cells by genetic manipulation. *Stem Cells, 25*, 2837–2844.
142. Xie, Q. P., Huang, H., Xu, B., et al. (2009). Human bone marrow mesenchymal stem cells differentiate into insulin-producing cells upon microenvironmental manipulation in vitro. *Differentiation, 77*, 483–491.
143. Zuk, P. A., Zhu, M., Ashjian, P., et al. (2002). Human adipose tissue is a source of multipotent stem cells. *Molecular Biology of the Cell, 13*, 4279–4295.
144. Timper, K., Seboek, D., Eberhardt, M., et al. (2006). Human adipose tissue-derived mesenchymal stem cells differentiate into insulin, somatostatin, and glucagon expressing cells. *Biochemical and Biophysical Research Communications, 341*, 1135–1140.
145. Trivedi, H. L., Vanikar, A. V., Thakker, A., et al. (2008). Human adipose tissue-derived mesenchymal stem cells combined with hematopoietic stem cell transplantatio synthesize insulin. *Transplantation Proceedings, 40*, 1135–1139.
146. Pessina, A., Eletti, B., Croera, C., Savalli, N., Diodovich, C., & Gribaldo, L. (2004). Pancreas developing markers expressed on human mononuleated umbilical cord blood cells. *Biochemical and Biophysical Research Communications, 323*, 315–322.
147. Soria, B. (2001). In vitro differentiation of pancreatic beta-cells. *Differentiation, 68*, 205–219.
148. Ende, N., Chen, R., & Redid, A. S. (2004). Transplantation of human umbilical cord blood cells improves glycemia and glomerular hypertrophy in type 2 diabetic mice. *Biochemical and Biophysical Research Communications, 321*, 168–171.
149. Yoshida, S., Ishikawa, F., Kawano, N., et al. (2005). Human cord blood derived cells generate insulin producing cells in vivo. *Stem Cells, 23*, 1409–1416.
150. Denner, L., Bodenburg, Y., Zhao, J. G., et al. (2007). Directed engineering of umbilical cord blood stem cells to produce C-peptide and insulin. *Cellular Proliferation, 40*, 367–380.
151. Gao, F., Wu, D. Q., Hu, Y. H., et al. (2008). In vitro cultivation of islet-like cell clusters from human umbilical cord blood-derived mesenchymal stem cells. *Translational Research, 151*, 293–302.
152. Parekh, V. S., Joglekar, M. V., & Hardikar, A. A. (2009). Differentiation of human umbilical cord blood derived mononuclear cells to endocrine pancreatic lineage. *Differentiation, 78*, 232–240.
153. Sun, N. Z., & Ji, H. S. (2009). In vitro differentiation of human placenta-derived adherent cells into insulin-producing cells. *The Journal of International Medical Research, 37*, 400–406.
154. Parolini, O., Alviano, F., Bagnara, G. P., et al. (2008). Concise review: isolation and characterization of cells from human term placenta: outcome of the first international Workshop on Placenta Derived Stem Cells. *Stem Cells, 26*, 300–311.
155. Chao, K. C., Chao, K. F., Fu, Y. S., & Liu, S. H. (2008). Islet-like clusters derived mesenchymal stem cells in Wharton's jelly of the human Umbilical Cord for transplantation to control type 1 diabetes. *PLoS ONE, 3*, e1451.
156. Palmer, J. P. (2009). C-peptide in the natural history of type 1 diabetes. *Diabetes/Metabolism Research and Reviews, 25*, 325–328.
157. Wu, L. F., Wang, N. N., Liu, Y. S., & Wei, X. (2009). Differentiation of Wharton's Jelly primitive stromal cells into insulin-producing cells in comparison with bone marrow mesenchymal stem cells. *Tissue Engineering Part A, 15*, 2865–2873.
158. Wang, H. S., Shyu, J. F., Shen, W. S., et al. (2010). Transplantation of insulin producing cells derived from umbilical cord stromal mesenchymal stem cells to treat NOD mice. *Cell Transplantation*. doi:10.3727/096368910X522270.
159. Kaneto, H., Matsuoka, T., Kawashima, S., et al. (2009). Role of MafA in pancreatic β-cells. *Advanced Drug Delivery Reviews, 61*, 489–496.
160. Faustman, D. L., & Davis, M. (2009). The primacy of CD8 T lymphocytes in type 1 diabetes and implications for therapies. *Journal of Molecular Medicine, 87*, 1173–1178.
161. Abdi, R., Fiorina, P., Adra, C. N., Atkinson, M., & Sayegh, M. H. (2008). Immunomodulation by mesenchymal stem cells: a potential therapeutic strategy for type 1 diabetes. *Diabetes, 57* (7), 1759–1767.
162. Eizirik, D. L., Colli, M. L., & Ortiz, F. (2009). The role of inflammation in insulitis and β cell loss in type 1 diabetes. *Nature Reviews Endocrinology, 5*, 219–226.
163. Brennand, K., & Melton, D. (2009). Slow and steady is the key to β-cell replication. *Journal of Cellular and Molecular Medicine, 13*, 472–487.
164. Granger, A., & Kushner, J. A. (2009). Cellular origins of b-cell regeneration: a legacy view of historical controversies. *Journal of Internal Medicine, 266*, 325–338.