Review
Special Focus Issue: Immunological challenges & opportunities

Regenerative Medicine

Immunogenicity of allogeneic mesenchymal stromal cells: what has been seen *in vitro* and *in vivo*?

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Mesenchymal stromal cells (MSC) are promising candidates for supporting regeneration and suppressing undesired immune reactivity. Although autologous MSC have been most commonly used for clinical trials, data on application of allogeneic MHC-unmatched MSC were reported. The usage of MSC as an 'off-the-shelf' product would have several advantages; however, it is an immunological challenge. The preclinical studies on the (non)immunogenicity of MSC are contradictory and, unfortunately, solid data from clinical trials are missing. Induction of an alloresponse would be a major limitation for the application of allogeneic MSC. Here we discuss the key elements for the induction of an alloresponse and targets of immunomodulation by MSC as well as preclinical and clinical hints on allo(non)response to MSC.

Keywords: antigen-presenting cells • direct/indirect alloimmunity • immunogenicity • immunomodulation • major histocompatibility complex • mesenchymal stromal cells

The potential of mesenchymal stromal cells for regeneration & immunomodulation

Mesenchymal stromal cells (MSC) are a heterogeneous population of cells that can be isolated from different tissues like bone marrow, adipose tissue, umbilical cord or placenta. They are multipotent cells since they have been shown to differentiate *in vitro* into different cells of the mesodermal lineage, such as adipocytes, chondroblasts and osteoblasts [1]. Although this differentiation capacity was not clearly shown *in vivo* so far, MSC are well known for their capacity to directly (by stimulating tissue repair processes and vascularization) and indirectly (e.g., by immunomodulation) support endogenous tissue regeneration [2,3]. Therefore, they are of great interest for the treatment of a broad panel of diseases ranging from acute injuries (e.g., bone fracture, muscle trauma, myocardial infarction), over chronic ischemic tissue injuries (e.g., critical limb ischemia, diabetes-related complications), to immune-mediated pathogenic processes (e.g., graft-versus-host disease or autoimmune diseases) [4–7]. However, the best route of administration for MSC still is questionable. The cells remain at the injection site when administered locally, for example, intramuscularly, and their concentration decreases within 2–4 weeks [8,9]. In contrast, intravenous injection leads to large sequestration of cells in the lung within hours, while their systemic effects can be observed for weeks [10,11]. Some authors noted that MSC indeed can engraft and differentiate *in vivo* with poor efficiency [12,13], yet in human trials there is no evidence for this phenomenon so far. Therefore, MSC rather serve as a biological drug delivery system by secreting soluble factors. The preparation of autologous MSC is logistically and regulatory challenging and time consuming. Moreover, autologous MSC can display disease and age-related impairments [14,15]. Therefore, the use of 'off-the-shelf' MHC-unmatched MSC would be of great advantage, provided that they are not immunogenic by themselves. Induced alloimmunity would lead to several problems for allogeneic MSC therapies:

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- Faster elimination of allogeneic MSC by donor cell-specific T-effector cells and antibodies, resulting in accelerated loss of efficacy;
- Altered homing of allogeneic MSC opsonized by allospecific antibodies ± complement, resulting in faster loss of efficacy;
- Triggering local inflammation following MSC administration by allospecific T-effector cells, complement-mediated lysis or antibody-dependent cellular cytotoxicity leading to adverse effects.

Undesired effects should be even more accelerated after repetitive injections of allogeneic MSC and boosting of memory alloresponse.

Several preclinical and some clinical studies addressed the question of humoral and cellular alloimmunity to MSC and reported contradictory results. While most studies show that application of allogeneic MSC is safe [16,17], others present clear evidence that MSC indeed can provoke alloimmunity and even stimulate graft rejection [18–20]. Unfortunately, clinical studies using allogeneic MSC did not adequately address alloreactivity by state-of-the-art test systems like memory T-cell Elispot and luminex HLA-antibody screening platforms. Thinking about multiple administration of MSC in patients, their immunogenicity is of special interest and needs to be clarified in more detail. In particular, it is important to understand under which conditions MSC can become immunogenic.

Recognition of alloantigens by the immune system

Alloimmunity can be triggered by different pathways:

- I. At T-cell level:
  - 1. Direct pathway by recognition of donor-derived antigen-presenting cells (APC);
  - 2. Indirect pathway by recognition of recipient-derived APC presenting donor-derived MHC I/II (and other allo) peptides;
  - 3. In a presensitized host, allogeneic MSC can be directly recognized by (pre)existing allospecific T-cells.
- II. At natural killer (NK) cell level:
  - 1. NK-cell-mediated recognition of missing self (no negative signal via killer-inhibitory receptors).
- III. At B-cell level:
  - 1. Allogeneic MSC can be directly recognized by (pre)existing allospecific B-cells, yet activation needs T-cell help (specific or bystander help).

In the case of MSC administration, direct T-cell alloimmunity (see No. I.1.) triggered by donor-derived APC is rather unlikely since most of the studies show that MSC are unable to stimulate allospecific naïve T-cells like professional APC. Even more, resting MSC do not express MHC class II and costimulatory molecules, such as CD40, CD80 or CD86 [21]. Interestingly, MSC upregulate MHC class II upon stimulation with IFN-γ, but still most of the studies did not observe activation of naïve T-cells even by preactivated MSC [21,22]. Nevertheless, some authors showed that preactivated MSC are indeed able to act as APC [23,24]. Chan *et al.* reported that only low levels of IFN-γ induce expression of MHC class II on MSC, while further increasing IFN-γ levels lead to a subsequent reduction of MHC class II [25]. However, under inflammatory conditions, MSC still do not express costimulatory molecules such as CD80 or CD86 *in vitro* [26,27], while they do upregulate CD40 expression [28]. Theoretically, MHC-mismatched MSC can trigger naïve T-cells via the indirect pathway (see No. I.2.) by delivering MSC-derived HLA-class I/II fragments on MHC class I/II of recipient APC. T-cells triggered via this pathway can help alloreactive B-cells to produce antibodies. This might explain the occurrence of donor MSC-specific alloantibodies observed in some studies. Finally, NK cells might be activated by stressed MSC if they cannot deliver an inhibitory signal via killer cell immunoglobulin-like receptors (KIR) due to missing self-MHC or MHC-like molecules. There are almost no data analyzing this possibility.

One important option of alloimmunogenicity of MSC has been neglected by most of the clinical studies so far – many patients, even healthy donors, express allospecific sensitized memory T/B-cells, which need less T-cell help and no professional APC for being rechallenged. Presensitization to alloantigens can be the result of priming by transfusion of blood components (e.g., thrombocytes, leukocytes), organ or cell transplantation, pregnancy or simply by unspecific cross-reactivity [29,30].

Without any doubt, the pre-existence of allospecific memory T/B-cells or antibodies before MSC administration needs to be addressed in much more detail in the future. Recently, we could show that HLA-specific memory T-cells could be detected in 6–12% of healthy donors, yet >50% of patients on transplant waiting lists [31]. Moreover, about 10–20% of patients suffering from critical limb ischemia showed T-cell and B-cell

sensitization to allo-HLA before treatment with MSC; fortunately, only very few of them reacted by amplified memory alloresponse (manuscript in preparation).

The immunological analyses need to be included in more clinical trials in order to better understand the *in vivo* mode of action and the conditions in which alloimmunogenicity is low.

Given the current state of research, there can be no doubt that MSC possess the capacity to modulate innate as well as adaptive immune responses. However, most of the data for human MSC derive from *in vitro* studies, while *in vivo* data are only available from preclinical trials, mainly in mouse or rat. Interestingly, several *in vitro* as well as *in vivo* studies reported that evasion from immune recognition is intrinsic for MSC, but not for other cells derived from the MSC donor [32–34].

As suggested above, we have to differentiate between direct and indirect allopresentation as well as between naïve (priming) and memory (boostering) response. Cross-reactivity and bystander activation of preactivated T/B-cells within inflammatory environment (both particularly for memory response) have also to be taken into account (sometimes named as heterologous immunity).

By presenting antigens to T-cells, professional APC provide the link between the unspecific innate and the specific adaptive immune system. Figure 1 illustrates the key checkpoints of triggering alloimmunity. DC are the most important professional APC and their progenitors originate from hematopoietic stem cells in the bone marrow. When these progenitors differentiate into immature DC, they leave the bone marrow to patrol the body. After antigen encounter in association with 'danger' signals in the periphery, DC start the maturation process, which leads to upregulation of costimulatory molecule expression, such as CD80 or CD86. Moreover, DC migrate in a CCR7-dependent manner toward draining lymph nodes where they encounter NK cells as well as naïve T-cells. IFN-γ derived from activated NK cells is essential for priming of Type 1 helper (Th1) cells [35,36]. Naïve T-cells must receive three signals from professional APC in order to get activated and lineage committed. The first one is presentation of their specific peptides on MHC molecules of APC, which is recognized by the T-cell receptor. The second signal is mediated via the costimulatory molecules CD80 and CD86 on APC and leading to CD28 signaling pathway on T-cells. Activated T-cells express CD40L (CD154), which interacts with CD40 on APC. This interaction leads to cytokine release and displays the third signal. In the presence of DC-activated NK cells, activated APC secrete IL-12 and IL-18, which drive naïve T-cells into Th1 lineage. In addition, CD40L+ (CD154+) activated T-cells can also cross-talk with B-cells – a key factor to activate their Ig class switch.

In case of prexisting memory, however, the response to rechallenge is less complex. Activated DC that migrated to the lymph nodes can also restimulate central memory T-cells, which are already committed to a particular T helper (Th) lineage, for example, Th1, without need for additional NK cell and cytokine support. Effector T-cells, which were recently activated, need even less signals (no need for CD28-CD80/86 interaction, etc.) and can migrate to nonimmune tissue and there be restimulated by other, even nonprofessional APC, including activated nonimmune cells expressing MHC class I and/or II molecules. In summary, the situation is completely different in sensitized patients or even more in patients with recent activation of alloreactive T/B-cells by different reasons – a situation completely overlooked for MSC therapies so far.

How can MSC suppress alloimmunogenicity?: induction of tolerogenic DC by MSC

DC are professional APC that patrol the body in an immature state. Once they encounter their antigen, which could also be alloantigens in the presence of 'danger' signals (triggering pattern recognition recep-

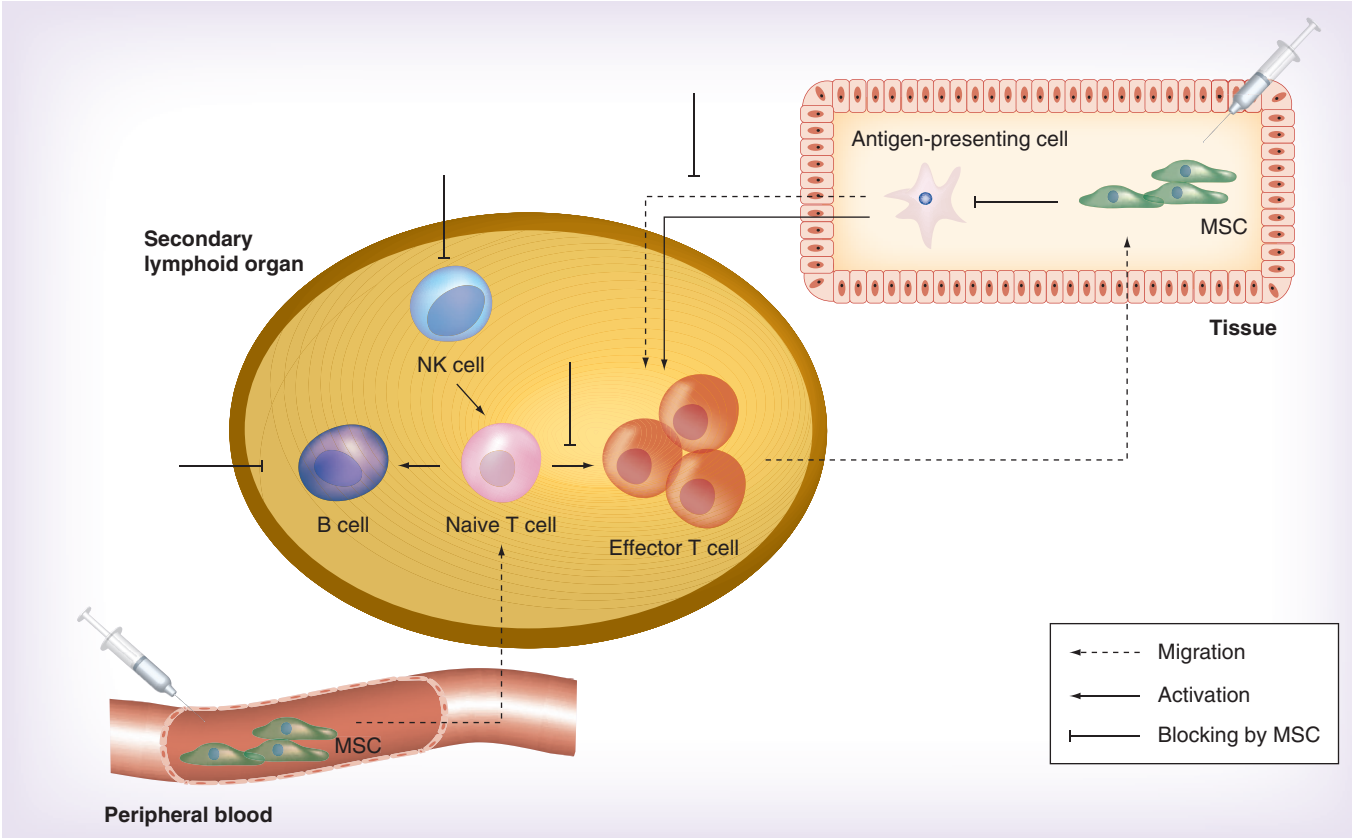

Figure 1. Mesenchymal stromal cells target different checkpoints of alloimmunity.
MSC: Mesenchymal stromal cell.

tors or TNF-α/IL-1 receptor pathways), they start the maturation process. The main function of DC is to process antigen and present the fragments on MHC class I/II to T cells. Antigen presentation by immature DC, however, mediates anergy and tolerance. Many *in vitro* studies show that MSC promote the generation of tolerogenic DC by inhibiting the differentiation of human CD14+ monocytes or hematopoietic CD34+ precursors into DC [37–39]. Normally, the differentiation of both precursors into DC goes along with downregulation of CD14 expression and upregulation of CD1a and other DC marker expression. In contrast, when they have been differentiated in the presence of MSC, DC still express high levels of CD14 and do not gain expression of CD1a [19,37,40]. As a consequence of the impaired differentiation, DC also express less HLA-DR as well as lower levels of costimulatory molecules, such as CD80, CD83, CD86 and CD40 after lipopolysaccharide (LPS) stimulation, a phenotype typical for tolerogenic DC. In contrast, it is less clear whether MSC interfere not only with the early steps of DC differentiation, but also with progression from immature to mature DC. So far, contradictory results were obtained on this topic. Jiang *et al.* showed that MSC could suppress LPS-induced maturation of *in vitro*-generated monocyte-derived DC (moDC), while Spaggiari *et al.* reported that MSC failed to inhibit maturation of moDC, but rather enhanced their ability to stimulate mixed lymphocyte reaction [37,40]. These studies have two limitations. First of all, *in vivo* MSC might not exclusively meet DC precursors, but very probably will also interact with already differentiated immature DC in immune and nonimmune tissues. And secondly, moDC do not represent all DC subsets seen *in vivo* [41,42] and sufficient data with freshly isolated immature DC are missing.

Therefore, it would be of great importance to perform further studies to address this question. Besides the upregulation of costimulatory molecules after activation and maturation, DC also have to acquire the expression of the chemokine receptor CCR7 allowing migration to the draining lymph nodes. To date, there are no data available about the influence of MSC on the migratory capacity of human DC, in particular of freshly isolated DC subsets. English *et al.* reported that murine MSC suppress the acquisition of CCR7 on bone-marrow-derived murine DC [43]. Furthermore, Chiesa *et al.* could demonstrate in a mouse model that intravenously injected MSC affect the expression of CCR7 and CD49dβ1 *in vivo* [11]. Both

surface molecules are involved in DC migration to the lymph nodes. Moreover, it has been shown that MSC not only interfere with the expression of cell surface markers on DC, but also with the secretion of soluble factors. Pro-inflammatory cytokines and chemokines released by DC play an important role for the recruitment and priming of other immune cells (third signal). Several authors reported that moDC generated in the presence of MSC produce lower levels of TNF-α as well as of IL-12 [11,39,40,44], the latter being a potent regulator of committing naïve T-cells into Th1 lineage and IFN-γ production by activated T and NK cells. In contrast, DC produced higher levels of the anti-inflammatory cytokine IL-10 when they have been generated in the presence of MSC [39,40,44]. This cytokine profile is equally typical for tolerogenic DC as the reduced expression of costimulatory molecules. Though there are only few data available that could give a broader overview about the influence of MSC on cytokine and chemokine production of DC. So far, several different factors and mechanistic details have been shown to play a role for the mechanism of immunomodulation by MSC. The inhibitory effects of MSC seem to be mainly dependent on soluble factors, as different authors could show that cell–cell contact is not necessary for the induction of tolerogenic DC by MSC [45]. For example, IL-10 and IL-6 have been reported to be involved in the generation of human tolerogenic DC by MSC *in vitro*, but only when present during early DC differentiation [37,46]. On the contrary, other studies could not confirm a role for IL-6 or IL-10, respectively, but showed that the effect of MSC on DC was rather mediated by prostaglandin E2 (PGE2) or the activation of Notch signaling [11,39,40]. The role of PGE2 could also be confirmed by other authors [47]. Nevertheless, it has been shown that not only soluble factors mediate MSC inhibition of DC. Zhang *et al.* proposed at least a partial role for contact-dependent activation of Jagged-2 [48]. Interestingly, Chiesa *et al.* [11] could show that MSC downregulate key molecules downstream of Toll-Like Receptor (TLR) 4 signaling [11]. The authors demonstrated that MSC inhibited phosphorylation of MAPK downstream of myeloid differentiation primary response gene 88 (MyD88), whose activation following TLR4 stimulation leads to nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB) mediated production of IL-12. Furthermore, they observed an enhanced activation of protein kinase B (Akt) on DC exposed to MSC, which impairs NF-κB signaling and therefore leads to inhibition of apoptosis. In conclusion, the mechanistic details of MSC induction of tolerogenic DC seem to be broad and complex and therefore need to be elucidated in more detail. The context in which MSC and DC encounter each other probably plays a crucial role for the specific mechanism.

Another limitation of most of the experimental studies addressing the effects of MSC on DC is the source of DC. Due to the low accessibility of DC in the body, most studies have been performed with *in vitro*-generated DC, either derived from murine bone marrow or from human monocytes. However, *in vivo,* monocytes give rise to a subset of macrophages and inflammatory DC that share many phenotypic and functional features with DC, yet it is now widely accepted that monocytes do not give rise to conventional mDC [41,42].

How MSC can suppress alloimmunogenicity?: MSC inhibit effector functions of NK cells but are not resistant to NK-cell-mediated killing

NK cells are another important cell type for alloimmunity as they can distinguish between self and non-self and are capable of lysing activated target cells expressing stimulatory ligands. Moreover, they can produce a number of cytokines, for example, TNF-α, IL-10 or IFN-γ. NK-cell-derived IFN-γ has been shown to be essential for Th1 priming [35,36]. NK cells can be activated by different pathways:

- Ligation of inhibitory or activating receptors, such as Ly49, NGK2 or KIR;
- Pro-inflammatory cytokines, such as IL-2, IL-12, IL-15 or IL-18, which can, for example, be produced by activated APC.

The balance between activating and inhibitory signals determines the NK-cell effector functions. Self-MHC class I molecules serve as ligands for inhibitory KIR. Since MSC have been shown to express low levels of MHC class I molecules [21] and are usually used from an MHC-unmatched donor, they might be susceptible for NK-cell-mediated lysis, particularly in an inflammatory environment triggering activating signals. Remarkably, Spaggiari *et al.* as well as Hoogduijn *et al.* reported that NK cells *in vitro* can kill allogeneic as well as autologous MSC to the same degree [49,50]. Furthermore, the authors could show that MSC even express some ligands for activating NK cell receptors, for example, NKG2D ligands. Nevertheless, the killing of MSC was only possible when NK cells have been activated by cytokines beforehand [49,51]. Different authors could show that MSC *in vitro* suppress cytokine-mediated NK-cell proliferation and reduce the expression of activation markers, such as NKp44, NKp30, NKG2D or CD132 [49,52]. In addition, NK cells displayed a reduced cytotoxic potential in the presence of MSC [51]. Chatterjee *et al.* could demonstrate that a subpopulation of NK cells acquires expression of

CD73 upon exposure to MSC, while the percentage of CD39+ NK cells remains unaltered [53]. Moreover, NK cells exposed to MSC during activation produce lower levels of IFN-γ, TNF-α and IL-10 [51].

The inhibitory effects of MSC on proliferation, expression of activation markers and cytokine production of NK cells were mediated by soluble factors as they could still be observed in transwell experiments. On the contrary, MSC required cell–cell contact to impair NK-cell cytotoxicity [51,52]. IDO, PGE2 and soluble HLA-G5 (sHLA-G5) have been demonstrated to play a role for the inhibition of NK-cell effector function by MSC [52,54].

So far, only few *in vivo* studies are available analyzing the influence of MSC on NK-cell activation and therefore, most of the data for MSC interactions derive from *in vitro* studies. Moreover, all of the studies only address the direct impact of MSC on NK cells and do not consider the possibility of NK-cell activation by DC, especially in inflamed peripheral tissues or secondary lymphoid organs. Our unpublished studies revealed an inhibition of cytokine secretion of NK cells triggered by activated DC that were tolerized by MSC.

IL-12 secreted by APC is a key cytokine driving NK-cell IFN-γ response and directly and indirectly the Th1 response [55]. To date, all studies only investigated the isolated impact of MSC on either DC, NK or T-cells *in vitro* [38,51,56] . However, these studies did not address the complex interaction of DC with NK and T-cells, especially considering their interaction in inflamed peripheral tissues or secondary lymphoid organs and the potential of MCS to interfere with this communication network. Our preliminary data suggest an important interplay of MSC in this cell–cell interaction network.

How MSC can suppress alloimmunogenicity?: MSC modulate T-cell function

The next check points after T-cell alloactivation are the clonal expansion of specifically triggered alloreactive T/B-cell clones, the maturation into effector T-cells, the migration from lymph nodes to the inflamed tissue and the intratissue reactivation and execution of effector functions (killing, cytokine secretion, Figure 1).

MSC have been shown to inhibit alloantigen-specific proliferation of CD4+ as well as CD8+ T-cells *in vitro* [56]. Most of these data were obtained from mixed lymphocyte reactions using complete peripheral blood mononuclear cells. Yet, there are studies reporting that the removal of monocytes from peripheral blood mononuclear cells reduces the suppressive effects of MSC [57,58], indicating that MSC rather modify APC than T-cells themselves. Furthermore, Aggarwal and Pittenger reported that isolated Th1 cells produced lower levels of IFN-γ in the presence of MSC, while Th2 cells increased IL-4 production [59]. This shift from a Th1 toward an anti-inflammatory Th2 response has also been observed in different animal models, showing an increased level of typical Th2 cytokines such as IL-4, IL-10 or IL-13 in spleens from MSC-treated mice and rats [60–62]. In other studies, it has been shown that MSC inhibited Th17 differentiation to the benefit of generation of Th2 cells [63–65]. Although most of the studies show an inhibitory effect of MSC on T-cell activation, there are still some publications reporting that MSC indeed can behave as APC under certain circumstances and thus activate T-cells [24,25]. For instance, Francois and colleagues reported that mouse MSC can cross-present soluble ovalbumin to naïve CD8+ T-cells and IFN-γ-preactivated MSC show an enhanced capacity for T-cell activation [23]. This potentially altered behavior of MSC under different cytokine milieus might lead to the activation of allospecific (memory) T-cells by MSC. However, most of the studies suggest that the presence of IFN-γ is essential for the immunomodulatory capacity of MSC and even enhances T-cell inhibition by MSC [26,66]. Mechanistically, PGE2 and IDO have been shown to mediate MSC inhibition of T-cell proliferation [67,68]. MSC do not constantly express IDO, but rather have to be stimulated by IFN-γ to produce IDO, which regulates T-cell proliferation [69]. Nevertheless, some authors reported at least a partial role of cell–cell contact for T-cell modulation by MSC, for example, mediated by the negative costimulatory molecule B7-H4, Fas-L/Fas interaction or the programmed death ligand-1 (PD-L1)/programmed death-1 (PD-1) pathway [26,64]. In addition, MSC have been shown to increase the frequency of Tregs both *in vitro* and *in vivo* [65,70]. The increased Treg generation by MSC has been associated with protection from different immune system-related diseases and even alloimmunity [71]. Moreover, when Treg were depleted in a mouse model of allergic airway inflammation, the anti-inflammatory effect of MSC was abrogated [72]. Mechanistically, it has been shown that MSC favor the generation of Treg by cell–cell contact, PGE2 and TGF-β [73].

However, robust data on MSC-treated patients are almost missing. One very interesting study in MSC-treated baboons analyzed immunological parameters and showed that T-cell responses to MSC were inhibited without reduction of the overall T-cell response to concanavalin A [74].

How MSC can suppress alloimmunogenicity?: contradictory effects of MSC on B-cells

B-cells can contribute significantly to the undesired alloreactivity and can mediate injury of allogeneic tissues [75,76]. They can have two different functions

for the alloimmune response. On the one hand, B-cells can act as professional APC in particular for memory T-cells, since they express high levels of MHC class I and II along with costimulatory molecules, such as CD80 or CD40. On the other hand, their main effector function is to differentiate into antibody-secreting cells upon binding of their specific antigen to the B-cell receptor together with adequate activation stimuli provided by CD4+ (CD8+) Th cells. In contrast to their influence on DC and T-cells, the interaction of MSC with B-cells has rarely been subject of research and the few data available reveal contradictory results. Some studies report that MSC suppress B-cell proliferation, differentiation and antibody production under different stimulatory conditions [77,78]. However, other authors rather observed enhancement of B-cell activation by MSC [66,79,80]. Recently, Rosado *et al.* could show that MSC inhibit proliferation and antibody production of B-cells, but only in the presence of T-cells [81]. On the contrary, Franquesa *et al.* showed that MSC could still inhibit B-cell differentiation in the absence of T-cells, whereas the authors could confirm that MSC only inhibit B-cell proliferation in the presence of T-cells [82].

Conclusion

In summary, there is clear evidence that MSC target different checkpoints for alloimmunity (Figure 1). Most importantly, MSC modulate APC function, especially when present during differentiation from precursor into DC. These DC display a phenotype of tolerogenic DC, characterized by lower expression of co-stimulatory molecules, reduced migration towards lymph nodes and secretion of anti-inflammatory cytokines. Furthermore, MSC reduce the cytotoxic potential, proliferation and cytokine production of NK cells that have been activated by cytokines beforehand. However, MSC are still susceptible for NK-cell mediated lysis. In addition, MSC have been shown to inhibit T-cell proliferation and to induce a shift from a Th1 towards an anti-inflammatory Th2 response. Moreover, there are contradictory publications regarding the effects of MSC on B-cell proliferation, differentiation and antibody production. The known mode of action used by MSC to modulate immune responses are extremely diverse. Most of the studies show that different soluble factors play a role. However, there are some puplication showing that MSC also use contact-dependent mechanisms for their immunomodulatory effects.

Future perspective

It is well established that MSC have a significant immunomodulatory capacity. This can have different implications – it might contribute to the stimulation of endogenous regeneration by adjusting inflammatory reaction to tissue injury into a supportive one; modulate undesired immune reactivity associated with immune diseases (graft-versus-host disease, autoimmunity) or result in low alloimmunogenicity. Although there are many reports on preclinical data, mostly *in vitro*, adequate *in vivo* studies, particularly in patients are missing. In addition, in most studies poorly defined MSC were used. The phenotype and function of MSC is strongly dependent on the source, cell isolation and expansion technology, 2D versus 3D culture, and application route. Moreover, MSC can adapt to the local environment (e.g., inflammation, hypoxia) making comparability of MSC application into healthy probands or distinct diseases heavily comparable. Finally, the aspect of presensitization of patients to different alloantigens has to be taken into account.

In summary, there is a high need to apply highly standardized immune monitoring on clinical trials, but also on preclinical mode of action *in vivo* studies.

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Executive summary

Are mesenchymal stromal cells feasible as 'off-the-shelf' product?

- The use of HLA-unmatched mesenchymal stromal cells (MSC) as 'off-the-shelf' product would have several advantages compared with autologous therapies.
- Induction of humoral and/or cellular alloimmunity by allogeneic MSC would limit their therapeutic efficacy and might provoke adverse effects.
- Robust immunological data from clinical trials using allogeneic MSC are missing.

Immunomodulation by mesenchymal stromal cells (MSC): what do we really know about it?

- There exist numerous reports on the immunomodulatory effects by MSC, mostly limited on *in vitro* studies and some *in vivo* studies in murine models.
- Data on induction of alloimmunity are contradictory.
- Taking into account the frequent situation of presensitization in patients is essential.

What is next?

- Implementation of standardized immune monitoring into clinical trials and mode of action *in vivo* preclinical studies is important.

involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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