Human Myeloid-Derived suppressor cells in solid organ transplantation

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Abstract

The balance between effector and regulatory immune cells needs a very exquisite balance in the context of solid organ transplantation to avoid the rejection of the organ while maintaining the more immune competence as possible. In the last two decades the role of regulatory immune cells has been extensively studied, mainly with regulatory T cells (Tregs), and in the last years another subset of regulatory cells, named myeloid derived suppressor cells (MDSC), has gained importance. These cells have different mechanisms of action and some of them are differentially regulated in a number of immune-mediated processes. Thus, MDSC, which play important roles in tolerance of experimental models of solid organ transplantation, have been proposed as biomarkers of the degree of immunosuppression and risk of rejection. Besides, they are also thought as therapeutic approach for the establishment of tolerance in human transplantation.

Introduction

Solid organ transplantation is a primary therapy in patients with end-stage disease. Throughout the years immunosuppressive protocols have clearly reduced the incidence of acute rejection, but current pharmacological protocols still result in undesirable side effects, such as infection and cancer among others, what results in a moderate long-term allograft survival [1,2]. As a consequence, the main goals in transplantation are to predict the risk of developing rejection and to find alternative tolerance approaches to allow immunosuppression withdrawal in order to minimize the adverse effects that have deleterious effects on long term graft survival. In this regard, myeloid cells, which are involved both in non-specific reactions and donor-specific adaptive responses during allograft rejection, play a main role starting and controlling immune responses. Under certain circumstances, they contribute to the inflammatory process, expanding disease pathology. However, myeloid cells with regulatory properties can protect the host from uncontrolled inflammation. These cells, known as myeloid regulatory cells (MRCs), have been described within all the major myeloid cell lineages. Among them, myeloid-derived suppressor cells (MDSCs) have been described as a heterogeneous group of myeloid cells known to accumulate under chronic pathological conditions [3]. As a reflection of their biology, these cells had been called "immature myeloid cells" or "myeloid suppressor cells" (MSC) but as neither term was considered as accurate, Gabrilovich DI, *et al.* [4] proposed the term "myeloid -derived suppressor cells' considering this term closer to reflect their origin and function. The first observations of suppressive myeloid cells were described more than 20 years ago in patients with cancer [5-7]. However, their functional importance in the immune system has only recently been appreciated due to the evidence that has demonstrated their contribution to the negative regulation of immune responses in cancer and other clinical settings, such as organ transplantation, infection and autoimmune diseases [3,8-12]. Initially MDSC have been described as immature cells that expand in the bone marrow in response to chronic inflammatory signals but evidence support in certain circumstances MDSC may represent monocytes and neutrophils that have been activated into immunosuppressive populations [13].

In transplantation the MDSCs are able to suppress adaptive and innate immune responses and they have been suggested as potential biomarkers for allograft tolerance as they can play a main role in the balance between graft acceptance and rejection [14,15]. The MDSCs were first described in mice as CD11b+ Gr1+ cells and experimental transplant models demonstrated they have an important role in the induction of tolerance [15,16]. As most of the published studies were performed in animal models, there is a paucity of data addressing MDSC features and their role in human transplantation. Human MDSCs in peripheral blood are classified in three main subsets: monocytic-MDSC (Mo-MDSCs: CD33+ CD11b+ CD14+ HLA-DR-/low), polymorphonuclear-MDSC (PMN-MDSCs:CD11b+ CD14- CD15+ HLA-DR- or CD11b+ CD14-+ CD66b+) [17] and a population lacking both differentiation surface markers classified as early- stage MDSC (e-MDSCs: CD33+ CD15- CD14-HLA-DR-) [17]. CD33 marker can be used instead of CD11b since very few CD15+ cells are CD11b-. While Mo-MDSC are CD33+, PMN-MDSC are CD33dim [18]. The features and clinical relevance of e-MDSC are not well stablished but limited suppression of T cell proliferation and cytokine expression was found by some authors [19]. Other suggested makers in human MDSCs include high levels of CD66b and low levels of CD62L and CD16, vascular endothelial growth factor receptor 1 (VEGFR1) (Flt-1) [20] and expression of CD124 [21]. Initially the term 'granulocytic MDSC' was used to describe PMN-MDSC [22,23] but since PMN-

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MDSC are phenotypically distinct from steady-state neutrophils lately V. Bronte, *et al.* proposed the term PMN as more accurate to define this subset [17]. Because these markers are not exclusively expressed by MDSCs, these regulatory cell subsets are best defined by their capacity to suppress T cell proliferation [24], which is associated with their ability to induce T cell apoptosis [25] and expand Treg cells [26] (Figure 1). Moreover, the interaction between MDSC and other immune cells has been described in recent years [27–30]. It is important to remark that assays of human MDSC function are difficult to implement due to their technical complexity and high variability. At the present time, the technique allowing for separation of neutrophils from PMN-MDSC is Ficoll gradient regularly used for the isolation of mononuclear cells. Low- density fraction contains PMN-MDSC and activated neutrophils. Therefore, CD11b+CD14-CD15+/CD66+ cells in low-density fraction contain both PMN-MDSC and neutrophils [17]. Hence, there is a need for reliable surrogate markers of human MDSC

function as gating criteria cannot discriminate monocytes from Mo-MDSCs and neutrophils from PMN-MDSC since at present there are no combinations of markers exclusive to MDSC. Human MDSCs exert their T cell suppressive actions through a wide variety of mechanisms, including production of anti-inflammatory cytokines and up-regulation of immune- regulatory molecules, including arginase 1 (Arg1) and indoleamine 2,3-dioxygenase (IDO) [31,32] (Figure 1). Conventional phenotyping of human Mo-MDSC subsets mainly relies upon HLA-DR expression; however, it is unclear to what extent HLA-DR expression is influenced by standard immunosuppression, especially glucocorticoids. The release of neutrophils from the bone marrow in response to glucocorticoids is well established [33] and it has been reported that glucocorticoids can induce anti-inflammatory monocytes resembling MDSC [34,35]. In previous experiments from our group (data not published) we observed a dose-dependent reduction in HLA-DR expression levels in monocytes
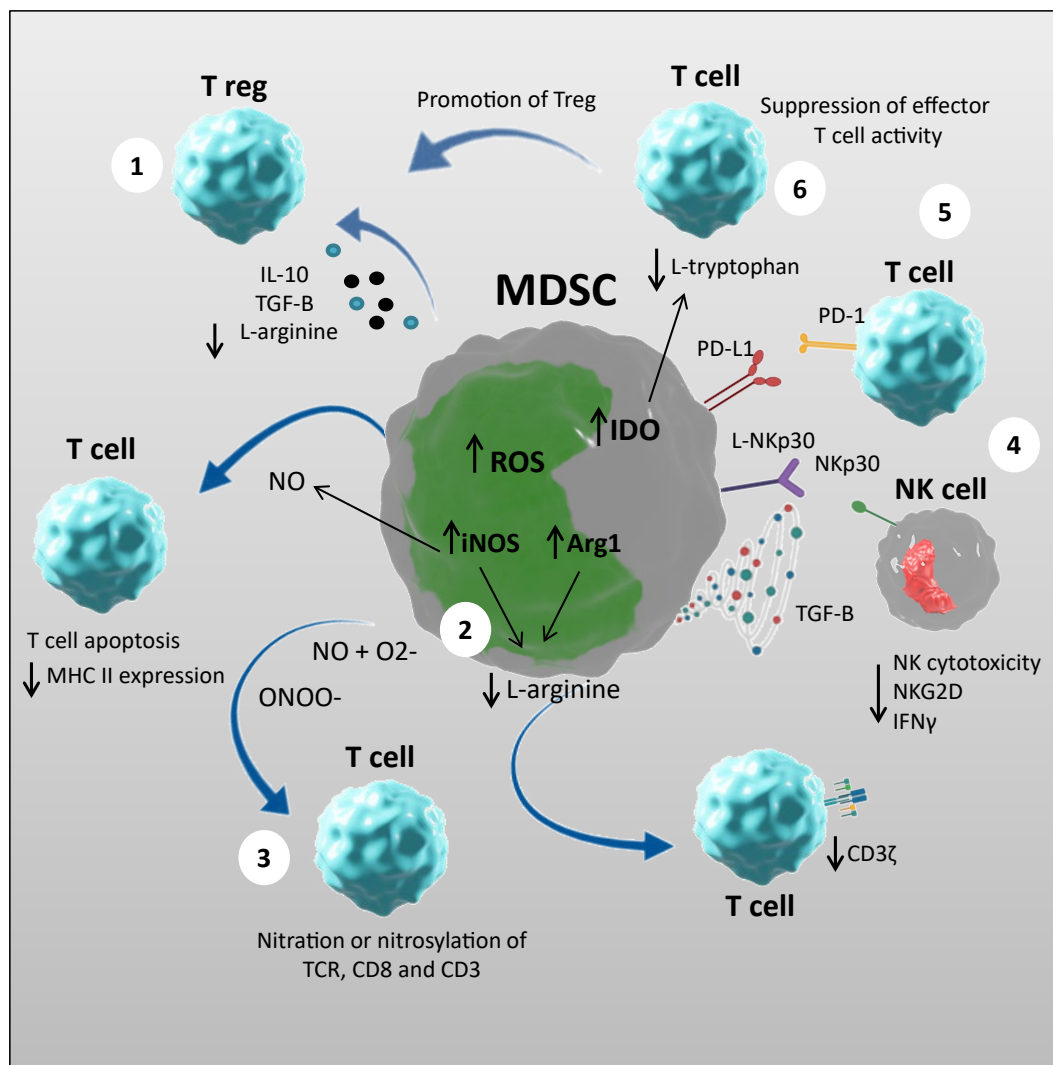

Figure 1. Mechanisms of MDSC suppressive activity

MDSC exert their suppressive function through a variety of mechanisms: (1) secretion of anti--inflammatory mediators, such as IL--10 and TGF--β that promote induction of T--regulatory cells; (2) increased arginase and iNOS: the increased activity of arginase leads to enhanced L--arginine catabolism. The lack of L--arginine inhibits T--cell proliferation through different mechanisms, including decreasing their CD3ζ expression; on the other hand iNOS generates NO which suppresses T--cell function inhibiting, MHC class II expression and inducing T--cell apoptosis; (3) increased production of ROS generates peroxynitrite which induces the nitration and nitrosylation of the amino acids and mediate MDSC suppression of T--cell function; (4) MDSCs can inhibit NK cell function by interacting with the NKp30 receptor; (5) inducing increased PD--1 expression; and (6) increased IDO activity which catabolizes tryptophan and limits T cell proliferation.

after dexamethasone exposure, monocytes were phenotypically indistinguishable from Mo-MDSC. In the critical illness context, Le Tulzo, *et al.* studied 48 septic patients and found an association between high levels of circulating cortisol and reductions in monocyte HLA-DR expression on day 6 of illness [36]. The authors then demonstrated *in vitro* that dexamethasone caused a down-regulation of a key transcription factor for HLA-DR in normal monocytes. They suggest that glucocorticoid action may represent another mechanism for the development of innate immune dysfunction. Similarly, Volk, *et al.* demonstrated that the administration of methylprednisolone in the setting of cardiopulmonary bypass resulted in an exacerbation of innate immunosuppression over that obtained with bypass alone [37]. An important goal for future studies is to define cell-surface markers and gating strategies that uniquely identify the different populations of MDSC. On the other hand, a major challenge in immune monitoring of transplant recipients is distinguishing between changes in biomarkers reflective of underlying alloimmune responses versus changes related to immunosuppressive therapy.

Kidney Transplantation

In kidney transplant models Dugast, *et al.* reported the role of MDSC in kidney transplant recipient rats [38]. In this model, MDSC in the recipient allograft were described for the first time in organ transplantation and their suppressive mechanism of tolerance was in part mediated by iNOS. In concordance, the role of NO (nitric oxide) in MDSC mediated suppression was first described by Mazzoni [39]. Another report from Vanhove's laboratory reported that secretion of CCL5 by MDSC was responsible for the accumulation of Treg into tolerized kidney allografts [40]. In subsequent studies the results indicate that a gradient of CCL5 might contribute to the intra graft localization of Treg in tolerant recipients controlled by MDSC [41]. In human kidney transplantation Luan, *et al.* found that the overall MDSC frequencies were elevated at 3, 6 and 12 months post-transplant [42]. Utrero-Rico, *et al.*, observed Mo-MDSC cells counts rapidly increase after kidney transplantation and remain high one year after transplantation [43]. Hock *et al.* showed that renal transplant recipients (RTR) had elevated frequencies of circulating MDSC [8], but they further found MDSC numbers had returned to normal levels 12 months post-transplantation [44]. However, in their previous study of RTRs with longer term transplants elevated MDSC numbers were detected in the majority of patients, suggesting that MDSC expand in the long-term, as the graft acceptance progresses. In a previous report from our group we evaluated the phenotype and function of different MDSCs subsets in 38 kidney transplant recipients (KTR) at different time-points and our data indicated that Mo-MDSC increase in KTR at 6 months and 12 months post-transplantation [45]. Moreover, the MDSCs were shown to expand early after transplantation, independently of using basiliximab or thymoglobulin during induction [44] and almost tolerant kidney transplant recipients (ATKTRs) had significantly higher levels of monocytic MDSCs and CD4+CD25+FoxP3+ Tregs than short-term graft survival kidney transplant recipients and healthy donors [46].

These observational studies suggest that MDSC numbers increase rapidly after transplantation and peak following immunosuppressive therapy. Moreover, analysis of the changes in MDSCs obtained from donors, provided strong evidence that the changes occurring in RTRs were likely due to the immunosuppressive regimens rather than the acute inflammation from surgery itself [44]. Although Mo-MDSC phenotype seems to be influenced by standard immunosuppression, especially glucocorticoids, whether MDSC subsets are differentially regulated by local conditions or treatments require further investigations.

Studies developed in mice suggest that MDSCs have an important role to induce T regulatory cells (Treg) after transplantation [16,47], but their role in human transplantation is under investigation. In KTR, Luan, *et al.* observed that CD33+ CD11b+ HLA-DR− MDSC are capable of expanding Treg, and they correlate with Treg increases *in vivo* (42). Consistent with this view, Meng, *et al.* [48] found that MDSCs isolated from transplant recipients were also able to expand regulatory T cells and were associated with longer allograft survival, and we also reported an increase in Treg expansion after Mo-MDSC coculture [45]. Further, the Mo-MDSC levels correlated positively with the survival rates, estimated glomerular filtration rates (eGFRs) of grafts, and the levels of CD4+CD25+FoxP3+ Treg in ATKTRs [46]. In two cohorts of patients with acute rejection the mRNA levels of S100A8 and S100A9 in biopsies predicted improved graft outcome. Expression of both proteins correlated with MDSC markers in PBMC and renal biopsies and higher expression of immune regulatory molecules [49]. Due to the lack of unique phenotypic markers functional studies have to be performed to identify MDSC subsets [24]. Murphy, et al. evaluated the capacity of blood derived CD11b+CD33+HLADR− MDSC from human KTRs to suppress CD4+T cells proliferation in vitro [42] demonstrating that CD11b+CD33+HLA-DR− myeloid cells from human KTR inhibit T cell proliferation, but no inhibition was found when CD11b+CD33+HLA-DR− cells were obtained from healthy donors [42]. Moreover, we observed that Mo-MDSC from KTR under tacrolimus treatment had increased suppressive activity compared to rapamycin [45] and we attribute loss of suppressive function to diminished IDO expression in rapamycin-exposed Mo-MDSC. However, another study addressing the murine MDSC response to acute kidney injury demonstrated that MDSC reduced the injury, and the effect was potentiated by MDSC induction and enhancement of the immunosuppressive activity promoted by mTOR [50]. More recently, a previously unrecognized mechanistic pathway associated with organ rejection identifies the expression of mTOR by graft infiltrating macrophages at the center of epigenetic and metabolic changes that correlate with graft loss [51]. This novel functional mechanism has been termed "trained immunity" [52]. Therefore, it seems that, while mTOR inhibition may prevent trained immunity and inflammatory pathways in myeloid cells [53,54], it may also interfere with tolerogenic programming and the ability of myeloid cells to expand Treg and suppress T-cellmediated immune responses. This dual effect of mTOR inhibition in vivo is likely to determine the outcome of the transplanted organ.

Liver Transplantation

In the 1990s, Settmacher, *et al.* described an association between aggressive calcineurin inhibition and a reduction HLA-DR expression in monocytes in the setting of induction therapy in adults following liver transplantation [55]: among 91 patients, those whose monocyte HLA-DR expression dropped below 30% experienced increased rates of bacteremia, viremia, and fungemia compared to those whose HLA-DR levels remained > 30%. In the same manner, Haveman JW, *et al.* monitored 20 liver transplantation recipients during the first month after transplantation and measured the expression of HLA-DR in monocytes. Seven out of 20 patients developed sepsis after a median of 15 days post-transplantation and HLA-DR expression was significantly lower in these patients. The expression of HLA-DR in monocytes remained low before onset of sepsis. On day 7 after transplantation, HLA-DR expression on 50% or less of monocytes had a positive predictive value for sepsis of 71%, whereas the negative predictive value was 85%. Furthermore, patients who received significantly more prednisolone

developed sepsis. The authors conclude that low HLA-DR expression on monocytes is a marker for a high risk of subsequent sepsis in liver transplantation patients and this high risk may be related to the dose of prednisolone [56]. It is known that under inflammation and fibrosis, MDSC are induced in the liver due to the local conditions [57]. MDSC are recruited in the liver and they differenciate by mechanisms that depend on contact between several cell types and on soluble mediators. For example, hepatic stellate cells promote MDSCs in mice and humans and mesenchymal stromal cells in human [58]. Bernsmeier, *et al.* reported that immunosuppressive CD14+HLA-DR- Mo-MDSCs, are expanded in patients with acute-on- chronic liver failure (ACLF) and TLR-3 agonists reversed Mo-MDSC expansion [59]. In a murine model, rapamycin induced the recruitment of MDSC and protected against immunological hepatic injury. Downregulating the mTOR activity in MDSCs induced iNOS and NO, and the pharmacological inhibition of iNOS completely eliminated the recruitment of MDSCs [60]. In another model of allogeneic liver transplantation, the authors observed an increase of regulatory T cell phenotypes and accumulation of MDSC in spleen [61].

Lung Transplantation

Hoffman, *et al.* monitored HLA-DR expression weekly after transplantation in 13 pediatric lung transplant recipients (LRT) [62]. Six out of seven patients who developed post-transplant pneumonia demonstrated lack of monocyte HLA-DR expression within the first two weeks of monitoring and those who developed pneumonia had lower monocyte HLA-DR expression over the four-week study period than those who remained infection-free. The authors propose that monitoring HLA-DR expression in monocytes may be useful to identify risk of infection and stratifying patients into higher and lower risk groups. Alingrin, *et al.* assessed the influence of early post-operative sepsis on T cell and monocyte reconstitution in anti- thymocyte globulin (ATG)-treated lung transplant recipients. Peripheral blood T-lymphocytes counts and monocyte HLA-DR (mHLA-DR) expression within 60 days post-transplant were analyzed. The authors found that sepsis is negatively correlated with the HLA-DR expression in monocytes [63]. These findings taken together highlight the importance of immunomonitoring after lung transplantation. Deshane, *et al.* found high numbers of CD11b+ CD14+ CD16− HLA-DR− NO-producing myeloid derived regulatory cells, in the airways of patients with asthma but not in patients with chronic obstructive pulmonary disease (COPD) or in healthy control subjects [64]. On the other hand Scrimini, *et al.* observed elevated levels of circulating-lineage HLA-DR− CD33+ CD11b+ MDSCs in patients with COPD [65]. Other researchers demonstrated that CCR2+ Mo-MDSCs inhibit collagen degradation and promote lung fibrosis by producing transforming growth factor-β1 (TGF-β1) [66]. The number of circulating activated MDSCs was found to be significantly increased in patients with pulmonary hypertension (PH) compared to control subjects, and was correlated with an increase in mean pulmonary artery pressure [67]. Sharma, *et al.* described the association of distinct MDSC sub-populations with the lung microbiome in LTRs. Their results suggested a functional link between the local microbiome and MDSC phenotype, which may play a role in the pathogenesis of BOS [68].

One from our group (unpublished) analyzed MDSC frequencies in 82 LTR were analyzed during the first year after transplantation. Percentages of total MDSC were increased in LTR 3 months after transplantation up to a year. When we studied the effect of transplantation on MDSC subsets in our cohort, Mo-MDSC percentages increased promptly after transplantation and decreased gradually during follow up. On the contrary, PMN-MDSC percentages decreased in the short term after transplantation, and increased during follow up although no changes compared to pre-transplant levels were observed. Compared to pre-transplant levels, e-MDSC percentages were significantly increased at 7 days, 21days and 360 days**.** We obtained similar results when we calculated MDSC absolute numbers. In previous experiments we observed a dose-dependent reduction in HLA-DR expression levels in monocytes after dexamethasone exposition, then monocytes were phenotypically indistinguishable from Mo-MDSC. On concordance with these results, some studies previously published [33,37,56] point corticosteroids are modulating MDSC levels then we hypothesize that corticosteroids are increasing Mo-MDSC populations in peripheral blood immediately after transplantation. We observed (unpublished data) the suppressive capacity of MDSC from Tacrolimus treated LTR is increased compared to the suppressive results when CD14+CD11b+CD33+HLA-DR- cells were obtained from healthy donors. Heigl, *et al.* characterized MDSCs in lung transplant recipients to assess if MDSCs can serve as a potential new research target in the field [69]. They observed that G-MDSCs obtained from LTR were functionally suppressive and showed a modest correlation with increasing CNI trough levels, a previously reported phenomenon [70,71]. Previous studies demonstrated that expression of FK binding protein FKBP in Mo-MDSCs and PMN- MDSCs from tumor-bearing mice is increased and regulates their suppressive function [72]. Altogether these results indicate that MDSC activity and numbers are modulated by immunossupressive treatments, such as CNI. In contrast, MDSC percentages in our study were not related to immunosuppressant levels in peripheral blood. As our cohort of LTR was under the same immunosuppressive regimen, potential differences between treatments, with respect to their effect on MDSC frequency or function cannot be determined which still remains a limitation. In contrast with previous reports [23,24] we observed that 90 and 180 days post-transplant Mo-MDSCs percentages were higher in patients who reject compared to those who do not reject.

Heart, Corneal, Pancreatic islets and Skin Transplantation

Ling Zhou, *et al.* found a cardioprotective role of MDSCs in heart failure [73] although the human MDSC response in heart transplantation remains unstudied. In murine models, several studies demonstrated MDSC were required for the induction of transplantation tolerance [47,74,75]. Some authors reported the development of MDSC and induction of tolerance after treating recipients with rapamycin and costimulatory blockade with anti-CD40L mAb [76,77] in contrast to mice treated with either rapamycin or anti-CD40L mAb alone [77]. Nakamura T, *et al.* further observed that rapamycin increased PD-L1 expression on MDSC that accumulate in the cardiac allograft [78]. The effect of dexamethasone for the induction of MDSC was also reported [79] by Zhao, *et al.* In corneal allograft animal models it was observed an increase in allograft survival after MDSC infusion [80]. These data suggest that transplantation may lead to recipient derived MDSCs able to suppress anti-donor responses [81]. Further, it was observed an expansion of Mo-MDSC after dexamethasone administration [82] and rapamycin nano-micelle (RNM) ophthalmic solution treatment delayed rejection and expanded MDSC in allografts [83]. In pancreatic Islet transplantation MDSC infusion prolonged allograft survival and increased the number of Tregs within the graft [84,85]. MDSC generated by hepatic stellate cells (HCS) increased islets allograft survival [84]. As well as heart, corneal and pancreatic islet transplantation there is a lack of studies regarding skin human transplantation but several animal models pointed the ability of MDSC to increase skin graft

survival [86,87]. Yang, *et al.* described that TNFα induced MDSC *in vitro* and the expression of iNOS was necessary for suppression of T cell proliferation [88]. The role of iNOS in MDSC function was also described by Wu, et al. [89]. Liao, *et al.* reported induced iNOS expression and NO production in MDSC after dexamethasone treatment [90]. Rapamycin downregulates iNOS expression in MDSC, and the suppressive activity and MDSC numbers are significantly reduced after rapamycin treatment in an allogeneic skin transplant model [91]. The results confirm the administration of glucocorticoids as a therapeutic approach by increasing the development of MDSC and point mTOR as an intrinsic factor essential for the differentiation and immunosuppressive function of MDSCs.

Conclusions and future directions

MDSC are a group of immunoregulatory myeloid cells that are gaining attention throughout the years in the field of transplantation. Several animal models have point them as important regulators in transplantation but in human transplantation their role as a biomarkers and their potential use as immunotherapy to promote tolerance remains under investigation.

A major challenge in immune monitoring of transplant recipients is distinguishing between biomarkers changes as a consequence of underlying alloimmune responses from those related to immunosuppressive therapy. Conventional phenotyping of human Mo-MDSC subsets relies upon HLA-DR expression [17]. Although it may be possible MDSC increase naturally after transplantation [15] provided evidence supports that HLA-DR expression is influenced by standard immunosuppression, especially glucocorticoids [33,34,37,56] which supports the need for new and specific markers to identify human MDSC. Then one of the more important goals for future studies is to define specific cell-surface markers and gating strategies that uniquely identify MDSC subpopulations [24]. The lack of specific markers also obliges to perform functional assays to check human MDSC function. In addition functional assays are difficult to implement due to their technical complexity and high intra-assay variability. In this regard, both the definition of specific markers and identification of the transcriptomic profile of human MDSC may shed light on the field [92]. Even though there are some studies regarding the effect of immunsuppressive drugs on human MDSC function, the effect of the current main immunosuppressive regiments on MDSCs should be further studied.

MDSC represent a promising therapeutic approach in solid organ transplantation but additional investigations are needed to fully understand their role in tolerance and to achieve immunosuppression withdrawal or minimization.

Funding

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