Transplant Immunology 56 (2019) 101225

Contents lists available at ScienceDirect

Transplant Immunology

journal homepage: www.elsevier.com/locate/trim

Preliminary assessment of the feasibility of autologous myeloid-derived suppressor cell infusion in non-human primate kidney transplantation

Mohamed B. Ezzelarab[a], Angelica Perez-Gutierrez[a,1], Abhinav Humar[a], Martin Wijkstrom[a], Alan F. Zahorchak[a], Lien Lu-Casto[a], Yu-Chao Wang[a], Roger W. Wiseman[b], Marta Minervini[a,c], Angus W. Thomson[a,d,*]

[a] Starzl Transplantation Institute, Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA
[b] Wisconsin National Primate Research Center, University of Wisconsin-Madison, Madison, WI, USA
[c] Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA
[d] Department of Immunology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

ARTICLE INFO

Keywords:
Myeloid-derived suppressor cells
Kidney transplantation
Non-human primate

ABSTRACT

Myeloid-derived suppressor cells (MDSC) are a heterogenous population of immunosuppressive myeloid cells now considered important immune regulatory cells in diverse clinical conditions, including cancer, chronic inflammatory disorders and transplantation. In rodents, MDSC administration can inhibit graft-versus-host disease lethality and enhance organ or pancreatic islet allograft survival. There is also evidence, however, that under systemic inflammatory conditions, adoptively-transferred MDSC can rapidly lose their suppressive function. To our knowledge, there are no reports of autologous MDSC administration to either human or clinically-relevant non-human primate (NHP) transplant recipients. Monocytic (m) MDSC have been shown to be more potent suppressors of T cell responses than other subsets of MDSC. Following their characterization in rhesus macaques, we have conducted a preliminary analysis of the feasibility and preliminary efficacy of purified mMDSC infusion into MHC-mismatched rhesus kidney allograft recipients. The graft recipients were treated with rapamycin and the high affinity variant of the T cell co-stimulation blocking agent cytotoxic T lymphocyte antigen 4 Ig (Belatacept) that targets the B7-CD28 pathway. Graft survival and histology were not affected by infusions of autologous, leukapheresis product-derived mMDSC on days 7 and 14 post-transplant (cumulative totals of 3.19 and 1.98 $\times$ 10^6 cells/kg in $n = 2$ recipients) compared with control monkeys that did not receive MDSC (n = 2). Sequential analyses of effector T cell populations revealed no differences between the groups. While these initial findings do not provide evidence of efficacy under the conditions adopted, further studies in NHP, designed to ascertain the appropriate mMDSC source and dose, timing and anti-inflammatory/immunosuppressive agent support are likely to prove instructive regarding the therapeutic potential of MDSC in organ transplantation.

1. Introduction

Current therapies used to prevent organ allograft rejection are dependent largely on pharmacologic agents that induce non-specific immune suppression, fail to induce donor-specific tolerance and cause significant side effects. Increased understanding of the functional biology of regulatory immune cells, their roles in promoting tolerance in the healthy steady-state, and their ability to induce transplant tolerance in rodent models [1–3] suggests their therapeutic potential. Both innate and adaptive regulatory immune cells have been identified, including forkhead box p3 (Foxp3$^+$) regulatory T cells (Treg), Foxp3$^-$ type-1 regulatory T cells (Tr1) cells, and regulatory myeloid cells, that include regulatory dendritic cells (DCreg), regulatory macrophages (Mreg) and myeloid-derived suppressor cells (MDSC). Some of these

Abbreviations: DC(s), dendritic cell(s); MDSC(s), myeloid-derived suppressor cell(s); NHP, non-human primate

* Corresponding author at: Starzl Transplantation Institute, University of Pittsburgh School of Medicine, 200 Lothrop Street, BST W1544, Pittsburgh, PA 15261, USA.

E-mail addresses: ezzemb@upmc.edu (M.B. Ezzelarab), humara2@upmc.edu (A. Humar), wijkstrommn@upmc.edu (M. Wijkstrom), zahor@pitt.edu (A.F. Zahorchak), lult@upmc.edu (L. Lu-Casto), wangy25@upmc.edu (Y.-C. Wang), rwwiseman@wisc.edu (R.W. Wiseman), minervinimi@upmc.edu (M. Minervini), thomsonaw@upmc.edu (A.W. Thomson).

[1] Present address: Department of Surgery, University of Chicago, Chicago, IL, USA.

https://doi.org/10.1016/j.trim.2019.101225
Received 22 May 2019; Received in revised form 15 July 2019; Accepted 16 July 2019
Available online 19 July 2019
0966-3274/

populations are undergoing early phase clinical testing. However, there are many unresolved questions regarding the appropriate cell source, dosage, timing and choice of concomitant agents (immunosuppressive drugs/cytokines/cytokine antagonists) that may be required to enable these cells exhibit their function in vivo and promote long-term graft survival/transplant tolerance [4–6].

MDSC are a heterogeneous population of immunosuppressive myeloid cells that undergo systemic expansion during inflammatory conditions or cancer [7,8]. They are currently subdivided into three subsets based on their cell surface phenotype,- in humans, polymorphonuclear (PMN)-MDSC, monocytic (m) MDSC and immature/early MDSC [9]. These cells suppress T cell responses via multiple mechanisms [10–13], including the activity of arginase-1, inducible nitric oxide synthase, heme oxygenase-1, reactive oxygen species, IL-10 and transforming growth factor β. MDSC participate in the induction of prolonged allograft survival in rodents [11,14–16]. In humans, mMDSC accumulate in renal transplant recipients and may mediate expansion of $Foxp3^+$ Treg [17], while they increase in number and suppress donor-reactive T cell responses to graft epithelium in intestinal allograft recipients [18]. On a per-cell basis, mMDSC are considered more potent than polymorphonuclear MDSC [19–21], suggesting that the former could be of potential therapeutic use, both in organ and hematopoietic stem cell (HSC) transplantation [22].

To our knowledge, no studies of autologous mMDSC infusion in human organ or HSC transplant recipients have been reported. Non-human primates (NHP) are important models for pre-clinical assessment of the feasibility, safety and efficacy of promising new approaches to the promotion of transplant tolerance [23,24]. Recently, we have characterized mMDSC in the peripheral blood of normal and hematopoietic growth factor-mobilized rhesus macaques [25]. In this study, we have conducted a preliminary investigation of autologous mMDSC isolation, sorting and infusion into MHC-mismatched rhesus renal allograft recipients, together with an assessment of graft function/survival and host T cell responses.

2. Materials and methods

2.1. Animals

Captive-bred, male Indian juvenile rhesus monkeys (*Macaca mulatta*; 5–7 kg) were obtained from the NIAID-sponsored NHP colony (AlphaGenesis; Yemassee, SC). Before shipment, they were screened for macaque viruses, i.e. herpes B, simian immunodeficiency virus, SRV, and STLV (BioReliance Corporation Rockville, MD). They were housed in environmentally-controlled rooms in an AAALAC-accredited facility, in strict conformance with the NIH Guide for Care and Use of Laboratory Animals. All procedures were carried out in accordance with the 'Principles of Laboratory Animal Care' formulated by the National Society for Medical Research and the 'Guide for the Care and Use of Laboratory Animals' published by the NIH (publication 80–23; revised 1978) and approved by the University of Pittsburgh IACUC. Specific environment enrichment was provided.

2.2. Hematopoietic growth factor mobilization and leukapheresis

Prior to leukapheresis, monkeys received recombinant human (rhu) granulocyte-macrophage (GM)-colony-stimulating factor (CSF) (Leukine; Genzyme, Cambridge, MA; 10 μg/kg/day for 4 days), followed by rhu granulocyte (G)-CSF (Neupogen; Amgen, Thousand Oaks, CA; 10 μg/kg/day for 4 days). Leukapheresis was performed on day 8, as described [26,27], using a NHP research-dedicated COBE® Spectra Apheresis System (Lakewood, CO).

2.3. Abs and FACS staining procedure for flow cytometric analysis

The following anti-human/rhesus monkey cross-reactive antibodies (Abs) were used to characterize mMDSC and lymphocyte subsets by multi-color fluorescence-activated cell staining (FACS) analysis: anti-CD3 (clone # SP34–2), -CD4 (L200), -CD8 (RPA-T8), -CD11b (ICRF44), -CD14 (M5E5), -CD127 (HIL-7R-M21), -CD62L (SK11), -CD45RA (5H9), -HLA-DR (G46–6), -CCR5 (3A9), -CD11a (HI111), -CD103 (Ber-ACT8) (all BD Pharmingen, San Diego, CA), anti-CD20 (2H7), -CD28 (CD28.2), -CD95 (DX2), -CCR6 (G034E3), -B7-integrin (FIB504) (Biolegend, San Diego, CA), anti-CD33 (AC104.3E3) (Miltenyi Biotec, Auburn, CA) and anti-CD25 (BC96) (eBioscience, San Diego, CA). Ab cross-reactivity was confirmed by vendor technical data sheets and by the NHP Reagent Resource website (www.nhpreagents.org). Briefly, PBMC were suspended in cell staining buffer (CSB; 1× PBS/1% denatured fetal calf serum [Atlanta Biologicals]). After addition of Abs, cells were incubated for 20 min at 4 °C protected from light, then washed with 2 ml cell staining buffer. After centrifugation, cell pellets were suspended in CSB and held on ice until analyzed. Isotype-matched irrelevant Abs were used as controls. Flow cytometry was performed using a LSR Fortessa II cytometer (BD Sciences). Data acquired were analyzed with FlowJo software (Tree Star Inc., San Carlos, CA).

2.4. Isolation of $CD14^+$ cells and flow sorting of mMDSC

One-two months before kidney transplantation, PBMC were harvested from single leukapheresis products of prospective graft recipients ($n = 2$) by Ficoll density gradient centrifugation. Anti-CD2 and -CD20 microbeads (Miltenyi Biotec) were used to positively select and remove T and B cells respectively. $CD14^+$ cells (monocytes) were isolated using CD14-specific immunobeads (Miltenyi Biotec) and cryopreserved. Seven and 14 days post-transplant, FACS-stained mMDSC ($CD3^-CD20^-HLA\text{-}DR^-CD33^+CD14^+$) were flow-sorted from the cryopreserved $CD14^+$ cells using a LSR ARIA II cell sorter (BD Sciences).

2.5. Renal transplantation, immunosuppressive regimen and MDSC infusion protocol

Orthotopic renal transplantation was conducted and the experimental end-point determined as described previously [26]. Transplants were performed from the same MHC-mismatched donor to both a control (no cell infusion) and a prospective MDSC recipient. Fig. 1 shows the experimental protocol and immunosuppressive drug regimen used, and the timing of autologous mMDSC infusion. Rapamycin (LC Laboratories, Woburn, MA) was administered i.m, starting 2 days before transplant and maintained at a whole blood level of 10–15 ng/ml. CTLA4Ig (Belatacept; Bristol Myers Squibb; New York, NY) was started on day −1 (20 mg/kg), then administered (10 mg/kg) on days 0, 2, 4, 7 and 14, and every 2 weeks thereafter. MDSC were infused i.v. on days 7 and 14 post-transplant.

2.6. Pre- and post-transplant monitoring of host effector T cell populations

Naïve and memory T cell (Tmem) subsets were identified based on CD45RA and CD62L expression and quantified in sequential peripheral blood samples, as described [28].

2.7. Graft histopathology

Histopathological analysis and Banff grading of renal biopsies was performed on all animals on post-operative day (POD) 42, and for all animals at the time of euthanasia by a board-certified transplant pathologist (MM).

2.8. Levels of circulating cytokines

Cytokine levels in serum were determined by the Southwest National Primate Research Center Luminex Core Laboratory (San

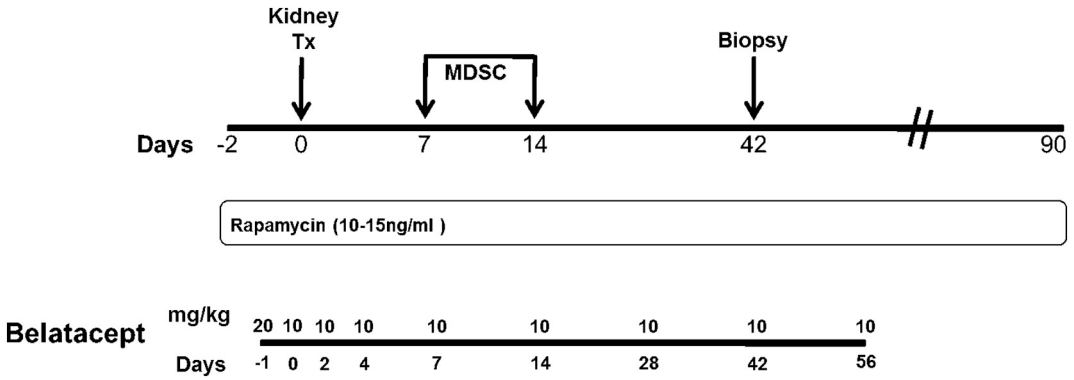

Fig. 1. Experimental protocol and immunosuppressive regimen used for evaluation of autologous mMDSC infusion in renal-allografted rhesus macaques. Leukapheresis was performed in GM- and G-CSF-treated animals at least 4 weeks before organ transplantation and $CD14^+$ cells isolated and cryopreserved. Kidneys from MHC-mismatched donors were transplanted on day 0 and flow-sorted autologous mMDSC infused on days 7 and 14. Immunosuppressive therapy comprised rapamycin starting on day -2 and Belatacept (CTLA4Ig), commencing on day -1. Graft biopsies were performed on day 42.

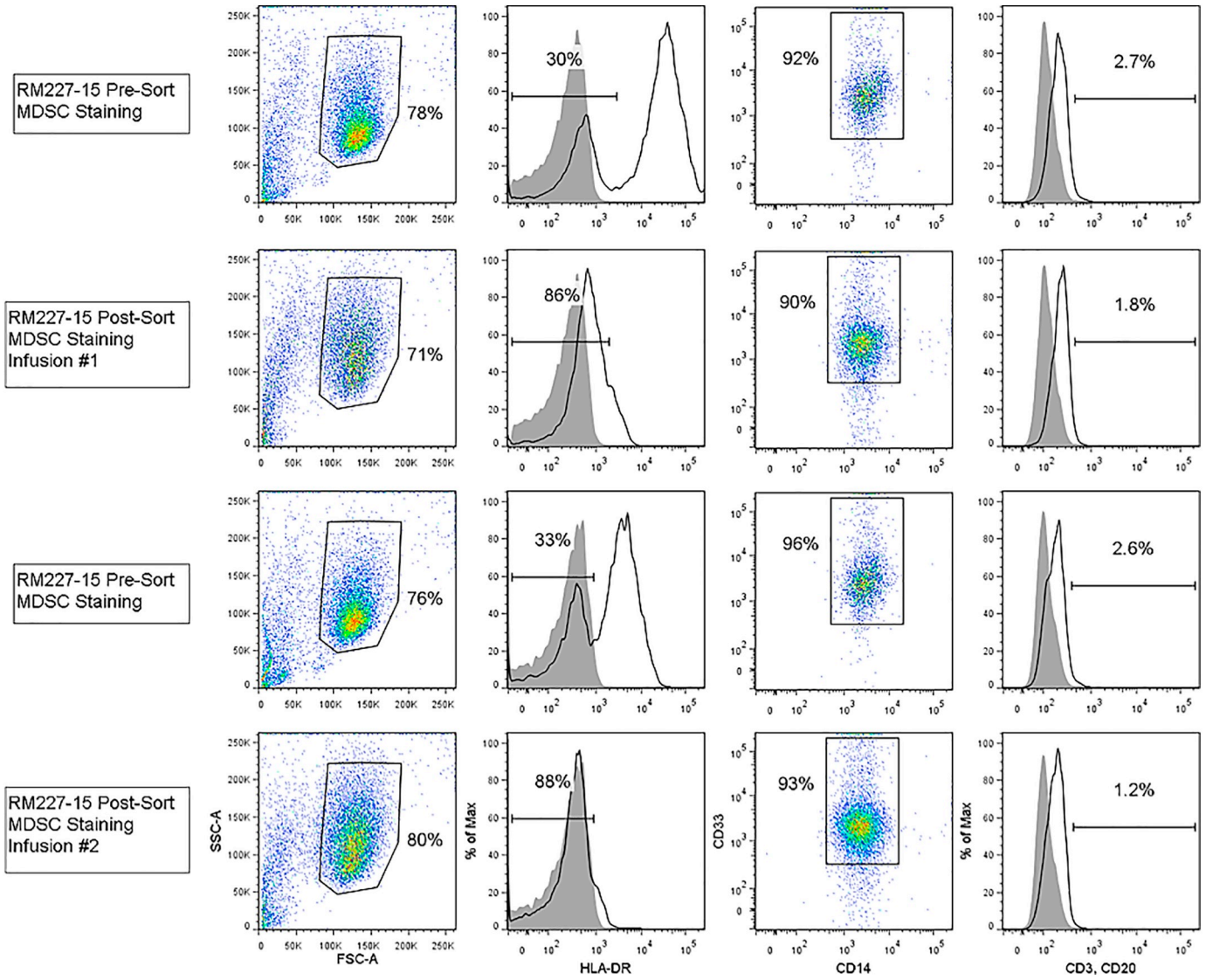

Fig. 2. Phenotypic characteristics of flow-sorted recipient mMDSC infused into renal allograft recipients.
The incidences of cells expressing HLA-DR, CD14, CD33, CD3 and CD20 are shown for each infusion (on day 7 and 14) both pre- and post-sorting for each rhesus monkey that received the cells ([A] monkey RM 227 and [B] monkey RM 230).

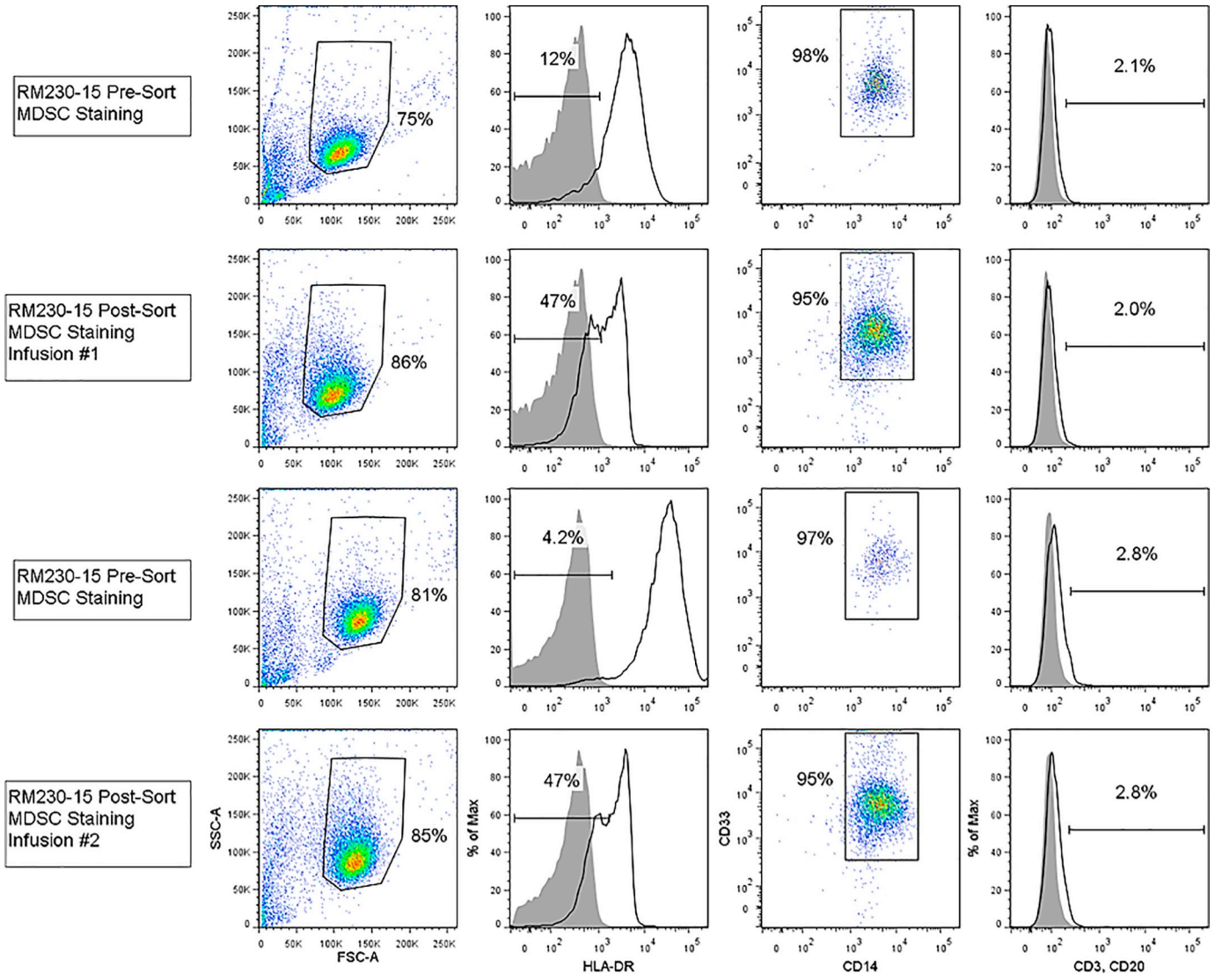

Fig. 2. (*continued*)

Table 1
$CD14^+$ cells obtained and cryopreserved from leukapheresis products and the number of flow-sorted MDSC infused into each graft recipient.

Transplant recipient	Total pheresis PBMC recovered ($\times 10^9$)	$CD14^+$ cells cryopreserved ($\times 10^6$)	Day of infusion	Total recovered cells for flow-sort ($\times 10^6$)	Flow-sorted MDSC ($\times 10^6$)	MDSC/kg ($\times 10^6$)
RM227	3.9	402	7	75	8.1	1.35
			14	72	9.2	1.84
RM230	3.8	410	7	238	5.3	1.06
			14	193	4.6	0.92

Antonio, TX) using MILLIPLEX NHP kits from Millipore (Billerica, MA) according to the manufacturer's instructions.

3. Results

3.1. Identification and flow sorting of autologous monocytic MDSC from leukapheresis products for infusion into graft recipients

mMDSC have been characterized previously in humans, including transplant patients [17,29] and in normal and vaccinated rhesus macaques [25,30] by cell surface expression of CD14, the integrin CD11b, the sialic acid binding lectin CD33 and by low expression of MHC class II surface receptor HLA-DR. Seven and 14 days after kidney transplantation (on day 0), half of the recipient's cryopreserved $CD14^+$ cells were thawed and mMDSC flow-sorted, then infused into each transplanted monkey. An aliquot of the thawed $CD14^+$ cells was stained pre- and post-flow sorting for HLA-DR, CD14, CD33, CD3 and CD20. As shown in Fig. 2, the flow-sorted cells infused into each transplanted monkey were $CD14^+HLA\text{-}DR^{-/lo}CD33^+$ and $CD3^-CD20^-$.

3.2. MDSC yield and cell dosage infused into graft recipients

An important goal of this study was to ascertain the total number of mMDSC that could be obtained from single apheresis products of growth factor-mobilized, prospective graft recipients. The total number of $CD14^+$ cells isolated from each prospective graft recipient and the

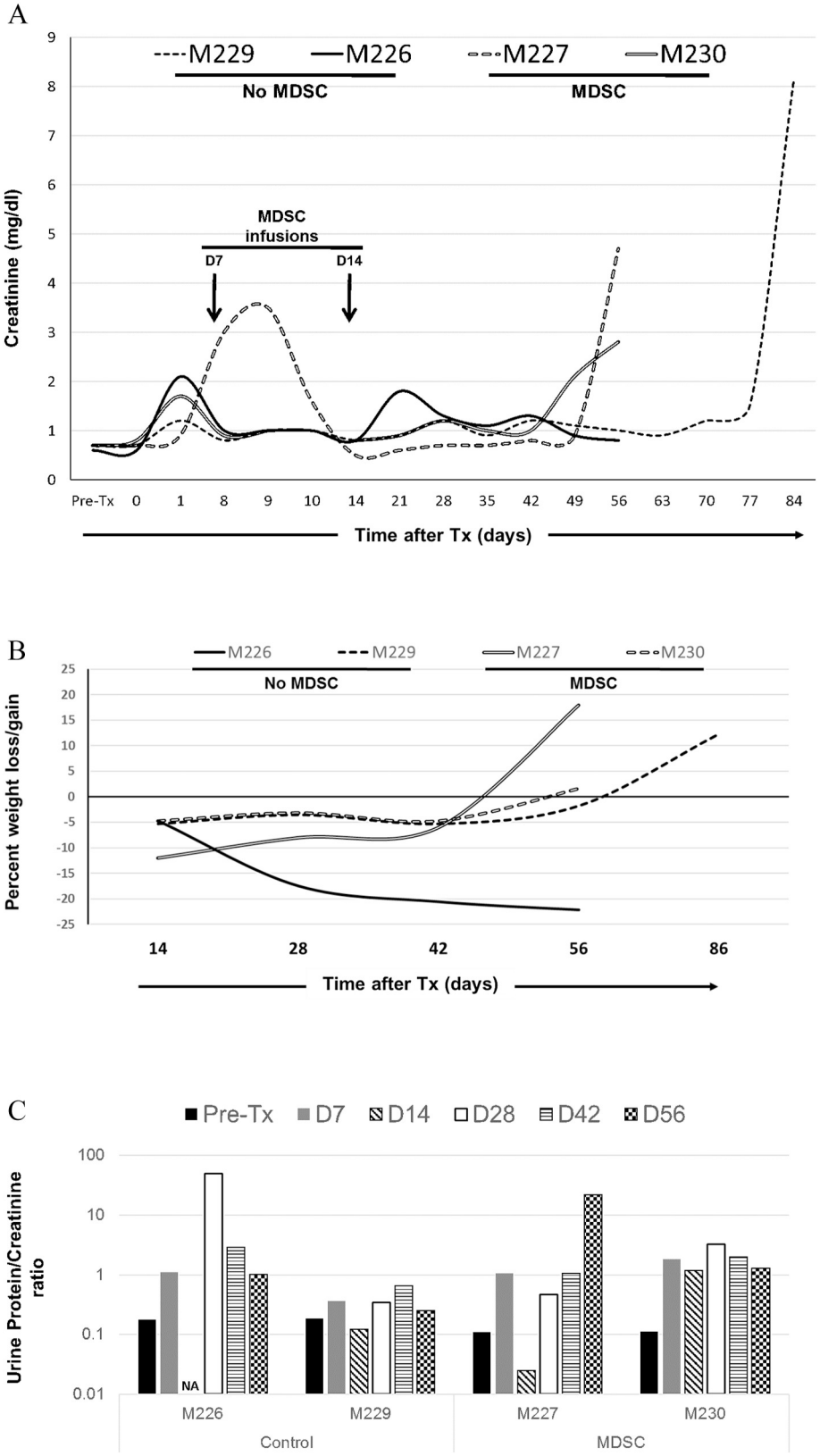

Fig. 3. Clinical monitoring of renal-allografted rhesus monkeys with or without autologous mMDSC infusion.
Data are shown for $n = 2$ monkeys given mMDSC i.v. on day 7 and 14 post-transplant, and for n = 2 control animals that received the same immunosuppressive drug regimen, but no cell infusion. (A), serum creatinine levels, (B) percent body weight loss/gain and (C), urine protein/creatinine ratio. D = days post-transplant.

Table 2
Graft survival and histopathological grades of rejected kidney allografts.

	MDSC infusion	Banff type		Graft survival (days)
M227	Yes	Biopsy	IB	55
		Euthanasia	IA	
M226	No	Biopsy	IA	58
		Euthanasia	IB	
M230	Yes	Biopsy	IA	55
		Euthanasia	IB	
M229	No	Biopsy	IB	85
		Euthanasia	IIA	

total number of flow-sorted cells infused subsequently on days 7 and 14 post-transplant are shown in Table 1. Autologous MDSC doses were 1.35×10^6/kg and 1.84×10^6/kg for the first recipient (RM227), and 1.06×10^6/kg and 0.92×10^6/kg for the second (RM230).

3.3. Renal transplant survival and graft histopathology

Serum creatinine levels and urine protein/creatinine ratios pre- and at various times post-transplant in control and MDSC-infused recipients, together with body weight loss/gain are shown in Fig. 3. Graft survival times in the control group were 58 and 85 days, and in the MDSC-infused group 55 and 55 days (Table 2). As also shown in Table 2, all transplanted monkeys displayed histological evidence of cellular rejection. Post-operative day 44 biopsies indicated either Banff grade 1A or 1B rejection in all recipients. At the time of euthanasia, control monkeys exhibited grades 1B and 11A rejection, while MDSC-infused animals exhibited grades 1A and 1B rejection.

3.4. Sequential analysis of naïve and memory T cell populations in graft recipients

Memory T cell populations (that are resistant to Belatacept [31,32]) constitute an important barrier to improved long-term organ allograft survival/tolerance induction, both in humans and NHP. We used flow cytometric analysis to monitor naive (Tn), central memory (Tcm), effector memory (Tem) and terminally-differentiated effector memory cells (Temra) in peripheral blood of the graft recipients pre- and 1, 2, 4 and 6 weeks post-transplant as described [28,33]. As shown in Fig. 4, no differences were observed in kinetics of these populations between the control and mMDSC-infused groups. $CD4^+$ and $CD8^+$ T cells expressed similar levels of other T cell surface markers, including CD25, CD127, CD28, CD95, CD11a, CD103, CCR5, CCR6, CD62L and B7 integrin, and the mean fluorescence intensity of expression of these markers was unaffected by MDSC infusion (data not shown).

3.5. Circulating cytokine levels

The levels of serum anti- and pro-inflammatory cytokines and chemokines were assessed before and after transplant, but no marked differences were observed between non-infused and MDSC-infused recipients (Supplementary Fig. 1).

4. Discussion

MDSC expand from common myeloid progenitors in response to hematopoietic growth factors (G-, M- and GM-CSF) and other molecules, that act together with pro-inflammatory cytokines to interrupt the differentiation of these progenitors into mature myeloid cells, including DC, macrophages and granulocytes [34,35]. MDSC are now considered important immune regulatory/suppressive cells that function in diverse clinical conditions, including HSC and organ transplantation [22,36–38]. As such, they are considered potential therapeutic targets. Moreover, it has been argued [39] that, since MDSC express minimal/no cell surface MHC class II or T cell co-stimulatory molecules, they are immunoprivileged and can consequently be used readily as cellular therapeutics across MHC barriers.

Testing of MDSC therapy either for (i) the prevention of graft-versus-host disease (GVHD) following HSC transplantation [39] or (ii) the prevention of organ allograft rejection, has been performed to date only in small animal models. Thus, in rodents, there is evidence that MDSC used as cellular therapeutics can enhance survival in an acute model of lethal GVHD [10], or prolong cardiac [16,40] or pancreatic islet allograft survival [15,41]. In mice, a single dose of $2–6 \times 10^6$ donor-derived MDSC infused together with allogeneic HSC can prevent GVHD [10]. On the other hand, administration of 2×10^6 mMDSC (via the inferior vena cava) [40] or 3×10^6 MDSC (intravenously; i.v.) [16] at the time of transplant, can markedly prolong vascularized heart allograft survival, while favoring the in vivo expansion of $Foxp3^+$ Treg. Moreover, co-transplantation of pancreatic islet allografts (300 islets) with 2.5×10^6 MDSC protects the islets from rejection, with augmentation of Treg in regional lymphoid tissue [41]. Alternatively, mouse islet allograft survival can be prolonged by a single systemic infusion of 2×10^6 MDSC immediately after transplantation [15]. Similar doses of related regulatory myeloid cell populations (regulatory or 'tolerogenic" DC, or regulatory macrophages) can inhibit chronic autoimmune inflammatory disease [42,43] or prolong allograft survival/ induce donor-specific tolerance in mice [44,45].

Given these findings in rodents, our purpose in this study was to conduct a preliminary feasibility assessment of the adoptive transfer of mMDSC in a clinically-relevant, life-sustaining NHP (rhesus) organ allograft model. We chose to infuse autologous mMDSC to graft recipients treated with the mechanistic target of rapamycin inhibitor rapamycin and the T cell co-stimulation blocking agent belatacept (CTLA4Ig). These immunosuppressive agents were selected since there is recent evidence that rapamycin prolongs heart allograft survival in mice by inducing MDSC [40], whereas, as we have shown previously [26], a related immunoregulatory myeloid cell (regulatory DC (DCreg); $2–10 \times 10^6$/kg i.v.) can prolong rhesus renal allograft survival significantly in NHP when combined with minimal immunosuppressive therapy consisting of rapamycin and co-stimulation blockade (CTLA4Ig).

Since there is recent evidence [46] that, under acute inflammatory conditions (such as exist following organ transplantation), mouse or human MDSC can rapidly lose their suppressive function (via inflammasome activation), we adopted a protocol in which MDSC administration was delayed until 7 and 14 days post-transplant. We infused the maximum number of purified autologous MDSC that we could flow-sort from a single leukopheresis product of the prospective graft recipients. No influence on graft survival or on graft biopsy or terminal graft tissue histology was observed compared with the control animals treated identically, but that did not receive the cell infusions. In addition, our sequential analyses of host T cell responses did not show any differences in circulating effector T cell populations between the two groups of graft recipients, nor did we observe any differences in systemic cytokine levels.

In this study, both GM-CSF and G-CSF were administered to mobilize MDSC in rhesus monkeys [25]. Rodent studies have demonstrated that monocytic suppressor function can be induced, in vitro and in vivo, by GM-CSF, but not G-CSF administration [47]. While the number of NHP in these preliminary studies is small, our observations suggest that numbers of autologous MDSC in excess of the dosages attained (i.e.$1.7–3.4 \times 10^6$/kg cumulative dose) may be required to achieve a therapeutic effect. Indeed, the numbers of MDSC that we infused into the monkeys was comparatively small, on a body weight basis, compared with cell doses shown to be effective in mice. Obtaining comparatively larger numbers of NHP MDSC may however, necessitate the generation of mMDSC from alternative sources to graft recipient blood, in particular bone marrow or spleen precursors, as described in mice [10,15,48] and more recently rhesus macaques [49]. Since, like

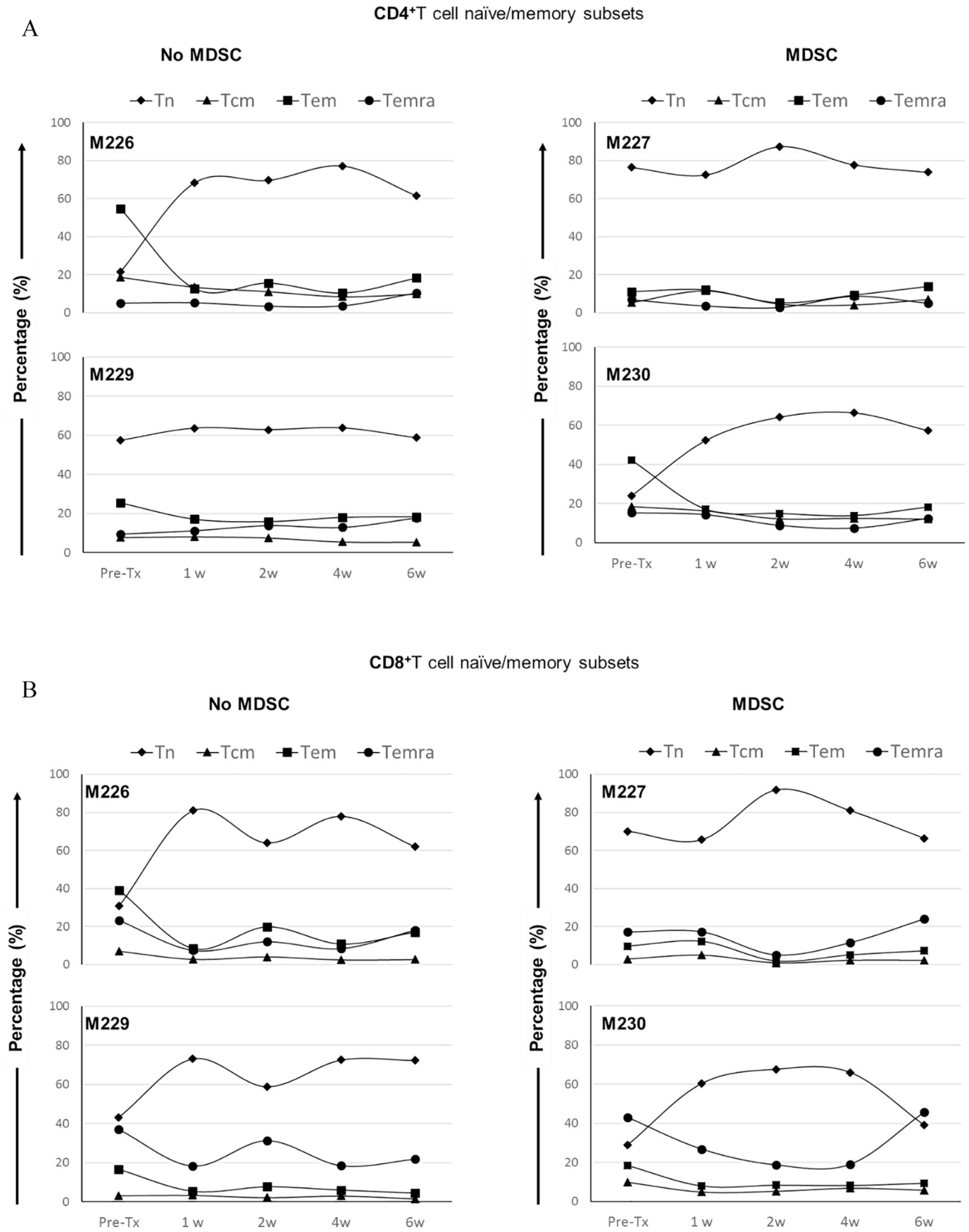

Fig. 4. Circulating levels (incidences) of naïve and memory T cell subsets in rhesus monkey renal allograft recipients before and at various times post-transplant. Data are shown for n = 2 monkeys given autologous mMDSC on day 7 and 14 post-transplant, and for n = 2 control animals that received the same immunosuppressive drug regimen, but no cell infusion. Results are percentages of peripheral blood $CD4^+$ T cells (A) and $CD8^+$ T cells (B).

mesenchymal stem cells, MDSC may be immunologically privileged, non-autologous (third party) MDSC, that could potentially be obtained in larger numbers, might prove as efficacious as autologous MDSC. Indeed, allogeneic G-CSF-mobilized $CD34^+$ cells containing $CD33^+$ $CD11b^+$ $CD14^+$ $HLA\text{-}DR^{lo}$ cells have been safely infused into humans [50]. Compared to other donor-derived regulatory immune cells, e.g. DCreg or Mreg, MDSC may exhibit a distinct pattern of migration following their infusion into immunosuppressed subjects [51].

Additionally considerations are the timing and choice of anti-inflammatory/immunosuppressive agents to use in combination with MDSC that are likely to spare/promote their in vivo immune regulatory function.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.trim.2019.101225.

Acknowledgements

This work was supported by a National Institutes of Health (NIH) NHP Transplantation Tolerance Cooperative Study Group Opportunities Pool award made to grant U01 AI1091197, sponsored by the National Institute of Allergy and Infectious Diseases. A. P-G. was in receipt of a Starzl Transplantation Institute post-doctoral fellowship in clinical and human translational research.

References

[1] J.A. Bluestone, A.W. Thomson, E.M. Shevach, H.L. Weiner, What does the future hold for cell-based tolerogenic therapy? Nat. Rev. Immunol. 7 (8) (2007) 650–654.
[2] A.E. Morelli, A.W. Thomson, Tolerogenic dendritic cells and the quest for transplant tolerance, Nat. Rev. Immunol. 7 (8) (2007) 610–621.
[3] K.J. Wood, A. Bushell, J. Hester, Regulatory immune cells in transplantation, Nat. Rev. Immunol. 12 (6) (2012) 417–430.
[4] P. Trzonkowski, R. Bacchetta, M. Battaglia, D. Berglund, H.R. Bohnenkamp, A. Ten Brinke, A. Bushell, N. Cools, E.K. Geissler, S. Gregori, S. Marieke van Ham, C. Hilkens, J.A. Hutchinson, G. Lombardi, J.A. Madrigal, N. Marek-Trzonkowska, E.M. Martinez-Caceres, M.G. Roncarolo, S. Sanchez-Ramon, A. Saudemont, B. Sawitzki, Hurdles in therapy with regulatory T cells, Sci. Transl. Med. 7 (304) (2015) 304ps18.
[5] J.A. Hutchinson, E.K. Geissler, Now or never? The case for cell-based immunosuppression in kidney transplantation, Kidney Int. 87 (6) (2015) 1116–1124.
[6] Q. Tang, F. Vincenti, Transplant trials with Tregs: perils and promises, J. Clin. Invest. 127 (7) (2017) 2505–2512.
[7] F. Veglia, M. Perego, D. Gabrilovich, Myeloid-derived suppressor cells coming of age, Nat. Immunol. 19 (2) (2018) 108–119.
[8] S. Ostrand-Rosenberg, C. Fenselau, Myeloid-derived suppressor cells: immune-suppressive cells that impair antitumor immunity and are sculpted by their environment, J. Immunol. 200 (2) (2018) 422–431.
[9] V. Bronte, S. Brandau, S.H. Chen, M.P. Colombo, A.B. Frey, T.F. Greten, S. Mandruzzato, P.J. Murray, A. Ochoa, S. Ostrand-Rosenberg, P.C. Rodriguez, A. Sica, V. Umansky, R.H. Vonderheide, D.I. Gabrilovich, Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards, Nat. Commun. 7 (2016) 12150.
[10] S.L. Highfill, P.C. Rodriguez, Q. Zhou, C.A. Goetz, B.H. Koehn, R. Veenstra, P.A. Taylor, A. Panoskaltsis-Mortari, J.S. Serody, D.H. Munn, J. Tolar, A.C. Ochoa, B.R. Blazar, Bone marrow myeloid-derived suppressor cells (MDSCs) inhibit graft-versus-host disease (GVHD) via an arginase-1-dependent mechanism that is up-regulated by interleukin-13, Blood 116 (25) (2010) 5738–5747.
[11] M.R. Garcia, L. Ledgerwood, Y. Yang, J. Xu, G. Lal, B. Burrell, G. Ma, D. Hashimoto, Y. Li, P. Boros, M. Grisotto, N. van Rooijen, R. Matesanz, F. Tacke, F. Ginhoux, Y. Ding, S.H. Chen, G. Randolph, M. Merad, J.S. Bromberg, J.C. Ochando, Monocytic suppressive cells mediate cardiovascular transplantation tolerance in mice, J. Clin. Invest. 120 (7) (2010) 2486–2496.
[12] V. De Wilde, N. Van Rompaey, M. Hill, J.F. Lebrun, P. Lemaitre, F. Lhomme, C. Kubjak, B. Vokaer, G. Oldenhove, L.M. Charbonnier, M.C. Cuturi, M. Goldman, A. Le Moine, Endotoxin-induced myeloid-derived suppressor cells inhibit alloimmune responses via heme oxygenase-1, Am. J. Transplant. 9 (9) (2009) 2034–2047.
[13] J. Yu, W. Du, F. Yan, Y. Wang, H. Li, S. Cao, W. Yu, C. Shen, J. Liu, X. Ren, Myeloid-derived suppressor cells suppress antitumor immune responses through IDO expression and correlate with lymph node metastasis in patients with breast cancer, J. Immunol. 190 (7) (2013) 3783–3797.
[14] A.S. Dugast, T. Haudebourg, F. Coulon, M. Heslan, F. Haspot, N. Poirier, R. Vuillefroy de Silly, C. Usal, H. Smit, B. Martinet, P. Thebault, K. Renaudin, B. Vanhove, Myeloid-derived suppressor cells accumulate in kidney allograft tolerance and specifically suppress effector T cell expansion, J. Immunol. 180 (12) (2008) 7898–7906.
[15] J. Qin, Y. Arakawa, M. Morita, J.J. Fung, S. Qian, L. Lu, C-C chemokine receptor type 2-dependent migration of myeloid-derived suppressor cells in protection of islet transplants, Transplantation 101 (8) (2017) 1793–1800.
[16] Y. Zhao, X.F. Shen, K. Cao, J. Ding, X. Kang, W.X. Guan, Y.T. Ding, B.R. Liu, J.F. Du, Dexamethasone-induced myeloid-derived suppressor cells prolong allo cardiac graft survival through iNOS- and glucocorticoid receptor-dependent mechanism, Front. Immunol. 9 (2018) 282.
[17] Y. Luan, E. Mosheir, M.C. Menon, D. Wilson, C. Woytovich, J. Ochando, B. Murphy, Monocytic myeloid-derived suppressor cells accumulate in renal transplant patients and mediate CD4(+) Foxp3(+) Treg expansion, Am. J. Transplant. 13 (12) (2013) 3123–3131.
[18] S. Okano, K. Abu-Elmagd, D.D. Kish, K. Keslar, W.M. Baldwin 3rd, R.L. Fairchild, M. Fujiki, A. Khanna, M. Osman, G. Costa, J. Fung, C. Miller, H. Kayashima, K. Hashimoto, Myeloid-derived suppressor cells increase and inhibit donor-reactive T cell responses to graft intestinal epithelium in intestinal transplant patients, Am. J. Transplant. 18 (10) (2018) 2544–2558.
[19] K. Movahedi, M. Guilliams, J. Van den Bossche, R. Van den Bergh, C. Gysemans, A. Beschin, P. De Baetselier, J.A. Van Ginderachter, Identification of discrete tumor-induced myeloid-derived suppressor cell subpopulations with distinct T cell-suppressive activity, Blood 111 (8) (2008) 4233–4244.
[20] J.I. Youn, S. Nagaraj, M. Collazo, D.I. Gabrilovich, Subsets of myeloid-derived suppressor cells in tumor-bearing mice, J. Immunol. 181 (8) (2008) 5791–5802.
[21] L. Dolcetti, E. Peranzoni, S. Ugel, I. Marigo, A. Fernandez Gomez, C. Mesa, M. Geilich, G. Winkels, E. Traggiai, A. Casati, F. Grassi, V. Bronte, Hierarchy of immunosuppressive strength among myeloid-derived suppressor cell subsets is determined by GM-CSF, Eur. J. Immunol. 40 (1) (2010) 22–35.
[22] J.R. Scalea, Y.S. Lee, E. Davila, J.S. Bromberg, Myeloid-derived suppressor cells and their potential application in transplantation, Transplantation 102 (3) (2018) 359–367.
[23] S.P. Montgomery, D.A. Hale, B. Hirshberg, D.M. Harlan, A.D. Kirk, Preclinical evaluation of tolerance induction protocols and islet transplantation in non-human primates, Immunol. Rev. 183 (2001) 214–222.
[24] Z. Fitch, R. Schmitz, J. Kwun, B. Hering, J. Madsen, S.J. Knechtle, Transplant research in nonhuman primates to evaluate clinically relevant immune strategies in organ transplantation, Transplant. Rev. 33 (3) (2019) 115–129.
[25] A.F. Zahorchak, M.B. Ezzelarab, L. Lu, H.R. Turnquist, A.W. Thomson, In vivo mobilization and functional characterization of nonhuman primate monocytic myeloid-derived suppressor cells, Am. J. Transplant. 16 (2) (2016) 661–671.
[26] M.B. Ezzelarab, A.F. Zahorchak, L. Lu, A.E. Morelli, G. Chalasani, A.J. Demetris, F.G. Lakkis, M. Wijkstrom, N. Murase, A. Humar, R. Shapiro, D.K. Cooper, A.W. Thomson, Regulatory dendritic cell infusion prolongs kidney allograft survival in nonhuman primates, Am. J. Transplant. 13 (8) (2013) 1989–2005.
[27] V. Pathiraja, A.J. Matar, A. Gusha, C.A. Huang, R. Duran-Struuck, Leukapheresis protocol for nonhuman primates weighing less than 10 kg, J. Am. Assoc. Lab. Anim. Sci. 52 (1) (2013) 70–77.
[28] M.B. Ezzelarab, H. Zhang, H. Guo, L. Lu, A.F. Zahorchak, R.W. Wiseman, M.A. Nalesnik, J.K. Bhama, D.K. Cooper, A.W. Thomson, Regulatory T cell infusion can enhance memory T cell and alloantibody responses in lymphodepleted non-human primate heart allograft recipients, Am. J. Transplant. 16 (7) (2016) 1999–2015.
[29] B.D. Hock, K.A. Mackenzie, N.B. Cross, K.G. Taylor, M.J. Currie, B.A. Robinson, J.W. Simcock, J.L. McKenzie, Renal transplant recipients have elevated frequencies of circulating myeloid-derived suppressor cells, Nephrol. Dial. Transplant. 27 (1) (2012) 402–410.
[30] Y. Sui, A. Hogg, Y. Wang, B. Frey, H. Yu, Z. Xia, D. Venzon, K. McKinnon, J. Smedley, M. Gathuka, D. Klinman, B.F. Keele, S. Langermann, L. Liu, G. Franchini, J.A. Berzofsky, Vaccine-induced myeloid cell population dampens protective immunity to SIV, J. Clin. Invest. 124 (6) (2014) 2538–2549.
[31] M. Cortes-Cerisuelo, S.J. Laurie, D.V. Mathews, P.D. Winterberg, C.P. Larsen, A.B. Adams, M.L. Ford, Increased pretransplant frequency of CD28(+) CD4(+) TEM predicts belatacept-resistant rejection in human renal transplant recipients, Am. J. Transplant. 17 (9) (2017) 2350–2362.
[32] D.V. Mathews, W.C. Wakwe, S.C. Kim, M.C. Lowe, C. Breeden, M.E. Roberts, A.B. Farris, E.A. Strobert, J.B. Jenkins, C.P. Larsen, M.L. Ford, R. Townsend, A.B. Adams, Belatacept-resistant rejection is associated with CD28(+) memory CD8 T cells, Am. J. Transplant. 17 (9) (2017) 2285–2299.
[33] M.B. Ezzelarab, L. Lu, H. Guo, A.F. Zahorchak, W.F. Shufesky, D.K. Cooper, A.E. Morelli, A.W. Thomson, $Eomesodermin^{lo}$ $CTLA4^{hi}$ alloreactive $CD8^+$ memory T cells are associated with prolonged renal transplant survival induced by regulatory dendritic cell infusion in CTLA4 immunoglobulin-treated nonhuman primates, Transplantation 100 (1) (2016) 91–102.
[34] T. Condamine, D.I. Gabrilovich, Molecular mechanisms regulating myeloid-derived suppressor cell differentiation and function, Trends Immunol. 32 (1) (2011) 19–25.
[35] Y. Zhao, T. Wu, S. Shao, B. Shi, Y. Zhao, Phenotype, development, and biological function of myeloid-derived suppressor cells, Oncoimmunology 5 (2) (2016) e1004983.
[36] D.I. Gabrilovich, S. Ostrand-Rosenberg, V. Bronte, Coordinated regulation of myeloid cells by tumours, Nat. Rev. Immunol. 12 (4) (2012) 253–268.
[37] J.G. Cripps, J.D. Gorham, MDSC in autoimmunity, Int. Immunopharmacol. 11 (7) (2011) 789–793.
[38] J. Ochando, P. Conde, V. Bronte, Monocyte-derived suppressor cells in transplantation, Curr. Transpl. Rep. 2 (2) (2015) 176–183.
[39] B.R. Blazar, K.P.A. MacDonald, G.R. Hill, Immune regulatory cell infusion for graft-versus-host disease prevention and therapy, Blood 131 (24) (2018) 2651–2660.
[40] T. Nakamura, T. Nakao, N. Yoshimura, E. Ashihara, Rapamycin prolongs cardiac allograft survival in a mouse model by inducing myeloid-derived suppressor cells, Am. J. Transplant. 15 (9) (2015) 2364–2377.
[41] H.S. Chou, C.C. Hsieh, R. Charles, L. Wang, T. Wagner, J.J. Fung, S. Qian, L.L. Lu, Myeloid-derived suppressor cells protect islet transplants by B7-H1 mediated

enhancement of T regulatory cells, Transplantation 93 (3) (2012) 272–282.
[42] B.G. Brem-Exner, C. Sattler, J.A. Hutchinson, G.E. Koehl, K. Kronenberg, S. Farkas, S. Inoue, C. Blank, S.J. Knechtle, H.J. Schlitt, F. Fandrich, E.K. Geissler, Macrophages driven to a novel state of activation have anti-inflammatory properties in mice, J. Immunol. 180 (1) (2008) 335–349.
[43] P. Riquelme, S. Tomiuk, A. Kammler, F. Fandrich, H.J. Schlitt, E.K. Geissler, J.A. Hutchinson, IFN-gamma-induced iNOS expression in mouse regulatory macrophages prolongs allograft survival in fully immunocompetent recipients, Mol. Ther. 21 (2) (2013) 409–422.
[44] F. Fu, Y. Li, S. Qian, L. Lu, F. Chambers, T.E. Starzl, J.J. Fung, A.W. Thomson, Costimulatory molecule-deficient dendritic cell progenitors (MHC class II^{+}, $CD80^{dim}$, $CD86^{-}$) prolong cardiac allograft survival in nonimmunosuppressed recipients, Transplantation 62 (5) (1996) 659–665.
[45] M.B. Lutz, R.M. Suri, M. Niimi, A.L. Ogilvie, N.A. Kukutsch, S. Rossner, G. Schuler, J.M. Austyn, Immature dendritic cells generated with low doses of GM-CSF in the absence of IL-4 are maturation resistant and prolong allograft survival in vivo, Eur. J. Immunol. 30 (7) (2000) 1813–1822.
[46] B.H. Koehn, P. Apostolova, J.M. Haverkamp, J.S. Miller, V. McCullar, J. Tolar, D.H. Munn, W.J. Murphy, W.J. Brickey, J.S. Serody, D.I. Gabrilovich, V. Bronte, P.J. Murray, J.P. Ting, R. Zeiser, B.R. Blazar, GVHD-associated, inflammasome-mediated loss of function in adoptively transferred myeloid-derived suppressor cells, Blood 126 (13) (2015) 1621–1628.
[47] E. Ribechini, J.A. Hutchinson, S. Hergovits, M. Heuer, J. Lucas, U. Schleicher, A.L. Jordan Garrote, S.J. Potter, P. Riquelme, H. Brackmann, N. Muller, H. Raifer, I. Berberich, M. Huber, A. Beilhack, M. Lohoff, C. Bogdan, M. Eyrich, H.M. Hermanns, E.K. Geissler, M.B. Lutz, Novel GM-CSF signals via IFN-gammaR/IRF-1 and AKT/mTOR license monocytes for suppressor function, Blood Adv. 1 (14) (2017) 947–960.
[48] Z. Zhou, D.L. French, G. Ma, S. Eisenstein, Y. Chen, C.M. Divino, G. Keller, S.H. Chen, P.Y. Pan, Development and function of myeloid-derived suppressor cells generated from mouse embryonic and hematopoietic stem cells, Stem Cells 28 (3) (2010) 620–632.
[49] A.F. Zahorchak, A. Perez-Gutierrez, M.B. Ezzelarab, A.W. Thomson, Monocytic myeloid-derived suppressor cells generated from rhesus macaque bone marrow enrich for regulatory T cells, Cell. Immunol. 329 (2018) 50–55.
[50] M. D'Aveni, J. Rossignol, T. Coman, S. Sivakumaran, S. Henderson, T. Manzo, E.S.P. Santos, J. Bruneau, G. Fouquet, F. Zavala, O. Alegria-Prevot, M. Garfa-Traore, F. Suarez, H. Trebeden-Negre, M. Mohty, C.L. Bennett, R. Chakraverty, O. Hermine, M.T. Rubio, G-CSF mobilizes CD34+ regulatory monocytes that inhibit graft-versus-host disease, Sci. Transl. Med. 7 (281) (2015) 281ra42.
[51] L. Carretero-Iglesia, L. Bouchet-Delbos, C. Louvet, L. Drujont, M. Segovia, E. Merieau, E. Chiffoleau, R. Josien, M. Hill, M.C. Cuturi, A. Moreau, Comparative study of the immunoregulatory capacity of in vitro generated tolerogenic dendritic cells, suppressor macrophages, and myeloid-derived suppressor cells, Transplantation 100 (10) (2016) 2079–2089.