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Regulatory Myeloid Cells in Transplantation

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Abstract

Regulatory myeloid cells (RMC) are emerging as novel targets for immunosuppressive (IS) agents and hold considerable promise as cellular therapeutic agents. Herein, we discuss the ability of regulatory macrophages (Mreg), regulatory dendritic cells (DCreg) and myeloid-derived suppressor cells (MDSC) to regulate alloimmunity, their potential as cellular therapeutic agents and the IS agents that target their function. We consider protocols for the generation of RMC and the selection of donor- or recipient-derived cells for adoptive cell therapy. Additionally, the issues of cell trafficking and antigen (Ag) specificity following RMC transfer are discussed. Improved understanding of the immunobiology of these cells has increased the possibility of moving RMC into the clinic to reduce the burden of current IS agents and promote Ag-specific tolerance. In the second half of this review, we discuss the influence of established and experimental IS agents on myeloid cell populations. IS agents believed historically to act primarily on T cell activation and proliferation are emerging as important regulators of RMC function. Better insights into the influence of IS agents on RMC will enhance our ability to develop cell therapy protocols to promote the function of these cells. Moreover, novel IS agents may be designed to target RMC *in situ* to promote Ag-specific immune regulation in transplantation and usher in a new era of immune modulation exploiting cells of myeloid origin.

Keywords

immune regulation; myeloid cells; transplantation

INTRODUCTION

Despite excellent short-term outcomes due to the prevention and successful treatment of acute rejection, late graft failure remains an important problem in organ transplantation (1). Moreover, current non-specific suppression of the immune system using anti-rejection drugs carries significant risks, including infection, malignancy and drug toxicity (2). Currently,

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there is increasing interest in the potential of regulatory innate or adaptive immune cells to control allograft rejection (3). Targeting myeloid cells with the goal of minimizing dependency on immunosuppressive (IS) drugs and promoting donor-specific tolerance represents a promising approach.

Herein, we discuss strategies to target regulatory myeloid cells (RMC) *in situ* and prospects for cell therapy in transplantation using RMC. Three RMC populations,- regulatory macrophages (Mreg), regulatory dendritic cells (DCreg) and myeloid-derived suppressor cells (MDSC) will be the focus of this review. Mreg will be discussed in the context of studies on peripheral blood mononuclear cell (PBMC)-derived cells differentiated in macrophage colony-stimulating factor (M-CSF) and then stimulated with interferon (IFN)-γ, since most work on Mreg in the field of transplantation has been focused on this population (4, 5). Dendritic cells (DC) are innate professional antigen (Ag)- presenting cells (APC) that serve as critical initiators and regulators of innate and adaptive immunity (6–8). For in-depth analysis of DC ontogeny and the mechanisms that underlie their immune regulatory capacity, please see recent comprehensive reviews (8–12). MDSC are a heterogeneous population of immature myeloid cells and myeloid progenitors that regulate anti-tumor immunity and share the ability to suppress effector T cell responses. The origin and suppressive mechanisms of MDSC have been reviewed in detail (13, 14).

RMC AS CELLULAR IMMUNOTHERAPEUTIC AGENTS

In Vitro Generation of RMC

RMC generated *in vitro* for therapeutic evaluation are propagated typically from rodent BM (BM) cells or human PBMC (Figure 1). Although differentiation procedures between species are similar, distinct starting cell populations make the translation of findings from rodents to humans difficult (15). Moreover, RMC therapy lacks standard differentiation protocols since the optimal immune regulatory properties of each RMC population are unknown (16). Although MDSC have not been evaluated for immune regulatory function in humans, protocols for the propagation and administration of Mreg and DCreg have been described in human renal transplantation and in healthy volunteers or type 1-diabetics, respectively (Table 1). Importantly, no adverse effects of RMC therapy have been reported in these limited clinical studies to date.

Human Mreg are differentiated from donor PBMC acquired by leukapheresis, in recombinant human M-CSF for 6 days, followed by 24h stimulation with IFN-γ (17). Human DCreg are typically differentiated from PBMC or purified monocytes in the presence of granulocyte-macrophage (GM)-CSF and interleukin (IL)-4, with the addition of one or more factors that promote their tolerogenicity (reviewed in (11, 18)). DCreg are typically immature myeloid DC and maturation-resistant, or ‘alternatively-activated’ (e.g. exposed to IL-10 and transforming growth factor β [TGFβ] during propagation, then stimulated with LPS), so that they maintain expression of major histocompatibility complex (MHC) molecules, but display low levels of co-stimulatory molecules and pro-inflammatory cytokines. Vitamin D3 (vitD3) and dexamethasone promote DCreg (19, 20). Thus, activation of human DC cultured in vitD3/dexamethasone with lipopolysaccharide (LPS) results in stable, ‘alternatively-activated,’ semi-mature DC (21). Addition of IL-10 (‘DC-10’) (22) or the mechanistic target of rapamycin (mTOR) inhibitor, rapamycin (RAPA) (23) to human monocyte cultures also produces DCreg. Non-human primate (NHP) monocyte-derived DCreg can be generated using vitD3 and IL-10 (24, 25). DCreg are also made using low dose GM-CSF in the absence of IL-4 (26). Thus, although Mreg differentiation is comparatively well-defined, there is significant variability in methods to generate DCreg. Importantly, generation of recipient-derived RMC for clinical use must be validated with PBMC from patients with pre-existing disease (27). In this regard, DCreg

generated from patients with rheumatoid arthritis (28) or relapsing-remitting multiple sclerosis (29) exhibit a similar phenotype and function to DCreg generated from healthy controls.

MDSC exhibit considerable phenotypic heterogeneity and are subdivided into those that resemble monocytes or are similar phenotypically to neutrophils (30). They require factors to induce their activation, in addition to their expansion (13). Thus, mouse monocytic MDSC are generated from BM cells in G-CSF, GM-CSF or both, and activated with IL-6 or IL-13 (31, 32). Table 2 outlines adoptive MDSC therapies that have been evaluated in mouse models of skin or pancreatic islet cell transplantation, graft-versus-host disease (GVHD) and type 1-diabetes. Human MDSC generated from PBMC with GM-CSF+IL-6 appear to exert the most potent suppressive capacity, but GM-CSF+IL-1β, prostaglandin (PG)E2, tumor necrosis factor α (TNFα) or vascular endothelial growth factor (VEGF) also induce suppressive MDSC (33). Similarly, GM-CSF and IL-6 can be used to generate suppressive human BM-derived MDSC (31). Addition of PGE2 to GM-CSF and IL-4-stimulated human PBMC cultures blocks DC differentiation and promotes MDSC generation (34).

RMC therapies need to be designed in conjunction with current IS protocols due to the success of the latter in achieving high short-term organ allograft survival rates (15). Thus, experimental RMC therapy needs to be undertaken with an appropriate IS agent(s) that maintains their tolerogenic properties. In rodent organ transplant models, Mreg (35) and DCreg (11) synergize with pharmacologic agents, anti-lymphocyte serum or co-stimulation blockade, but the impact of IS agents on MDSC is largely unknown.

Selection of Donor or Recipient RMC for Therapy

Mouse Mreg prolong allograft survival only when donor-derived (35). Although there is a potential risk of sensitizing the recipient to donor, this has not been observed in the human renal transplant recipients given Mreg to date (36, 37). The risk is mitigated by infusing the cells one week before transplantation (to avoid surgically-induced inflammation) and choosing IS agents that are likely to maintain the tolerogenic properties of RMC in the face of inflammation.

Both donor- and recipient-derived DCreg have been investigated extensively in rodent transplant models (11, 15, 18, 38). While allogeneic DC trafficking from rodent organ grafts may survive in lymphoid tissue for several days in unmanipulated hosts or even weeks in immunosuppressed recipients (39, 40), these donor DC may be also killed by host natural killer (NK) cells (41) and reprocessed by endogenous DC able to present donor alloAg (42). Donor Mreg survive in humans for at least 30 hours in the spleen, liver and BM (17) and 2 weeks in mice in the lung (35). Although DCreg can be generated from the graft recipient at any time, the optimal method of loading donor alloAg (donor cell lysate, exosomes, apoptotic cells) has not been established (18). One group has used unpulsed autologous DCreg to promote long-term rodent allograft survival, thus maturation-resistant DCreg are given in the peri-transplant period and acquire donor alloAg *in situ* (43–45).

Similar events could accompany cell therapy with MDSC, since these cells are also able to process and present Ag (46, 47). As precursors of myeloid cells, MDSC can differentiate into DC and macrophages (31, 48–50), but MDSC have not been found to potentiate immunity following their adoptive transfer (Table 2) and retain immune regulatory function, even if they do differentiate (31, 50). On the other hand, cyclooxygenase (COX)2 activation by inflammatory mediators such as IL-1β and IFN-γ prevents the differentiation of MDSC into DC (51), while IFN-γ is an important stimulator of MDSC suppressive function (52). These properties resemble those of Mreg that are activated by IFN-γ (36) and provide the

advantage that inflammatory conditions such as occur in organ transplantation may reinforce the suppressive activity of MDSC. Thus, selection of donor or recipient RMC presents its own distinct challenges, such as circumventing allosensitization, and the need for/nature of Ag pulsing.

Ag Specificity

The ability of RMC to regulate immune responses in an Ag-specific manner is an important consideration to avoid global immunosuppression. Mouse (35) and human (17) Mreg suppress mitogen-activated $CD4^+$ and $CD8^+$ T cell proliferation, and mouse Mreg delete alloreactive T cells specifically *in vitro* (35). Moreover, donor-, but not recipient- or third party-derived Mreg, prolong mouse cardiac allograft survival (35), suggesting that Mreg can regulate alloAg-specific immunity *in vivo*. Administration of transplant acceptance-inducing cells (TAIC), i.e. unpurified Mreg, to human renal transplant recipients has been reported to promote donor-specific hyporesponsiveness, even in a pre-sensitized recipient (37, 53).

Donor- and host-derived DCreg promote long-term allograft survival or donor-specific tolerance in rodent transplant models when combined with anti-lymphocyte serum (ALS), anti-CD40L (CD154) mAb or cytotoxic T lymphocyte Ag (CTLA)4-Ig (54–58). Importantly, local administration of immature autologous DC to healthy human volunteers results in inhibition of Ag-specific $CD8^+$ T cell effector function (59) and generation of regulatory $CD8^+$ T cells (60). These latter findings provide proof-of-principle that DC have the capacity to regulate Ag-specific responses in humans. Recently, donor-derived DCreg have been shown to prolong organ allograft survival in a robust pre-clinical NHP renal transplant model accompanied by reduction in donor-reactive Tmemory cell responses (25).

The Ag specificity of MDSC suppressive function depends on the model, microenvironment and activation of target lymphocytes (61). MDSC can inhibit both $CD4^+$ and $CD8^+$ T cell reactivity (46, 52, 62–64). They can suppress Ag-specific $CD8^+$ T cell responses (46), but it is not known whether they are capable of Ag-specific $CD4^+$ T cell suppression (13), especially in view of their low or absent MHC class II expression (65). Importantly, MDSC generated *in vitro* can promote Ag-specific $CD8^+$ T cell hyporesponsiveness (31). In a mouse model of cardiac allograft tolerance induced by donor-specific transfusion (DST) and anti-CD40L mAb, suppression of T cells by graft-infiltrating MDSC was non-specific, and BM and splenic monocytes did not suppress (66). Taken together, DCreg and Mreg have Ag-specific regulatory capacity in transplantation, but the conditions under which MDSC suppress alloimmunity in an Ag-specific manner need to be better understood in order to harness these cells for therapeutic application.

Trafficking and Migration of RMC under Inflammatory Conditions and Following their Adoptive Transfer

There is evidence that human Mreg administered via central venous access migrate to the lungs and then distribute to the liver, spleen and BM within 30h of their infusion (17). Murine Mreg demonstrate a similar distribution pattern following intravenous (i.v.) injection and notably do not migrate to lymph nodes (35). Little is known about chemokine receptor expression on Mreg and the location(s) where they exert their regulatory function *in vivo* is not known (4).

Expression of CCR7 by DC directs them to secondary lymphoid organs where they interact with T cells. Adoptively-transferred, IL-10-expressing DC require CCR7 to prolong mouse cardiac allograft survival (67), suggesting that DCreg, and likely Mreg, must traffick to secondary lymphoid for their regulatory function. Notably, IL-10 reduces DC CCR7 expression and lymph node homing ability (68). Upregulation of CCR7 following activation

of DCreg by Toll-like receptor (TLR) ligation *in vitro* may be required to improve the migratory function of these cells (69). Following i.v. injection, rodent host-derived DCreg migrate rapidly to the spleen (70, 71), while RAPA-conditioned DC migrate to the lymph nodes following intramuscular injection (72). The route of DCreg administration may be critical to optimize their function *in vivo* (69). While i.v. DCreg injection prolongs cardiac allograft survival in mice, subcutaneous injection of the same DCreg does not affect graft survival (73). Similarly, in a NHP model, i.v. administration of DCreg results in immune regulation (24), whereas intradermal injection may boost the immune response (15). In human cancer patients, intradermal injection increases the migration of immature DC to the draining lymph nodes compared to subcutaneous administration (74); however, subcutaneous administration of immature DC has been shown to regulate $CD8^+$ T cell responses to model Ags in humans (59, 60). Together, these studies suggest that optimization of delivery route is critical to DCreg function and that directing their migration to secondary lymphoid organs is important.

MDSC express chemokine receptors, such as CX3CR1 (75) or CCR2 (46, 76), that direct them towards sites of inflammation, but they can also be directed towards secondary lymphoid organs by expression of CD62L (32, 46) and CCR7 (32). It is unknown whether MDSC migration to the allograft, secondary lymphoid organs or both is preferable following their adoptive transfer; however, MDSC are required to migrate to the graft and not lymph nodes for experimental transplant tolerance induced by donor-specific infusion and anti-CD154 mAb (66). The complement component C5a participates in the recruitment of MDSC to tumors and peripheral lymphoid organs in mice (77). Thus, it will be of interest to determine whether C5a plays a similar role in transplant rejection, since C5 is integral to Ab-mediated rejection (78). *In vitro*-generated MDSC traffic to peripheral lymphoid tissue and sites of inflammation in GVHD, including the liver and spleen (79) or spleen and lymph nodes (32). MDSC expanded *in vivo* in response to LPS that inhibited alloimmunity migrated to the spleen when transferred to skin transplant recipients, but their migration to the graft was not assessed (80). MDSC accumulate within tumors (50) and at sites of inflammation in murine experimental allergic encephalomyelitis (EAE) (52) and chronic contact eczema (63). They also accumulate within the spleen (50, 63, 81) and lymph nodes (50, 63) in inflammatory disease and cancer. Following transplantation, rodent MDSC are found in the allograft and peripheral blood (66, 82, 83) as the result of migration from the BM (66). Although human MDSC were reported to be elevated in the peripheral blood of renal transplant recipients, they were not assessed in biopsy tissue (84).

In summary, RMC therapies have demonstrated promising immune regulatory capacity. However, it will be necessary to rationally design protocols in transplantation that optimize *in vitro* generation of RMC whose *in vivo* migration (to the appropriate sites) and function are supported by the IS regimen. Further pre-clinical studies are warranted to optimize each parameter in increasingly stringent models from rodent to NHP, while also continuing to progress RMC therapy in human transplant recipients.

TARGETING RMC WITH THERAPEUTIC AGENTS

This section summarizes reports concerning the influence of IS drugs, specific therapeutic Abs and novel immunoregulatory strategies on DC, macrophages and MDSC (Table 3).

Conventional IS Drugs

Transplant recipients receive pharmacologic and biological agents to control graft rejection, and although the principal mechanism of action of these agents is inhibition of T cell responses, they also modulate RMC. The influence of anti-inflammatory agents, IS drugs

and biologic IS, on DC function *in vivo* has been reviewed in detail elsewhere (10, 85, 86). Studies of their influence on Mreg and MDSC are limited.

The most extensively-studied IS drugs that target DC *in vivo* are glucocorticoids (GC), calcineurin inhibitors (CNI), RAPA (sirolimus) and mycophenolate mofetil (MMF) (10). The *in vivo* effects of GC on DC have been reviewed by van Kooten *et al* (87). Specifically, GC reduce peripheral DC numbers and inhibit their maturation and production of pro-inflammatory cytokines, while enriching for Mreg (88, 89). Endogenous GC promote the expansion of MDSC in a murine model of trauma (90), and exposure of monocytes to GC induces $CD11b^{+}Gr\text{-}1^{+}CD124^{+}Ly6C^{med}$ MDSC (91). Administration of dexamethasone to glioblastoma patients increases circulating $CD14^{+}HLA\text{-}DR^{lo/neg}CD80^{-}$ immunosuppressive cells, that resemble MDSC (92).

CNI, i.e. cyclosporine A (CsA) and tacrolimus (FK506), are front-line anti-rejection agents used in combination with an anti-proliferative agent, in particular MMF. CsA and tacrolimus, but not RAPA, inhibit MHC-restricted Ag presentation by DC *in vitro* (93) and *in vivo* (94). Tacrolimus treatment of mice reduces responsiveness of macrophages and DC to LPS (95). Numbers of thymic DC and macrophages are decreased in rats during CsA treatment (96–98); however, their function appears to be unaffected (96). On the other hand, increased numbers of DC have been reported in NHP with long-surviving renal allografts treated with both tacrolimus and sirolimus (99). CsA combined with CCR5 blockade increases cardiac graft survival in NHP, an effect that is associated with generation of alternatively-activated macrophages through activation of the peroxisome proliferator-activated receptor (PPAR)γ (100). Additionally, CsA inhibits the phenotypic maturation, endocytic activity and allostimulatory function of human peripheral blood DC (101). CsA or tacrolimus increases the incidence of mDC in peripheral blood of human heart transplant recipients, but no difference in expression of the DC maturation marker CD83 is observed (102). To our knowledge, direct effects of CNI on MDSC have not been studied; however, expression of the immunophilin FK506 binding protein 51 is increased in monocytic and granulocytic MDSC isolated from tumor-bearing mice and regulates their suppressive function (103). Additionally, calcineurin and nuclear factor of activated T cells (NFAT) signaling are negative regulators of myelopoiesis, and CsA augments numbers of differentiated DC *in vitro* (104). Therefore it appears likely that CNI impact MDSC.

MMF is an anti-proliferative pro-drug of mycophenolic acid (MPA) that inhibits B and T cell proliferation (105). MPA also suppresses DC maturation and reduces Ag presentation to T lymphocytes (106–109). As MPA has been reported to suppress granulopoiesis, it is possible that it also affects MDSC.

RAPA inhibits the serine threonine kinase mechanistic target of rapamycin (mTOR) (110). Its administration to mice impairs DC costimulatory molecule up-regulation, production of proinflammatory cytokines, and T cell allostimulatory function (111–113). Moreover, RAPA induces apoptosis in DC, but not in monocytes or macrophages (114). Haidinger et al (115) found that DC in kidney transplant patients treated with RAPA displayed increased immunostimulatory potential compared with those in patients treated with CNI and in healthy controls. Interestingly, RAPA prevents the anti-inflammatory effects of GC on human monocytes as well as myeloid DC (116). Moreover, RAPA conditioning augments IL-12 production by mouse BM-derived DC or human monocyte-derived DC stimulated with LPS or pro-inflammatory cytokines, respectively (117, 118). Thus, under different circumstances RAPA can exert pro- or anti-inflammatory effects on DC. mTOR is required for DC development, so it will be interesting to determine whether RAPA affects MDSC due to its ability to inhibit myelopoiesis (119).

Thus, in addition to the ability of conventional IS agents to inhibit B and T cell activation, these drugs exert profound, but variable, effects on macrophage and DC differentiation and function.

Experimental IS Agents

Histone deacetylase (HDAC) inhibitors (including suberoylanilide hydroxamic acid, trichostatin A and valproic acid) are anti-tumor agents that also have anti-inflammatory properties. HDAC inhibitors reduce TLR-induced costimulatory molecule expression and pro-inflammatory cytokine release by DC and their T cell allostimulatory activity *in vitro* and *in vivo* (120–122). HDAC inhibition blocks GM-CSF-dependent function in macrophages and their differentiation to DC (123), but there are contradictory reports regarding its influence on cytokine secretion (124, 125), that may reflect the specific HDAC inhibitor or dose used. We have demonstrated recently (126) that HDAC inhibitors augment GM-CSF-mediated murine MDSC expansion *in vitro* and *in vivo*, and that these MDSC exhibit similar suppressive potency to control MDSC.

Proteasome inhibitors, such as bortezomib, are believed to block the activation and nuclear translocation of NF-κB, a transcription factor central to DC maturation and inflammatory responses (127). In experimental hematopoietic stem cell transplantation, bortezomib attenuates GVHD, yet preserves graft-versus-leukemia activity (128, 129). Administration of bortezomib to mice results in a more immature DC phenotype (130). Bortezomib reduces the phagocytic capacity of human monocyte-derived DC, skews their phenotypic maturation and reduces their cytokine production and immunostimulatory capacity. It also reduces their chemokine secretion and migration (131), while promoting their apoptosis and reducing the yield of viable DC (131), preferentially targeting immature DC (127).

There are also anti-inflammatory agents that modulate RMC function. NF-κB inhibitors block DC maturation and can induce tolerance in murine cardiac transplantation (132–134). Interestingly, NF-κB is implicated as a critical regulator of MDSC suppressive function (135). Furthermore, COX-2 inhibitors prevent production of PGE2 and reduce numbers of MDSC (136), and can prolong murine cardiac allograft survival (137). There is also evidence that a PGE2 receptor (EP4) agonist suppresses the activation of macrophages and prolongs mouse cardiac allograft survival (138).

Thus, various experimental IS agents currently under investigation are capable of modifying RMC function. Typically, they reduce DC maturation, but appear to have varying effects on MDSC expansion and function.

In Vivo RMC Targeting with Abs and Other Novel Approaches

T cell-depleting Abs also target RMC. Thus, polyclonal anti-thymocyte globulin (ATG) inhibits human DC Ag uptake and maturation, induces complement-mediated lysis of DC, and decreases the capacity of DC to stimulate allogeneic T cells *in vitro* (139). Additionally, ATG polarizes DC towards expression of indoleamine dioxygenase (IDO) (140) that inhibits T cell proliferation. Anti-CD52 mAb (Alemtuzumab; Campath-1H) depletes peripheral blood DC, but not tissue DC, due to differential expression of CD52 on DC in these sites (141, 142). It causes a sustained reduction of total peripheral DC in kidney transplant recipients (143). In addition to T cells, human DC express CD25 after stimulation (144, 145), making them a potential target for anti-CD25 (IL-2 receptor α subunit) mAb. Furthermore, anti-CD25 mAb treatment diminishes the ability of human DC to stimulate T helper cells (144), but does not affect HLA-DR or costimulatory molecule expression by the DC after LPS stimulation (145). Recent work using daclizumab (humanized anti-CD25 mAb) has shown that it potently inhibits Ag-specific T cell activation by human mature DC

in vitro (146). Interestingly, anti-CD25 mAb combined with IL-12 depletes MDSC in a mouse model of colon carcinoma (147).

Co-stimulation blockade is an emerging strategy to promote graft survival by interfering with T cell activation, in which APC play an important role. Development of co-stimulation blockers has focused mainly on targeting T cell surface co-stimulatory molecules, although some also target APC (148, 149). Notably, anti-CD28 mAb induces tolerance to rat kidney allografts in association with accumulation of circulating and graft-infiltrating MDSC that suppress effector T cell expansion (82). Belatacept (CTLA4-Ig) is the first costimulation blocker approved for renal transplantation. There is evidence that CTLA4-Ig binding to CD80/CD86 molecules provides a reverse signal to DC that results in the induction of indoleamine dioxygenase (150), and that enhanced secretion of inhibitory products by CTLA4Ig-exposed DC promotes alloantigen-specific transplant tolerance (151). However, it has been reported recently that CTLA4-Ig immunosuppressive activity may not depend on a DCreg phenotype, but on its presence during DC/T cell interaction (152). Interestingly, Ab blockade of CTLA-4 reduces the suppressive potential of MDSC in tumor-bearing mice (153). Anti-CD40 mAbs prolong renal and islet allograft survival in NHP (154, 155), while mouse mDC under CD40 blockade have a tolerogenic profile in vivo (156) and are responsible for inducing peripheral Treg and delaying cardiac allograft rejection (157).

Gene silencing of TLR adaptors, namely myeloid differentiation primary response gene (MyD) 88 and TIR-domain-containing adapter-inducing interferon-β (TRIF), using siRNA reduces DC maturation and prolongs murine cardiac allograft survival (158). Administration of recombinant G-CSF (Neupogen) prolongs skin transplant survival in mice and induces MDSC in peripheral lymphoid compartments (159). Suppressive granulocytic and monocytic MDSC are expanded in human stem cell donors during G-CSF-mobilization protocols for allogeneic hematopoietic stem cell transplantation (160). Furthermore, human inhibitory receptor ILT2, expressed on activated T cells and engaged by HLA-G on DC, has been shown to amplify MDSC and to promote long-term allograft survival (75).

Thus, although previously thought to act primarily on T cells, T cell-depleting inhibitory Abs also profoundly affect DC function, and novel approaches using costimulation blockade, siRNA or recombinant growth factors can promote MDSC.

CONCLUSION

RMC constitute an important, heterogeneous innate immune cell population with considerable promise for cell therapy. The influence of IS agents on these cells is becoming increasingly apparent. While the use of RMC as cellular therapeutics is beginning to advance from pre-clinical models to patients with inflammatory diseases, further insights into the differentiation and function of Mreg, DCreg and MDSC are required in order to maximize the utility of these cells. In addition to conventional IS drugs, novel therapeutic agents can promote the regulatory function of RMC, while preventing their immunostimulatory potential. These agents are likely to prove of considerable importance in exploiting the properties of RMC to promote transplant tolerance.

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Abbreviations

Ag	antigen
ALS	anti-lymphocyte serum
APC	antigen-presenting cell
BM	bone marrow
CNI	calcineurin inhibitor
COX	cyclooxygenase
CsA	cyclosporine A
CTLA4	cytotoxic T lymphocyte Ag 4
DC	dendritic cell
DCreg	regulatory dendritic cell
DST	donor-specific transfusion
EAE	experimental allergic encephalomyelitis
GC	glucocorticoids
GM-CSF	granulocyte macrophage colony stimulating factor
GMP	good manufacturing practice
GVHD	graft-versus-host disease
HDAC	histone deacetylase
IFN	interferon
IL	interleukin
ILT2	inhibitory receptor Ig-like transcript 2
IS	immunosuppressant/immunosuppressive
i.v.	intravenous
LPS	lipopolysaccharide
MDSC	myeloid-derived suppressor cell
M-CSF	macrophage colony stimulating factor
MHC	major histocompatibility complex
MMF	mycophenolate mofetil
Mφ	macrophage
MPA	mycophenolic acid
Mreg	regulatory macrophage
mTOR	mechanistic target of rapamycin
MyD88	myeloid differentiation primary response gene 88
NFAT	nuclear factor of activated T cells
NHP	non-human primate
NK	natural killer

PBMC	peripheral blood mononuclear cell
PG	prostaglandin
RAPA	rapamycin
RMC	regulatory myeloid cell
TAIC	transplant acceptance-inducing cell
TLR	Toll-like receptor
TNF	tumor necrosis factor
Tx	transplantation
vitD3	vitamin D3

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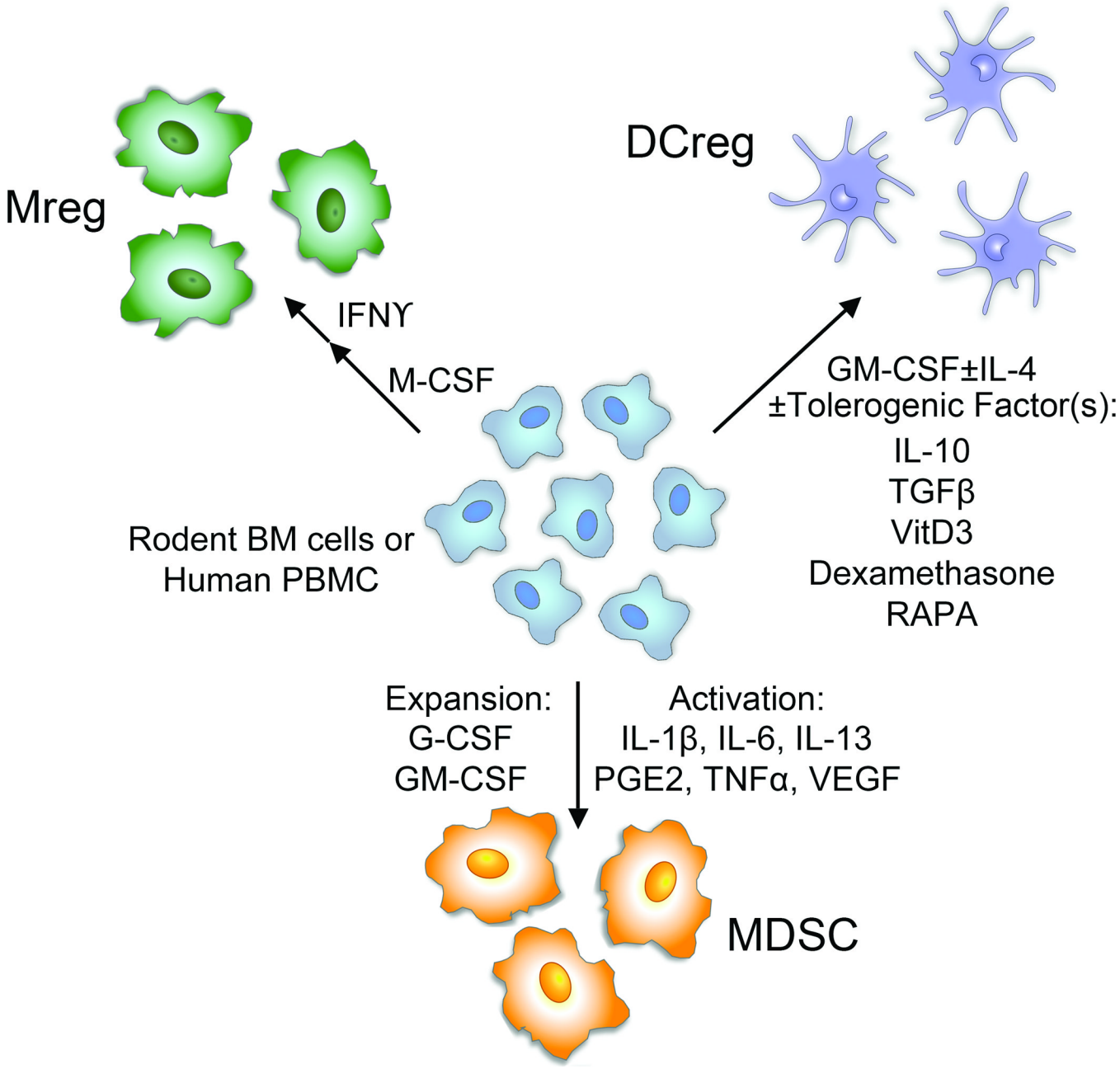

Figure 1.

Generation of RMC *in vitro* from rodent BM cells or human PBMC. Mreg, DCreg and MDSC can be generated *in vitro* from precursors in rodent BM or human PBMC exposed to specific growth factors. In some cases, RMC (Mreg and MDSC) are also activated *in vitro* by the addition of inflammatory cytokines or other soluble factors. DCreg are often generated in the presence of anti-inflammatory cytokines or agents that suppress their activation into stimulatory DC.

Table 1

Influence of RMC administration in humans.

RMC	Source	Study Name	Clinical	Protocol	Outcome	Reference
Immature DC	Autologous blood monocytes cultured in GM-CSF and IL-4 pulsed with Ag	N/A	N/A	2×10^6 s.c.	Ag-specific inhibition of $CD8^+$ effector T cell function and generation of $CD8^+$ Treg	(59, 60)
Immature or tolDC	Autologous unmanipulated DC (n=3) or DC treated with antisense oligonucleotides for CD40, CD80 and CD86 (n=7)	N/A	Type-1 diabetes (Phase I safety study)	10^7 cells intradermally every 2 weeks for 4 doses	10/10 no adverse events; Significant increase in peripheral $B220^+CD11c^-$ B cell frequency	(161)
Mreg (TAIC)	Donor splenic mononuclear cells cultured in M-CSF and stimulated with IFN-γ	TAIC-I	Deceased donor renal transplantation	$1.0–7.52\times10^6$ cells/kg by central venous infusion d5 (Patient receiving 0.55×10^6 cells/kg excluded); tacrolimus, sirolimus and glucocorticoid triple therapy for first month then weaned to tacrolimus monotherapy with progressive tacrolimus weaning	8/10 weaned from steroids; 6/10 weaned to low dose tacrolimus monotherapy	(162)
Mreg (TAIC)	Donor PBMC cultured in M-CSF and stimulated with IFN-γ then co-cultured with recipient PBMC prior to infusion of all cells	TAIC-II	Living donor renal transplantation	$1.74–10.39\times10^7$ co-cultured cells/kg by central venous infusion d-5; ATG (d0, 1 and 2), prednisolone and tacrolimus started at 8–12 ng/ml trough levels and weaned to 5–8 ng/ml; prednisolone stopped by 10 weeks if graft function stable	5/5 no adverse events; 3/5 on low-dose tacrolimus monotherapy; 1/5 withdrawn from all immunosuppression for 8 months	(53)
Mreg (TAIC)	Donor PBMC cultured in M-CSF and stimulated with IFN-γ then co-cultured with recipient PBMC prior to infusion of all cells	Case Study (TAIC-II)	Presensitized living-related renal transplantation	6.9×10^7 co-cultured cells/kg by central venous infusion d-17; ATG (d0, 1 and 2), prednisolone and tacrolimus started at 8–12 ng/ml trough levels and weaned to 4–8 ng/ml at week 35	No acute rejection episodes up to 27 months; donor-specific hyporesponsiveness and loss of donor-specific Ab	(37)
Mreg	Donor PBMC cultured in M-CSF and stimulated with IFN-γ	N/A	Living-donor renal transplantation	2 mg/kg/d AZA beginning 3d prior to central venous infusion of Mreg (7.1 or 8×10^6 cells/kg) until 8 week post-Tx; Mreg given 6–7d before Tx; tacrolimus and prednisolone begun day of Tx and prednisolone weaned by 10 weeks resulting in tacrolimus monotherapy (4–8 ng/ml trough)	n=2 patients maintained on low dose tacrolimus monotherapy with excellent graft function and no rejection episodes >3 year post-Tx	(17)

Abbreviations: Ab, antibody; ATG, anti-thymocyte globulin; AZA, azathioprine; GM-CSF, granulocyte macrophage colony stimulating factor; IFN, interferon; IL, interleukin; N/A, not applicable; PBMC, peripheral blood mononuclear cells; RMC, regulatory myeloid cell; TAIC, transplant acceptance-inducing cell; Tx, transplant

Table 2

MDSC transfer for immune modulation or cell therapy of allograft rejection.

Condition	Source of MDSC	Cell Dose and Route	Model	Mechanism and Outcome	Reference
(i) No transplant	Tumor-bearing mouse splenocytes	$3–5\times10^6$ i.v. 2–3d after T cell transfer	Ag-specific transgenic $CD8^+$ T cell	Ag-specific $CD8^+$ T cell tolerance but T cells remained responsive to non-specific αCD3 stimulation	(50)
	Tumor-bearing mouse BM	5×10^6 i.v. + 5μg/mouse peptide Ag d1 after T cell transfer	Transgenic T cell induction of diabetes	75% diabetes-free at d30 (Ag-specific) with T cell anergy and induction of Treg	(64)
	Tumor-bearing mouse BM	2×10^7 i.v. with T cells	NOD/SCID with transfer of diabetogenic T cells	60% diabetes-free at d100 with reduced lymphocyte infiltration and insulitis	(64)
(ii) Skin transplant	Transplant recipient splenocytes	2×10^5 i.v. on d-1 and d3	MHC class II-mismatched skin allograft	50% long-term survival when transplant-activated MDSC transferred from ILT2 (HLA-G receptor) transgenic mice but not wild-type mice	(75)
	Splenocytes from LPS-treated mice	5×10^6 i.v. on d-1	Male to female or MHC class II-mismatched skin allograft	Prolonged allograft survival dependent on heme oxygenase-1	(80)
(iii) Islet cell transplant	BALB/c BM cultures with GM-CSF+IL-6 or GM-CSF+G-CSF	10^7 i.v. on d0, 7, 14 and 21	Islet allograft (B6 to BALB/c)	Long-term survival in ~75% (GM-CSF+IL-6 MDSC) or ~40% (GM-CSF+G-CSF MDSC) without generalized immune suppression	(31)
	B6 BM cultures with GM-CSF with liver stellate cells (B6, BALB/c or C3H)	2.5×10^6 mixed with islets	Islet allograft (BALB/c to B6)	~45–65% long-term survival, B7-H1-dependent increase in Treg that mediate T cell hyporesponsiveness	(163)
(iv) GVHD	B6 BM cultures with GM-CSF+G-CSF+IL-13	2 or 6×10^6 i.v. with donor cells	GVHD (B6 to BALB/c)	Cell dose- and arginase-1-dependent improved survival with inhibition of $CD4^+$ and $CD8^+$ T cell responses and maintained graft-versus-leukemia effect	(32)
	129SvEv embryonic stem cell line cultured with KL, VEGF, Flt3L, TPO and M-CSF	2×10^6 i.v. with donor cells, d4 and d10	GVHD (129SvEv to BALB/c)	82% long-term survival	(79)

Abbreviations: G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte macrophage colony stimulating factor; ILT, immunoglobulin-like transcript; KL, c-Kit ligand; M-CSF, macrophage colony stimulating factor; NOD/SCID, non-obese diabetic/severe combined immune deficiency; VEGF, vascular endothelial growth factor; Flt3L, fms-like tyrosine kinase 3 ligand; TPO, thrombopoietin

Table 3

Influence of immunosuppressive drugs, biologic agents and novel immunoregulatory agents on myeloid DC, macrophages and MDSC *in vivo*.

Therapeutic Agent	Type of cell	Species/Model	Effect/s on cells	Reference
GCs, Dexamethasone	mDCs & Mφ	Delayed-type hypersensitivity mice	Depletion of mDC and pDC, and Mφ enrichment	(89)
	MDSC	Trauma model mice	Expansion of MDSC	(90)
1α,25(OH)$_2$D$_3$ (VitD3)	DCs	-	Modulation of phenotype and function towards tolerogenic DC	(164, 165)
	MDSC	Tumor-bearing mice	Diminished presence of MDSC within regional lymph nodes, spleens and tumors, restoration of their Ag-presenting ability and differentiation towards a DC phenotype	(166–168)
Cyclosporine A	DC	D-type hypersensitivity mice	Defective Ag acquisition and MHC-restricted Ag presentation	(169)
	DC & Mφ	Rat	Reduced numbers in thymus	(96–98)
	Mφ	Cardiac Tx in NHP (combined with CCR5 blockade)	Generation of alternatively-activated Mφ	(100)
	DC	Heart Tx patients	Increase in circulating mDC percentage	(102)
	DC & Mφ	Mouse kidney Tx model and humans	Reduction in bacterial phagocytosis	(170)
Tacrolimus	DC & Mφ	LPS-induced inflammatory response (mouse)	Decreased responsiveness to LPS, and blocking of MHC-restricted Ag presentation	(94, 95)
	Mφ	Brain-injured rats	Reduction in the number recruited to the inflammatory site, and their proliferative activity	(171)
	DC	Atopic dermatitis patients	Decrease in IgE receptors	(172)
Rapamycin	DC	-	Impairment of DC costimulatory molecule up-regulation, production of pro-inflammatory cytokines, and T cell allostimulatory capacity, and induction of apoptosis	(113, 114)
		Kidney transplant patients	Increased immuno-stimulatory potential	(115)
Mycophenolate Mofetil(MMF)	DC	Contact hypersensitivity (mice)	Impaired Ag-presenting capacity	(109)
	Mφ	Renal Tx rats	Inhibition of Mφ infiltration	(173)
HDAC inhibitors	DC	Graft-versus-host-disease mice, and humans	Reduced costimulatory molecule expression, pro-inflammatory cytokine release, and T cell allostimulatory activity	(120, 121)

Therapeutic Agent	Type of cell	Species/Model	Effect/s on cells	Reference
Proteasome inhibitors	DC	Mice	Impairment of DC maturation and cytokine production, as well as DC-mediated T cell stimulation	(130)
NFκB inhibition:				
- Azithromycin	DC	Murine histo-incompatibility model	Inhibition of DC maturation	(174)
- Liposomes containing NFκB decoy oligodeoxynucleotides (ODN)	Mφ	Kidney transplantation (rats)	Reduction of periarterial Mφ infiltration	(175)
PGE2 receptor (EP4) agonist	Mφ	Cardiac Tx mice	Suppression of Mφ activation	(138)
Polyclonal antithymocyte globulin (ATG) Ab	mDC	Allogeneic stem cell Tx patients	Reduction of circulating mDCs	(176)
Anti-CD52 mAb	mDC	Kidney transplant patients	Strong and sustained reduction in the total number of peripheral DC and a significant shift from myeloid to plasmacytoid DC subsets	(143)
CTLA4Ig	DC	Cardiac Tx rats	Secretion of inhibitory products that suppress alloAg-induced T cell proliferative responses	(151)
Anti-CD154	DC	Cardiac Tx mice	Potentiation of DC tolerogenicity	(156)
Anti-CD28 mAb	MDSC	Kidney Tx rat	MDSC accumulation in the blood and allograft	(82)
siRNA gene silencing of MyD88 and TRIF	DC	Cardiac Tx mice	Reduction of DC maturation	(158)
Recombinant G-CSF (Neupogen)	MDSC ($Gr\text{-}1^{+}CD11b^{+}$)	Skin Tx mice	Induction of a high frequency of MDSC in the peripheral lymphoid compartments	(159)
Human ILT2	MDSC	Skin Tx mice	Increased MDSC ($CD11b^{+}Gr\text{-}1^{+}$) and enhanced long-term survival of allografts	(75)

Abbreviations: DC, dendritic cell; GCs, glucocorticoids; HDAC, histone deacetylase; ILT2, immunoglobulin-like transcript 2; mDC, myeloid dendritic cell; Mφ, macrophage; MyD88, myeloid differentiation primary response gene 88; NHP, non-human primate; TRIF, Toll-IL-1 receptor domain containing adaptor-inducing interferon-β; tx, transplant; vitD3, vitamin D3