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Orchestration of transplantation tolerance by regulatory dendritic cell therapy or in situ targeting of dendritic cells

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Abstract

Purpose of review—Extensive research in murine transplant models over the past two decades has convincingly demonstrated the ability of regulatory dendritic cells (DCreg) to promote long-term allograft survival. We review important considerations regarding the source of therapeutic DCreg (donor or recipient) and their mode of action, in situ targeting of DCreg, and optimal therapeutic regimens to promote DCreg function.

Recent findings—Recent studies have defined protocols and mechanisms whereby ex vivo-generated DCreg of donor or recipient origin subvert allogeneic T cell responses and promote long-term organ transplant survival. Particular interest has focused on how donor antigen (Ag) is acquired, processed and presented by autologous DCs, on the stability of DCreg, and on in situ targeting of DC to promote their tolerogenic function. New evidence of the therapeutic efficacy of DCreg in a clinically-relevant non-human primate organ transplant model and production of clinical grade DCreg support early evaluation of DCreg therapy in human graft recipients.

Summary—We discuss strategies currently used to promote DC tolerogenicity, including DCreg therapy and in situ targeting of DC, with a view to improved understanding of underlying mechanisms and identification of the most promising strategies for therapeutic application.

Keywords

dendritic cells; cell therapy; transplant tolerance

Introduction

Dendritic cells (DC) have inherent tolerogenic properties that can be harnessed to promote long-term organ allograft survival and reduce transplant recipients' dependence on immunosuppressive drugs. New mechanistic insights have recently been gained into the

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Conflict of Interest

One of the authors (AWT) is co-inventor of US patents for generation of DCreg to improve organ transplant survival.

underlying basis of regulatory DC (DCreg) therapy and into how in situ targeting of DC can be exploited to promote tolerance. Important steps have also been taken to evaluate the safety and efficacy of DCreg in a clinically-relevant non-human primate (NHP) organ transplant model and to generate clinical grade DCreg that have entered phase 1 clinical trials.

Donor-derived versus Ag-pulsed autologous (AT) DCreg therapy in transplantation

During the '90s, growing understanding of the role of DC of myeloid lineage in peripheral T cell tolerance [1,2], together with advances in methods to generate large numbers of DC in the laboratory, led to the possibility of “negative vaccination” with regulatory DC (DCreg). These cells could be shown to restrain immune responses to cell and organ allografts [3,4] and to promote long-term transplant survival [5]. Numerous techniques have been described subsequently to generate DCreg from peripheral blood monocytes, CD34⁺ hematopoietic progenitors, or bone marrow precursors in human, NHP and murine models, and to render DC stably immature or maturation-resistant by pharmacologic, anti-inflammatory cytokine (most recently IL-27 [6]) or genetic manipulation in vitro (reviewed in [7-13]). These investigations include studies on rarer, non-conventional plasmacytoid DC, that also offer potential as regulatory innate immune cells in transplantation [14*].

Early studies in murine models demonstrated that systemic administration of donor-derived DCreg, 7 days before transplant, decreased the anti-donor adaptive immune response and prolonged the survival of solid organ allografts in otherwise untreated recipients (reviewed in [7-9,11,12]). Prospective clinical use of donor-derived DCreg was restricted to live-donor organ transplantation and could entail the risk of host sensitization. Thus an important alternative approach, that would allow inclusion of deceased donor transplantation, was to develop negative vaccines based on AT DCreg loaded with donor Ag. The administration of AT DCreg pulsed ex vivo with donor Ag in the form of soluble peptides, sonicates, or cell-derived vesicles has proved effective in murine models of organ transplantation (reviewed in [7,8,11]). Moreover, studies in murine models have shown that, when combined with low-dose pharmacologic immunosuppression (IS), donor- or recipient-derived DCreg administration leads to long-term graft survival in most cases (reviewed in [7,11]). This is significant, since an achievable goal in transplantation is to administer DCreg, not alone, but in combination with reduced doses of immunosuppressive drugs. In a recent review of 13 mouse and rat studies [10] it was observed that DCreg induced by 5 different methods prolonged major histocompatibility complex (MHC) -mismatched islet allograft survival to various degrees,- and that allopeptide-pulsed host DC performed best. In addition, immunosuppressive drug administration or costimulatory blockade was synergistic with the DCreg and also importantly, multiple injections were not superior to single injection.

The predominant concept underlying negative vaccination with donor DCreg in transplantation is that the injected DCreg migrate to secondary lymphoid organs of the recipient where they down-regulate alloimmunity by interacting directly with donor-reactive T cells. However, this idea is based on the impact of DCreg therapies on anti-donor responses measured using ex vivo assays and their influence on allograft survival. Interestingly, there is recent evidence that intravenously (i.v.)-administered donor DCreg, or

AT DCreg loaded with donor Ag, exert their immunosuppressive effects, not by themselves, but through host DC [15**,16]. The injected DCreg are short-lived and are processed by the recipient's quiescent conventional DC in secondary lymphoid organs. These host DC in turn, promote deletion of donor-specific effector T cells with consequent increases in the relative number of donor-specific CD4⁺ forkhead box P3 (Foxp3)⁺ regulatory T cells (Treg) [15**] (Fig. 1). Indeed, transient depletion of host conventional DC at the time of DCreg administration prevents the beneficial effect of donor- or recipient DCreg therapies in mouse cardiac transplantation [16**]. Processing of the injected donor-derived DCreg by AT DC can also explain why therapy with donor-derived DCreg restrains the rejection of corneal allografts, that occurs predominantly via the indirect pathway of allorecognition [17,18]. Thus, the quiescent/pro-tolerogenic status of host conventional DC in lymphoid organs that process injected DCreg seems to be key to the success of DCreg therapies. This raises a cautionary note, however, since the quiescent status of host DC may be altered during end-stage diseases that necessitate organ transplantation. Moreover, in certain mouse models, DCreg therapies have been shown to sensitize recipients via the transfer of donor alloAg to host Ag-presenting cells (APC), followed by priming of indirect T cells and the production of IgG allo-Abs [19*,20]. Host sensitization has not however, been observed in NHP given donor-derived DCreg that prolong renal allograft survival [21**].

Non-Ag pulsed AT DCreg therapy in transplantation

While systemic administration of AT DCreg represents a more generalizable approach to the control of anti-donor responses than injection of donor-derived DCreg, it has been thought that in vitro-generated AT DCreg need to be loaded ex vivo with donor Ag. Due to time constraints, this represents a limitation in the case of deceased donor transplantation as the cells can then only be administered after transplant surgery. Besides, the source of donor Ag and the method for ex vivo-loading of AT DCreg requires standardization between laboratories. There is evidence however, that in certain murine models, systemically-administered AT DCreg do not need to be loaded with donor Ag ex vivo to prolong graft survival (reviewed in [9,22]). Thus, i.v. injection one day before transplant of AT DCreg (generated in vitro with low dose granulocyte-macrophage colony-stimulating factor [GM-CSF]) and not exposed to donor Ag before administration, prolongs the survival of heart allografts in rats [23]. Moreover, in combination with the nuclear factor (NF)κβ inhibitor LF 15-0195, therapy with non-Ag-pulsed AT DCreg promotes donor-specific graft acceptance in most recipients [23]. The injected AT DCreg induce interferon (IFN)-γ production by CD4 CD8 double negative T cells, that accumulate in the spleen of tolerant rats [24]. In this model, expression by AT DCreg of the cytokine chain Epstein-Barr virus-induced gene 3 (EBI3), a member of the IL-12 family, is critical for differentiation of IFN-γ⁺ double negative T cells [24]. Interestingly, blockade of IFN-γ leads to allograft rejection, suggesting that IFN-γ plays a suppressive role in this model [24]. Recently, the same group has demonstrated that i.v administration of non-Ag-pulsed AT DCreg, one day before transplant and in combination with peri-transplant anti-CD3 Ab therapy, prolongs the survival of minor histocompatibility complex-mismatched male skin grafts in female recipients [25]. The beneficial effect of AT DCreg therapy on graft survival is associated with expansion of a subset of donor-specific CD8⁺ CD11c⁺ Treg in the graft-draining lymph nodes [25**]. Indirect evidence suggests that the injected non-Ag-pulsed AT DCreg capture donor Ag

within the skin grafts before migrating to draining lymph nodes [25**] (Fig. 1). The immunosuppressive effect of these AT DCreg *in vivo* is dependent on cross-presentation of internalized donor Ag to CD8⁺ T cells [25**]. Unlike donor DCreg or AT DCreg loaded with donor Ag, that need to be injected 7 days before transplant to have maximal effect, non-Ag pulsed AT DCreg prolong graft survival when administered peri-transplant. Since these non-Ag-pulsed AT DCreg are not exposed to donor Ag before injection, the potential risk of sensitization against donor Ag is minimized.

In a different context, non-Ag-pulsed AT DCreg, conditioned *in vitro* with transforming growth factor (TGF) β and impaired in their ability to produce proinflammatory cytokines, have been used to control T cell autoimmunity against syngeneic pancreatic islets transplanted in diabetic (non-obese diabetic [NOD]) mice. In this case, the non-Ag-pulsed AT DCreg were mixed directly with the islet grafts transplanted under the kidney capsule, which allowed the DCreg to acquire islet-derived Ag locally [26*]. Addition of AT DCreg promoted long-term survival of the islet grafts in most recipients. This occurred despite the presence of a non-pathogenic leukocyte infiltrate in the long-accepted grafts, that showed no reduction in the number of CD8⁺ T cells or increased presence of FoxP3⁺ Treg [26*]. Notably, the immature AT DC could delay rejection of islet grafts even when not exposed to TGF β .

In situ targeting of quiescent DC in secondary lymphoid organs to promote transplant tolerance

Presentation of Ag by immature or semi-mature conventional DC in lymphoid organs provides insufficient co-stimulation. This results in Ag-specific down-regulation of the T cell response by means of defective T cell proliferation, followed by apoptosis, T cell anergy, and/or generation of adaptive Treg [27]. Therefore, targeting donor Ag to host conventional DC in secondary lymphoid organs, without altering the quiescent/inherent protolerogenic status of the APC, constitutes an alternative approach to administration of *in vitro*-generated DCreg to promote donor-specific immune suppression. In most such experimental protocols, exogenous Ag are delivered in association with vehicles that bind the recipient's conventional DC *in situ*, without altering their quiescent status. These vehicles can be particles (i.e. apoptotic cell vesicles or immature APC-derived exosomes) or recombinant monoclonal Ab directed against DC surface markers.

Following internalization of cells in early apoptosis, DC reduce their expression of surface MHC and costimulatory molecules, decrease secretion of pro-inflammatory and T helper (Th)1-driving cytokines, and become weak stimulators of T lymphocytes. Splenic DC rapidly internalize apoptotic cells from the circulation and the vesicles shed by apoptotic splenocytes bear the entire repertoire of MHC molecules. Thus, systemic administration of donor splenocytes in early apoptosis was one of the first approaches used to deliver donor Ag to host DC for the purpose of restraining the anti-donor T cell response in transplantation [28] (Fig. 1). In murine models, *i.v.* injection of donor splenocytes in early apoptosis, 7 days prior to transplant, prolongs the survival of fully MHC-mismatched cardiac grafts in otherwise untreated recipients. Combination treatment with low dose blocking anti-CD154 mAb, results in long-term survival of most grafts [29,30]. The effect is donor-specific,

dependent on phagocytosis of the apoptotic cells by host splenic conventional DC and results from the deletion of indirectly-reactive T cells with a consequent relative increase in the percentage of indirectly alloreactive CD4⁺ FoxP3⁺ Treg [29-31]. Interestingly, apoptotic cell therapy also restrains the direct T cell response, possibly through its immunosuppressive effect on indirectly alloreactive T cells. Due to its potent regulatory effect on the indirect pathway, therapy with donor apoptotic splenocytes down-regulates the production of IgG alloAb and decreases the development of chronic allograft vasculopathy [30,31]. A limitation to this approach is that leukocytes in early apoptosis are difficult to preserve and when stored, their progression to late apoptosis diminishes their immunoregulatory potential.

Exosomes released by in vitro-generated immature DC have been employed as an alternative vesicle to deliver donor Ag to recipient APCs in situ. Exosomes are nanovesicles (100 nm in size) derived from the endocytic compartment of living cells and released by most, if not all cell types. Interestingly, APCs secrete exosomes bearing MHC class-I and -II molecules on their membrane, and exosomes injected i.v. are internalized rapidly by splenic conventional DC. After processing the vesicles, these DC present exosome MHC-derived peptides to T cells [32]. The composition of the exosomes depends on the stage of maturation/activation of the parent APC. Exosomes released by immature DC (immature exosomes) exhibit less T cell allostimulatory activity and greater capacity to prolong mouse cardiac allograft survival than exosomes secreted by mature DC (mature exosomes) [33]. Indeed, i.v. injection of exosomes released by donor immature DC before and after transplant, plus low dose rapamycin, prevents cardiac allograft rejection and induces donor-specific tolerance in mice [34]. Unlike apoptotic cell-derived vesicles, exosomes are highly stable, but more difficult to produce in sufficient quantities for systemic administration in humans. This may limit their potential therapeutic use in transplantation.

A more specific method to deliver Ag to DC and to simultaneously promote endocytosis, processing and presentation of the administered Ag via the endocytic route, is to couple the Ag to Ab directed against DC lectin-like receptors (Fig. 1). One of these is 33D1 (DC inhibitor receptor-2) that is expressed by CD4⁺CD8⁻CD11c^{hi} DC, or CD205 (DEC-205) that is present on CD4⁻CD8⁺CD11c^{hi} DC in mouse secondary lymphoid organs. Targeting mouse CD8⁺ DC via administration of Ag coupled to CD205 Ab promotes MHC class-I-restricted CD8⁺ T cell responses primarily, whereas in situ delivery of Ag to CD8⁻ DC using 33D1 Ab elicits MHC class-II-restricted CD4⁺ T cell responses [35]. In the absence of DC maturation stimuli, in vivo targeting to both DC subsets in the steady-state promotes T cell tolerance. By contrast, in situ DC targeting in combination to agonistic CD40 ligation leads to potent T cell immunity [35]. Importantly, administration of Ag coupled to CD205 Ab has been shown to induce Ag-specific CD8⁺ T cell deletional tolerance, not only in naïve mice [36], but also in animals with ongoing CD8⁺ T cell immunity [37]. In transplantation, pretreatment of C57BL/6 (B6) mice with a 33D1 Ab conjugated to the H2 K^d monomer prevented the development of CD4⁺ T cell indirect alloresponses and IgG alloAb against partially MHC class-I mismatched skin B6.K^d grafts [38]. However, long-term skin graft survival was achieved only when in situ targeting of DC was combined with abrogation of the direct CD8⁺ T cell alloresponse by co-administration of a depleting CD8 Ab [38]. These findings suggest that, unlike in autoimmune disorders, down-regulation of direct and indirect

T cell responses in transplantation by in situ targeting of recipient DC will require the delivery of a broad repertoire of donor Ag/peptides. The extensive variability in MHC alleles between donors makes this potential therapeutic approach challenging in the transplantation arena.

More recently, mAb directed against other lectin-like molecules have been tested for delivery of Ag to conventional DC in vivo. Thus, delivery to DC of influenza virus hemagglutinin 1 (HA1) or the self-Ag prostate-specific Ag (PSA) fused to a recombinant mAb against the lectin-like DC-asialoglycoprotein receptor (DC-ASGPR) generated Ag-specific, IL-10-producing suppressive CD4⁺ T cells in vitro in humans and in vivo in NHP [39*]. These suppressive CD4⁺ T cells were FoxP3⁻, likely derived from previously-polarized Th1 cells, and their regulatory effect was IL-10-dependent [39*]. Delivery of Ag via DC-ASGPR Ab promoted IL-10 secretion in the target DC, which was associated with polarization of Ag-specific CD4⁺ T cells into IL-10-producing cells [39*]. In situ delivery of Ag to conventional DC through different lectin-like receptors elicits qualitatively and quantitatively different CD4⁺ T cell responses. For instance, targeting of HA1 or PSA to DC through DC-ASGPR generated significantly more IL-10-secreting CD4⁺ T cells than delivery of the same Ag to DC through other lectin-like receptors, including lectin-like oxidized-LDL receptor (LOX-1), Dectin-1, or DC-specific intercellular adhesion molecule (ICAM)-3-grabbing non-integrin (DC-SIGN) [39*]. Furthermore, delivery of Ag to DC via recombinant mAb directed against lectin-like receptors was more efficient at eliciting CD4⁺ T cell responses than loading the corresponding Ag-derived peptide pools to DC [39*].

In some cases, the objective of targeting conventional DC in secondary lymphoid organs with recombinant mAb is to arrest the quiescent status of the DC, without delivering exogenous Ag, for the Ag are provided by another source (i.e. a transplant). Treatment with the mAb MD-3 directed against domain 2 of human and NHP ICAM-1 molecules prevents the maturation of human myeloid DC in response to LPS stimulation, without affecting critical functions of ICAM-1, such as adhesion of leukocytes to endothelial cells [40*]. Humanized mice rendered diabetic by streptozotocin and injected i.v. with 3 doses of MD-3 Ab, 9 days prior to transplantation of porcine islets, showed no evidence of xenograft rejection up to the experimental endpoint at 42 days. Ex vivo-analysis indicated that the lack of graft rejection was due to development of xeno-specific T cell tolerance [40*]. In rhesus macaques rendered diabetic by streptozotocin, MD-3 Ab monotherapy before pig islet transplantation promoted pig islet-specific T cell tolerance, although the islet grafts were quickly rejected. However, long-term survival of the pig xenoislets was achieved when MD-3 Ab therapy was combined with low-dose rapamycin and CD154 blocking Ab [40*].

Modulation of alloimmunity and prolongation of organ transplant survival by DCrege in NHP

NHP are important pre-clinical models for testing promising new strategies for the promotion of organ transplant tolerance. NHP DC have been well-characterized [41,42] and shown to modulate alloimmune reactivity in vitro [43,44] and in vivo [45]. Rhesus macaque monocyte-derived DC propagated in vitamin (Vit)D3 and IL-10 are stably immature, resistant to maturation following potent pro-inflammatory cytokine stimulation, and induce T cell hyporesponsiveness to alloAg in vitro [45]. When administered systemically to

normal allogeneic rhesus macaques, in combination with the costimulation blocking agent CTLA4Ig, these DCreg modulate alloimmune reactivity, with resulting T cell hyporesponsiveness to donor alloAg, and no detectable circulating IgM or IgG anti-donor alloAb [45]. These rhesus DCreg were examined for their influence on the survival of renal transplants in combination with minimal IS therapy [21**]. Thus, donor-derived DCreg were infused i.v. ($3.5\text{--}10\times 10^6/\text{kg}$), together with CTLA4Ig into quiescent, prospective graft recipients, 7 days before renal transplantation. CTLA4Ig was given for up to 8 weeks and rapamycin, started on day -2, was maintained with tapering of blood levels until full withdrawal at 6 months. Median graft survival time was 39.5 days in control monkeys (no DC infusion; $n=6$) and 113.5 days ($p<0.05$) in DCreg-treated recipients ($n=6$). No adverse events were associated with the DCreg infusion. Moreover, there was no evidence of induction of host sensitization based on circulating donor-specific alloAb levels. Significantly, immunologic monitoring revealed regulation of donor-reactive memory CD95^+ T cells and reduced memory/regulatory T cell ratios in the DCreg-treated monkeys compared with controls ([21**] Fig. 2). Termination allograft histology showed moderate combined T cell- and Ab-mediated rejection in both groups.

These findings provide the first evidence of safety and the immunosuppressive efficacy of DCreg in a stringent, pre-clinical NHP transplant model, and suggest that using DCreg, long-term use of immunosuppressive drugs in transplant recipients could be minimized or even eliminated. The data support further testing of DCreg in organ transplantation together with co-stimulation blockade and mTOR inhibition in a calcineurin inhibitor-free and steroid-sparing regimen to reduce host dependence on IS agents and promote rejection-free allograft survival.

Clinical application of DCreg

Extensive efforts are currently being made to generate DCreg for clinical use. As in murine models, human DCreg have been propagated in vitro from DC precursors using a wide range of agents, including anti-inflammatory cytokines (in particular IL-10), VitD3, neuropeptides and anti-inflammatory/IS drugs, such as dexamethasone (Dex) and rapamycin. However, from the perspectives of DCreg tolerogenicity, stability and capacity for migration to secondary lymphoid tissue, the agent(s) most suitable for clinical application has not been defined. Matsumoto et al [46] have recently reported that protein kinase C inhibitor (PKCI)-treated human monocyte-derived DC have a semi-mature phenotype, and produce low IL-12, but high levels of IL-10. They also induce IL-10-producing T cells and functional Foxp3^+ Treg from naïve CD4^+ T cells. Moreover, they are very stable when exposed to potent pro-inflammatory stimuli, express the lymphoid tissue homing receptor CCR7 and migrate towards CCR7 ligands. Such conventional PKCI-treated human DC therefore appear to fit the criteria for clinically-applicable DCreg. In this regard, an important additional consideration relevant to the improvement of human organ allograft survival, is the need to overcome memory CD8^+ T cell resistance to immunosuppression. In a recent study, Kleijwegt et al [47] show that (re)activation of human CD8^+ T cells by islet Ag-pulsed monocyte-derived DCreg (VitD3 and Dex-treated) impedes CD8^+ T cell immunity and depletes memory CD8^+ T cells, an effect ascribed to the absence of costimulation and the secretion of regulatory cytokines (IL-10/TGF β).

Despite the challenges associated with translation of DCreg therapy from rodents and NHP to humans, advances such as closed cell culture systems and GMP-compliant reagents have allowed strategies for the production of clinical grade DCreg to advance [48-50]; reviewed by Raich-Regue et al [51]. This has led to early (Phase I) clinical trials of DCreg in type-1 diabetes and rheumatoid arthritis [52-54], although clinical testing in organ or hematopoietic stem cell transplantation has not yet been reported. There does, however, appear to be adequate justification for moving forward with testing of human DCreg in organ transplantation.

Conclusion

While intensive study in murine models has generated important insight into the mechanisms whereby DCreg therapy or in situ targeting of tolerogenic DC promotes long-term allograft survival, as with other forms of regulatory immune cell therapy, the optimal protocol that may achieve this effect in the clinic remains uncertain. Nevertheless, cautious evaluation in phase I/II trials would appear to be justified.

Acknowledgments

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Key points

- In addition to negative vaccination with donor-derived DCreg, new therapies with recipient-derived DCreg (loaded or not with donor Ag before injection and administered peri-transplant) have broadened horizons for the clinical use of DC-based therapies in transplantation using live and deceased donors.
- In mouse models of heart transplantation, i.v. injected donor- or recipient-derived DCreg function as “Ag-transporting cells”, rather than immunosuppressive APC, by delivering donor Ag to host quiescent pro-tolerogenic DC in secondary lymphoid organs.
- Variability and extent of the donor Ag repertoire represent limitations for in situ-targeting of quiescent DC with mAb coupled to donor Ag/peptides.
- NHP studies demonstrate the safety and efficacy of donor-derived DCreg in prolonging renal allograft survival.
- Evaluation of DCreg therapy in clinical transplantation is justified.

I.V. administration of DCreg

Targeting of recipient's DCs

Figure 1.

DCreg-based negative vaccination in organ transplantation. Donor-derived DCreg (represented in black) or recipient-derived DCreg (in gray) loaded in vitro with donor Ag are injected systemically into the graft recipient. Once the injected DCreg home to secondary lymphoid organs, exogenous APC are processed by the recipient's quiescent conventional DC that present the donor Ag to anti-donor T cells in a pro-tolerogenic fashion. More recently, recipient-derived DCreg not exposed to donor Ag in vitro and injected i.v. have been shown to acquire donor Ag in vivo and cross-present donor peptides to anti-donor Treg. Targeting of host quiescent conventional DC in secondary lymphoid organs is achieved by injecting donor leukocyte-derived vesicles, donor MHC molecules or MHC-derived peptides coupled to monoclonal (m)Ab directed against surface DC markers. Alternatively mAb with regulatory effects on conventional DC can be administered in the absence of donor Ag, which is provided by the transplant itself.

Figure 2.

How donor DCreg could promote transplant survival. (a) In the absence of DCreg therapy, host T cells recognize donor peptides (Ag) loaded on MHC class II molecules expressed by donor graft 'passenger' DC. Inflammatory stimuli, graft ischemia–reperfusion injury and cognate interaction with T cells activate DC, leading to enhanced costimulatory molecule expression. Activated CD4⁺ T cells proliferate, differentiate into Th cells and provide help to B cells and CD8⁺ T cells. Effector mechanisms (cytotoxic, granzyme B⁺CD8⁺ T cells and Abs) induce graft injury. CD28/B7 costimulation blockade with CTLA4–Ig and immunosuppression with rapamycin delay graft rejection transiently. (b) With DCreg therapy, donor monocytes are mobilized by cytokines, isolated by leukapheresis, and differentiated into DC using GM-CSF and IL-4. DCreg are conditioned with vitamin D3 and IL-10 to prevent their maturation and then injected into kidney allograft recipients, 7 days before transplantation. Use of DCreg increases graft survival and inhibits alloAb production. This is associated with decreased Th cell proliferation and granzyme B expression, as well as upregulation of negative costimulatory molecules, - CTLA4 and PD-1 (B7-H1; CD274) [55].