Review Article

Dendritic cells and regulation of graft-versus-host disease and graft-versus-leukemia activity

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Abstract

Hematopoietic stem cell transplantation (HSCT) is the only curative treatment for many malignant hematologic diseases, with an often critical graft-versus-leukemia (GVL) effect. Despite peri-transplant prophylaxis, graft-versus-host disease (GVHD) remains a significant cause of post-HSCT morbidity and mortality. Traditional therapies have targeted T cells, yet immunostimulatory dendritic cells (DC) are critical in the pathogenesis of GVHD. Furthermore, DC also have tolerogenic properties. Monitoring of DC characteristics may be predictive of outcome and therapies that target DC are innovative and promising. DC may be targeted in vivo or tolerogenic (tol) DC may be generated in vitro and given in the peri-transplant period. Other cellular therapies, notably regulatory T cells (T_{reg}) and mesenchymal stem cells (MSC), mediate important effects through DC and show promise for the prevention and treatment of GVHD in early human studies. Therapies are likely to be more effective if they have synergistic effects or target both DC and T cells in vivo, such as tolDC or T_{reg}.

Given the effectiveness of tolDC in experimental models of GVHD and their safety in early human studies for type 1 diabetes, it is crucial that tolDC be investigated in the prevention and treatment of human GVHD, while ensuring conservation of GVL effects.
Introduction

Hematopoietic stem cell transplantation (HSCT) remains the only curative therapy for many high-risk malignant hematologic diseases, as well as numerous life-threatening genetic and hematological disorders. However, despite peri-transplant prophylaxis, HSCT is frequently complicated by graft-versus-host disease (GVHD), which leads to significant morbidity and mortality. The risk of GVHD limits the broader application of HSCT where it has the potential to cure autoimmune diseases, facilitate transplant tolerance, and correct immunological deficiencies, including HIV infection. Conventional immunosuppressants remain the mainstay of treatment for GVHD, yet they frequently fail and carry a significant risk for infection. It is therefore of significant interest to identify new, effective and safe prophylactic and therapeutic approaches, particularly those that maintain the critical graft-versus-leukemia (GVL) effect of HSCT. In this review, we consider advances that have been made in understanding the role of dendritic cells (DC) in GVHD and address the challenge of monitoring, targeting and exploiting these cells to improve therapeutic outcomes.

Our understanding of the pathogenesis of GVHD has advanced significantly over the past forty-five years, since Billingham proposed that GVHD is due to immunocompetent donor cells recognizing recipient antigens (Ags) in an immunocompromised host unable to reject donor cells. The principal immunocompetent donor effector cells are T cells, and the vigor of the immune response is driven by differences in major histocompatibility complex (MHC) and minor histocompatibility antigens (miHA). Furthermore, the crucial role of Ag-presenting cells (APC), in particular uniquely well-equipped donor and recipient DC, has begun to be elucidated, not only in GVHD, but also in the GVL effect of HSCT.

DC hematopoiesis and immunobiology

DC are rare, heterogenous bone marrow (BM)-derived professional APC, first characterized in mouse spleen by Steinman & Cohn, that are distributed ubiquitously in blood, lymphoid and peripheral tissues, especially at portals of entry. They arise from hematopoietic stem cells through specialized progenitor subsets and are important in innate and adaptive immune function and in determining the balance between immunity and
tolerance. In the normal steady-state, DC reside in ‘immature’ or ‘semi-mature’ states in the periphery where they constantly take up and process self-Ags and maintain self-tolerance. Immunostimulatory DC have undergone maturation following recognition of exogenous and endogenous alarmins/danger signals by Toll-like receptors (TLR). These signals include pathogen-associated molecular patterns (PAMPS) in the form of microbial products and danger-associated molecular patterns (DAMPS), such as products of damaged or dying cells (e.g. high-mobility group protein B1 or DNA). DC are also matured by CD40 ligation and by pro-inflammatory cytokines that can induce DC maturation ex vivo, independent of CD40 ligation. Maturation is associated with upregulation of cell surface MHC gene products, co-stimulatory molecules (CD40, CD80 and CD86, in addition to CD83 in humans) and appropriate chemokine receptors (in particular CCR7) that enhance the ability of DC to home to secondary lymphoid tissue. Therein they present Ag to Ag-specific T cells and induce T cell activation/proliferation. In turn, activated T cells drive DC towards terminal maturation. These aspects of DC immunobiology have been reviewed in detail.

DC develop from HSC in the BM and are derived from both myeloid and lymphoid progenitors (FIGURE 1). This has been demonstrated in both mouse and human studies, in which all DC subsets can be generated from either a common lymphoid progenitor (CLP) or common myeloid progenitor (CMP). The hematopoietic growth factor fms-like tyrosine kinase 3 ligand (Flt3L) plays a central role in steady-state DC development; this is evidenced by the majority of DC precursors being Flt3+ (CD135+) and culture with Flt3L resulting in all major DC subsets. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is also important in DC hematopoiesis, as it gives rise to DC from monocytes and early progenitors in the absence of intact Flt3L signaling and produces DC under inflammatory conditions. Monocyte/macrophage (M)-CSF is also a DC poietin and can drive DC generation in mice independently of Flt3L.

DC Subsets

(i) Overview of subsets
DC can be broadly categorized as conventional DC (cDC) and precursor DC (FIGURE 1). In the steady-state, cDC exhibit typical DC features (e.g. cytoplasmic dendrites) and function (e.g. Ag uptake, processing, and presentation). cDC can be subdivided into migratory DC, such as skin epidermal Langerhans cells (LC) and dermal DC, that present Ag in lymph nodes following its uptake in peripheral tissue, and resident DC that take up and present Ag within a lymphoid organ, such as splenic or thymic DC (TABLE 1). Resident DC can be further categorized in the mouse as CD8α–, which is the predominant splenic population, and CD8α+, which is the major thymic population. CD8α+ DC are involved in Ag cross-presentation and show high IL-12 secretion. Thymic cDC primarily present self-Ag and are important in self-tolerance through T cell negative selection and the production of naturally-occurring regulatory T cells (Treg). Plasmacytoid DC (pDC) are a subset of precursor DC which have an immature phenotype in the steady-state and plasma cell morphology (e.g. lack dendrites). Upon activation, pDC closely resemble cDC in form and function. Monocyte-derived DC or ‘inflammatory DC’ are similar to cDC in form and function and correlate with in vitro GM-CSF generated DC.

(ii) Function of DC subsets

DC subsets differ in their immune functions, which has important implications for HSCT. Under steady-state conditions, human pDC display lower levels of MHC and co-stimulatory molecules compared to conventional myeloid (m)DC. Also, since their Ag processing and loading ability is less efficient, pDC stimulate T cells less effectively than mDC. Following their activation via TLR, pDC secrete high levels of type-1 interferon (IFN) and stimulate CD4+ and CD8+ T cells. This is in contrast to activated mDC, which secrete IL-12 and promote T-helper type-1 (Th1) cell differentiation and CD8+ CTL responses. pDC have intrinsic tolerogenic properties; in the steady-state, human thymic pDC induce Treg, while liver and airway pDC regulate oral and mucosal tolerance, respectively. pDC have also been implicated in the regulation of disease activity in experimental models of autoimmunity and shown to exert disease-suppressing ability. This may be important following HSCT in terms of donor BM engraftment (tolerance), as well as for chronic GVHD (cGVHD) which
has clinical features that overlap with autoimmune disease. Epidermal LC may be immunostimulatory or tolerogenic, depending on their state of maturity, inciting immunogen, and the cytokine environment.29

(iii) Mouse versus human DC subsets

DC subsets have been well-characterized, especially in mice, and also in humans and other species (FIGURE 1).13,14,30-32 Most human work has been conducted in vitro, thus knowledge of the in vivo function and development of human DC subsets is lacking. Direct comparisons between mouse and human DC subsets can be problematic, due to phenotypic differences between the species (e.g. multiple human DC subsets are capable of Ag cross-presentation and display high IL-12 secretion, and are thus comparable to the mouse CD8α+ DC subset).33 Likewise, flow cytometric characterization of DC subsets has been refined over time, and the evolution of “standard” DC markers makes comparisons between present and past studies difficult. Currently, in mice, pDC are identified as CD11cloCD11b− sialic acid binding immunoglobulin-like lectin H (Siglec-H)+PDCA-1+, while in humans, they are lin−MHC II+CD11c+CD123(IL-3Rα)+BDCA2(CD303)+. Mouse mDC are identified as CD11c+CD11b+B220− (CD45R−) NK1.1−, whereas human mDC are lin−MHCII+CD11c+CD123−BDCA1 (CD1b/c)+.

Although characterization of human DC subsets has been more difficult due both to their rarity and difficulty of access, DC subsets in the skin have been well-characterized, while recent studies have begun to elucidate subsets in the blood and other organs. The epidermis contains Langerin+ LC, while the dermis contains CD1a+ and CD14+ DC, the former of which has an unknown function.34 CD14+ DC are involved in B cell differentiation both by activation of naïve B cells to plasma cells35,36 and induction of naïve CD4+ T cells to T follicular helper cells.37 Compared to other human skin DC subsets, LC are potent activators of Ag-specific CD8+ T cells, which may be explained in part by their production of IL-15.34,35 Conventional DC subsets appear to be comparable in human blood and spleen in the steady-state, with 3 major populations described, all of which are lin−CD11c+HLA-DR+, and unlike mouse subsets, cannot be further distinguished by CD8α.33 BDCA3(CD141)+ DC are thought to be the human equivalent of mouse CD8α+ DC, based upon cell adhesion
molecule 1 (CADM1) expression and their ability to cross-present Ag and secrete high IL-12, although more recent studies indicate that other human cDC subsets share these abilities.\(^{33,38}\) BDCA1(CD1b/c)\(^{+}\) DC may be comparable to mouse CD8\(^{α}-\) DC, while CD16\(^{+}\) DC have been termed inflammatory monocytes, based upon their high tumor necrosis factor (TNF)\(α\) and low IL-10 expression.\(^{33}\)

**DC tolerogenicity**

In addition to their capacity to stimulate innate and adaptive immunity, DC can induce and maintain tolerance (for reviews, see \(^{28,39,40}\)). Tolerogenic (tol) DC present Ag to T cells, but lack adequate co-stimulatory ability, deliver inhibitory signals (e.g. via the programmed death [PD] pathway), and produce tolerance-promoting cytokines (IL-10).\(^ {39}\) TolDC do not support Ag-specific T cell activation and proliferation, but instead facilitate T cell anergy/apoptosis and/or the generation or expansion of T\(_{reg}\).\(^ {39}\) Importantly, bidirectional feedback between tolDC and T\(_{reg}\) has been demonstrated in humans and mice, whereby tolDC promote the generation of T\(_{reg}\) from naïve T cells and T\(_{reg}\) generate tolDC from DC progenitors.\(^ {41}\) Regulation of immunosuppressive tryptophan catabolism in DC via activation of indoleamine 2,3-dioxygenase (IDO) may be an important mechanism of action of Treg\(^ {42}\) and may underlie transplant tolerance in vivo. The close relationship between T\(_{reg}\) and DC is illustrated by the observation that increases in DC lead to increases in T\(_{reg}\), whereas constitutive absence of DC leads to fatal autoimmunity.\(^ {43-45}\)

**Role of DC in the pathogenesis of GVHD**

Mouse studies have demonstrated that CD4\(^{+}\) T cell-dependent (MHC-mismatched) acute GVHD (aGHVD) can be induced by either host or donor APC, whereas host APC are required for the initiation of CD8\(^{+}\) T cell-dependent (MHC-matched, multiple miHA-mismatched) aGVHD and donor APC amplify the process.\(^ {46-48}\) Additional studies have tried to further characterize the contribution of different APC populations to the development of aGVHD. While earlier mouse studies implicated host LC in the pathogenesis of skin GVHD, more recent experiments using mice deficient in LC question the relevance of LC in the development of aGVHD.\(^ {49,50}\)
Less is known about the role of DC in cGVHD due to variability in clinical presentation (de novo cGVHD versus cGVHD evolving from aGVHD) and the lack of relevant mouse models. Both host and donor APC have been implicated, but with target organ specificity; skin cGVHD can be induced by either donor or host APC, while donor APC are dominant in intestinal cGVHD.\textsuperscript{51,52} Thymic independent and dependent pathways likely contribute to cGVHD. Autoreactive CD4\(^+\) T cells have been implicated in cGVHD, and cGVHD occurs in patients with little thymic function or in those with intact thymic negative selection.\textsuperscript{52,53} Mouse studies have implicated engrafted donor anti-host CD4\(^+\) T cells in the evolution of acute to cGVHD, whereby donor CD4\(^+\) T cells are generated in the milieu of CD8\(^+\) T cell-mediated aGVHD thymic damage, likely due to failure of thymic DC to delete autoreactive CD4\(^+\) T cells.\textsuperscript{54} This has important implications, both in the pathogenesis of cGVHD, but also in its prevention, as keratinocyte growth factor prevents cGVHD likely due to thymic protection.\textsuperscript{54}

The development of GVHD, particularly aGVHD, has been divided traditionally into three phases (FIGURE 2). Phase I involves activation of APC, particularly DC, by cytokines released following recipient tissue damage. These DC present acquired and processed Ag to T cells, which, in combination with simultaneous co-stimulation, leads to the second phase,- donor T cell activation. Mouse studies suggest that donor T cell activation in GVHD requires co-stimulation via B7 family molecules (CD80/86)/CD28 and B7H/inducible costimulator (ICOS) and is inhibited by B7/cytotoxic T lymphocyte Ag (CTLA)-4 and PD-L1/PD interactions.\textsuperscript{55,56} Following HSCT, DC can present host Ag to donor T cells, either directly or indirectly. In the direct pathway, donor T cells are stimulated by allogeneic MHC or miHA molecules (in the more common MHC-matched setting) present on host APC, whereas the indirect pathway involves presentation of acquired host Ags by engrafted donor APC, particularly CD11c\(^+\) DC.\textsuperscript{57} Mouse models have shown that the indirect pathway or ‘cross-presentation’ of host Ag does not initiate aGVHD, but that direct presentation by host DC resistant to conditioning is required.\textsuperscript{46,48} Wang et al\textsuperscript{57} expanded knowledge of cross-presentation in an experimental model, demonstrating that donor APCs are activated by donor CD4\(^+\) T cells (initially activated by...
host APC) dependent on CD40L and type I IFN, then cross-present acquired host hematopoietic and non-hematopoietic transmembrane proteins to donor CD8\(^+\) T cells. Utilizing monoclonal antibodies (mAbs) and/or transgenic/knock-out donor mice, Markey et al\(^{58}\) examined the role of donor APC subsets and showed that donor cDC are critical for cross-presentation of alloAg immediately following HSCT. Donor T cell activation leads to the third phase, in which cytokines and cellular effectors, particularly cytotoxic T lymphocytes (CTLs), natural killer (NK) cells and macrophages mediate target cell injury and apoptosis.\(^{57}\)

**Role of DC in GVL**

Host DC are required for full GVL effects, although donor APC can initiate GVL activity when low levels of tumor are present.\(^{48,59,60}\) Li et al\(^{61}\) found that depletion of donor BM CD11b\(^+\) myeloid cells in an experimental model enhanced survival of recipients with tumor; more recently, they reported that, conversely, addition of CD11b\(^-\) cells, which were primarily precursor pDC, augmented GVL without concomitant increase in GVHD.\(^{62}\) Clinical studies have demonstrated that increased graft pDC content is associated with relapse and decreased patient overall survival.\(^{63}\) Low CD11c\(^+\) DC, but not CD123\(^+\) DC, in peripheral blood (PB) at the time of engraftment has also been associated with death and relapse.\(^{64}\) New insights concerning the role of both donor and recipient DC subsets in GVL, including graft precursor pDC content, and the impact of pre-transplant manipulation of these subsets are clearly needed.

The role of DC in GVL following donor leukocyte infusion (DLI) has also been examined. In murine and clinical studies, GVL effects can be seen following DLI without GVHD.\(^{65}\) The presence of host APC and allo-MHC I has been shown to be critical for GVL effects in mixed chimeras created in the fully MHC-mismatched setting, although results are not as clear in the MHC-matched minor Ag mismatched and clinical settings.\(^{66-68}\) Furthermore, there is evidence that DLI-induced GVL effects in mice are dependent on MHC alloAg, but not miHC or tumor-associated Ags, in a CD4\(^+\) and CD8\(^+\) T cell-dependent manner, and that MHC class II-expressing host APCs are required for maximal GVL activity.\(^{60}\) Unfortunately, while DLI enhances the GVL effect, it is often complicated by GVHD.\(^{69,70}\)
Influence of transplant factors on DC in relation to GVHD

Given the role of host tissue damage in the pathogenesis of GVHD, it was thought that reduced intensity conditioning (RIC) would lead to less GVHD. However, while RIC has reduced transplant-related mortality, the incidence of aGVHD, while delayed, remains unchanged. In mouse HSCT following RIC, Turner et al demonstrated that, while the onset of GVHD was delayed, it was equally severe. The authors hypothesized that delays in GVHD were due to maintenance of absolute numbers of host DC and decreased TNFα production, promoting T<sub>reg</sub> responses. As donor chimerism increased, donor-activated DC increased and elevated TNFα led to decreased T<sub>reg</sub> and onset of delayed, yet equally severe GVHD.

Conditioning regimens can differ in host irradiation, however some human and mouse DC are resistant to radiation, particularly dermal DC and epidermal LC. In an experimental model, total body irradiation (TBI) led to complete depletion of splenic and BM pDC after 24 hours, whereas mDC were maintained, but in decreased numbers. Additionally, TBI is important in DC activation; studies in mice have shown that inflammation from irradiation is critical for pDC but not mDC activation.

HSC may be obtained either directly from BM or from the PB following expansion with granulocyte colony-stimulating factor (G-CSF). As reviewed by Korbling et al, despite a significant increase in donor T cells in PB stem cell (PBSC) grafts, there is surprisingly no increase in aGVHD, although there seems to be an increase in cGVHD. Numerous studies have documented increased graft pDC with G-CSF mobilization, with potential implications for outcome of HSCT, including that these cells may favor Treg function. G-CSF treatment has also been associated with decreased pro-inflammatory IL-12 production. In a mouse model of PBSC transplantation, G-CSF treatment of donors rather than recipients significantly reduced levels of TNFα, likely via decreased donor DC TNFα and IL-12 production. mDC IL-12 production was also significantly decreased in pediatric HSCT recipients who received G-CSF post transplantation. These differences must be taken into account when interpreting clinical studies.
DC analyses in relation to outcome of HSCT (summarized in Table 2)

(i) DC engraftment

Clinical studies have revealed an association between low total DC numbers at the time of engraftment and decreased patient overall survival (OS), increased relapse, and increased aGVHD. Neither host DC count pre-transplant nor graft DC count was associated with death or relapse. Though neither was independently significant, lower mDC count at engraftment was associated with decreased survival, increased relapse, and increased incidence of aGVHD. Lower circulating pDC count correlated only with increases in aGVHD. Skin GVHD has been associated with decreased human LC engraftment. However, this is thought to be a secondary effect related to steroid treatment and GVHD effector cells, as experimental studies have shown that donor T cells promote donor LC engraftment. Prospective studies are indicated to determine if low DC count at the time of engraftment can be used as a predictive tool for GVHD.

(ii) DC chimerism

There are conflicting data on DC chimerism following HSCT. Early human studies demonstrated an association between full donor chimerism and cGVHD as compared to mixed chimerism in some control patients without cGVHD. Chan et al assessed DC chimerism 100 days post transplant and found that host DC persistence correlated with severe aGVHD and cGVHD. There were significant differences between the two studies, however, with the latter involving primarily RIC regimens and samples differentiated in vitro and analyzed by DNA PCR banding, rather than by conventional flow cytometry. Given the differential effects of MAC versus RIC, further studies on DC chimerism in both populations are warranted to resolve the impact of DC chimerism on development of acute and cGVHD.

(iii) DC activation status

The activation status of DC is likely important in and potentially predictive of GVHD. Lau et al examined expression of CMRF-44, a cell surface marker which is expressed early during the activation and maturation of
human mDC, but not on freshly-isolated DC from healthy controls. The incidence of circulating CMRF-44^CD11c^ DC correlated with onset and severity of aGVHD and was found to be predictive when used as a screening test before the onset of GVHD. In the same study, cell surface expression of CD83 and CD86, both of which are increased upon human DC activation and are important in T cell co-stimulation, were not predictive of GVHD. Larger studies investigating the predictive role of these DC activation/maturation markers and their anti-inflammatory versus pro-inflammatory cytokine production, such as IL-10 or IL-12 respectively, should be performed. Analysis of donor versus host origin DC expression of these immunoregulatory molecules could enhance the insights from these further evaluations.

(iv) DC subsets

Prior to current immunophenotyping of DC subsets, Waller et al demonstrated an association between high BM graft presumptive pDC progenitors and decreased cGVHD, although the incidence of leukemic relapse was increased. A more recent clinical study did not find an association between G-CSF-mobilized PB graft pDC content and GVHD, however it confirmed the increased incidence of relapse, as well as decreased OS and event-free survival. Since these studies utilized different stem cell sources, the difference in the incidence of GVHD may be accounted for, in part, by differences in cytokine release and/or DC activation status following G-CSF administration. The association between relapse and graft pDC highlights the importance of preserving the GVL effect with any intervention to decrease GVHD.

Human DC subsets have also been examined in the post-transplant period. Reddy et al documented a dependent association between low CD11c^ DC in PB at the time of engraftment and death, relapse and aGVHD; low CD123^ DC count was associated with aGVHD only. More recent studies in patients following MAC and RIC found a significant correlation between pDC count and increased GVHD, as well as pDC and mDC count and increased GVHD severity. Low pDC count 3 months following RIC transplant was also associated with severe aGVHD, decreased OS, and increased non-relapse mortality, notably from GVHD and late infections. As with other analyses, there have been conflicting reports, as an earlier study associated high
pDC count with cGVHD, although this was at a median of 14.5 months post transplant. Larger studies comparing DC subsets following various conditioning regimens may help elucidate the differences between studies.

DC subsets have been examined extensively in experimental GVHD. By adoptively transferring DC into MHC II-deficient recipient mice, both pDC and cDC were found to be sufficient to induce comparable donor CD4+ T cell-dependent GVHD, although pDC required an inflammatory environment created by host irradiation for activation and donor T cell priming. Thus, pDC expressing alloAg were sufficient to prime alloreactive T cells and induce GVHD. Similar to human studies, low pDC (depleted by 120G8 Ab to BM stromal cell Ag-2) in the BM graft led to increased aGVHD, while there was no association between GVHD and pDC count in G-CSF mobilized grafts. The authors indicated that these latter pDC were mature, which may account for the difference in incidence of GVHD. Additionally, while cDC reconstitution did not differ between control and GVHD mice, pDC maturation was abrogated in GVHD. Interestingly, GVHD led to a suppressive precursor DC population that may contribute to immune paralysis post transplant. These findings concerning the role of DC subsets provide important insight into potential strategies for tolerance induction in HSCT.

Influence of current GVHD therapies on DC

Many current therapies significantly affect DC phenotype and function. More precisely, calcineurin inhibitors (CNI; cyclosporine or tacrolimus) suppress Ag presentation, while glucocorticosteroids inhibit DC maturation, activation and production of TNFα, IL-1β, and IL-12 following stimulation. DC generated in the presence of CNI or rapamycin (sirolimus; the serine-threonine kinase inhibitor of the mammalian target of rapamycin [mTOR]) have decreased costimulatory molecule expression and T-cell allostimulatory capacity. In addition, epidermal LC exposed to glucocorticosteroids are phenotypically immature and expand Treg via transforming growth factor (TGF)-β production.
Ab therapy directed against immune cells is used both in the prevention and treatment of GVHD. Polyclonal anti-thymocyte globulin (ATG) Ab has been used pre-transplant for T-cell depletion for decades. However, as reviewed by Mohty, ATG has diverse immunological effects, including its impact on DC. ATG inhibits experimental DC Ag uptake and maturation, induces complement-mediated lysis of DC, and decreases the capacity of DC to stimulate allogeneic T cells. In humans, ATG decreases DC Ag uptake, PB mDC and pDC, and mDC IL-12 production and allogeneic T cell proliferation.

Alemtuzumab (Campath-1H), a lymphocyte-depleting humanized anti-CD52 mAb, has been used for both GVHD prevention and treatment. As well as depleting donor T cells, alemtuzumab may also target host DCs. Although its effects on DC are not well-studied, alemtuzumab depletes human PB DC in vivo, but has few significant effects on LC or dermal DC which only weakly express the epitope. Multiple mAbs against the IL-2 receptor (R) (CD25) have shown efficacy in second-line treatment of GVHD. While much of these effects have been attributed to direct binding to T cells, recent work using daclizumab (humanized anti-CD25 mAb) has shown that it potently inhibits Ag-specific T cell activation by mature DC.

Prevention or treatment of GVHD by (via) targeting DC (summarized in Table 3)

Historically, T cells have been the primary target in GVHD, but given the important role of DC in its pathogenesis, APC also represent an important target. DC may be manipulated using multiple approaches in vivo or in vitro, in the latter case for the production of tolDC vaccines with the ability to regulate immunity and suppress GVHD. Approaches being evaluated include the following.

(i) Pharmacologic interventions

Histone deacetylase (HDAC) inhibitors, used clinically as anti-cancer drugs, reduce DC TLR-induced costimulatory molecule expression, proinflammatory cytokine release, and T cell allostimulatory activity. Further, they increase T<sub>reg</sub> number and function via increased IDO expression in a signal transducer and activator of T cells (STAT)3-dependent manner. HDAC inhibition decreases GVHD in several
Proteasome inhibitors have been studied in cancer and auto-immunity and are thought to induce apoptosis by blocking the degradation of pro-apoptotic proteins. Bortezomib, approved for the treatment of multiple myeloma, is believed to block the activation and nuclear translocation of nuclear factor (NF)-κB, a transcription factor central to DC maturation and inflammatory responses. Thus, inhibition of DC NF-κB activation with bortezomib or other inhibitors is an attractive strategy for GVHD prevention.\textsuperscript{106} Immature DC treated with bortezomib fail to upregulate MHC-II and co-stimulatory molecules in response to maturation signals, have decreased T cell allostimulatory capacity, and are more susceptible to apoptosis.\textsuperscript{106,107} In experimental HSCT, bortezomib attenuates aGVHD, yet preserves GVL.\textsuperscript{106,107} While early treatment post-HSCT prevents mild aGVHD in mice, later treatment increases mortality significantly,\textsuperscript{108} which may reflect loss of early effects on immature DC. Notably, histopathological observations in later bortezomib treatment have implicated severe colonic damage in increased GVHD-dependent mortality.\textsuperscript{106}

RelB, an NF-κB family subunit, has been shown to be critical within both host and donor APCs for the induction and maintenance of experimental GVHD.\textsuperscript{109} RelB in APC is required for differentiation of Th1 effectors, but not for expansion or function of donor T\textsubscript{reg}.\textsuperscript{109} Inhibition of nuclear RelB translocation, with RelB inhibitors targeted to DC using Ab, thus appears to be an attractive strategy for therapy of GVHD.\textsuperscript{109} Although these studies confirm NF-κB in DC as an important therapeutic target, they also urge caution when considering bortezomib for the treatment of established GVHD given that late (versus early) treatment in an experimental model significantly increased mortality.

(ii) Biologic interventions
Activated DC may be targeted by mAbs against cell surface molecules, including CD83, which is upregulated upon DC maturation. There is recent evidence that anti-CD83 (polyclonal Ab) decreases T cell proliferation induced by DC, while maintaining anti-viral T cell memory.\(^1\) In an experimental model, anti-CD83 therapy prevented GVHD, while preserving HSC engraftment and GVL.\(^1\) Co-stimulatory signal blockade also prevented experimental GVHD, with the most significant effect achieved by blocking ICOS (using mAb) and CD28 (CD28\(^{-}\) donor T cells) with intact CTLA-4 signaling.\(^5\) Further mechanistic and therapeutic studies of mAbs directed against activated DC are clearly justified.

**Generation of tolDC for prevention or treatment of GVHD** (summarized in Table 4)

DC can be manipulated in vitro to produce tolDC or ‘negative DC vaccines’ for control of alloimmunity or allograft rejection. TolDC may be produced under specific culture conditions, by pharmacological modification, or by cell sorting. Early studies showed that immature DC generated from BM cells in GM-CSF, and with weak allostimulatory T cell capacity, could prolong organ allograft survival.\(^\text{111,112}\) Subsequent reports have verified and extended these findings to show that immature or maturation-resistant tolDC can promote tolerance in experimental organ and HSCT,\(^\text{39,113,114}\) while still protecting against leukemia relapse.\(^\text{114}\)

Pharmacologic manipulation of DC (e.g. using dexamethasone, rapamycin or IL-10) renders DC maturation-resistant and enhances their tolerogenic potential for inhibition of allograft rejection and GVHD. As an example, rapamycin-treated DC (RAPA-DC) resist maturation and have impaired capacity to stimulate allogeneic effector T cells, yet promote T\(_{\text{reg}}\).\(^\text{93}\) When adoptively transferred to organ graft recipients, RAPA-DC promote transplant survival and, in conjunction with a short course of host immunosuppression, can induce indefinite graft survival.\(^\text{93,115,116}\) When administered systemically in experimental GVHD, host-derived RAPA-DC traffic to secondary lymphoid tissue and improve both survival and histopathological grade of GVHD.\(^\text{117}\)

Similarly, vasoactive intestinal peptide (VIP) is an immunosuppressive neuropeptide that has been used to generate host-derived tolDC that increase T\(_{\text{reg}}\) and abrogate aGVHD, while maintaining GVL.\(^\text{118}\) Interestingly,
early administration (by day +5) of these tolDC is critical in the MHC-mismatched model. They were more effective in the miHA mismatched model regardless of timing.\textsuperscript{118}

IDO is an important enzyme in tryptophan catabolism that is thought to be critical for control of T\textsubscript{eff} responses.\textsuperscript{119} Following experimental GVHD, IDO expression in host APC is increased via IFN\textsubscript{γ} release by donor T cells. IDO\textsuperscript{-/-} recipients have accelerated colonic GVHD and mortality, with enhanced T cell proliferation and decreased apoptosis.\textsuperscript{120,121} Specific culture conditions (e.g. low tryptophan or LPS and IFN\textsubscript{γ}) can be used to generate tolDC with increased IDO expression.\textsuperscript{122,123} While these tolDC have not been studied directly in experimental GVHD, DC treated with the HDAC inhibitor SAHA display enhanced IDO expression and suppress experimental GVHD in an IDO-dependent manner.\textsuperscript{104} Additionally, increasing colon IDO expression via the injection of kynurenine (tryptophan breakdown product) or a TLR7/8 agonist (3M-011) abrogates experimental GVHD mortality.\textsuperscript{121}

Cell sorting can be used to isolate/purify tolDC. Murine CD8\textalpha\textsuperscript{+} DC are the principal DC subset involved in cross-presentation (Table 1) and have tolerogenic properties.\textsuperscript{124} In both MHC- and miHA-mismatched models of aGVHD, immunization of recipients with ex vivo-generated and FACS-sorted autologous CD8\textalpha\textsuperscript{+} DC pre-transplant reduces GVHD in an IL-10-dependent, Ag-specific manner.\textsuperscript{124} These results confirm the therapeutic ability of CD8\textalpha\textsuperscript{+} DC to modify aGVHD, as shown in earlier studies in which Flt3L administration expanded CD8\textalpha\textsuperscript{+} DC in vivo and reduced aGVHD.\textsuperscript{125} Ildstad and colleagues have also described how CD8\textalpha\textsuperscript{+}/TCR\textsuperscript{-} ‘facilitating cells,’ with a critical component of plasmacytoid precursor-like CD11c\textsuperscript{+/B220\textsuperscript{-}/CD11b\textsuperscript{-}} cells, enhance HSC engraftment in mice without increased GVHD.\textsuperscript{126,127} This effect was attributed to the induction of Ag-specific chimeric T\textsubscript{reg} that suppress effector T cells. Murine CCR9\textsuperscript{+} pDC, obtained via Flt3L-induced mobilization and cell sorting, display an immature phenotype and prevent experimental aGVHD via induction of T\textsubscript{reg} and suppression of IL-17-producing effector T cells, while maintaining IFN-γ-producing effector T cells.\textsuperscript{128} Overall, distinct subsets of ex vivo-fashioned tolDC or endogenous DC have potential for therapy of GVHD and an important question is which subset is best-suited for therapeutic application.
So called “regulatory DC” (DC\textsubscript{reg}), generated by culturing BM in GM-CSF, IL-10, and TGF-β, are proposed to have greater therapeutic efficacy than conventional tolDC\textsuperscript{129}. There is evidence that DC\textsubscript{reg} exclusively express CD200R3 and that naturally-occurring mouse CD49\textsuperscript{+}CD200R3\textsuperscript{+} DC are identical phenotypically and functionally\textsuperscript{130}. Both BM-derived and naturally-occurring recipient-type DC\textsubscript{reg} protect against cutaneous cGVHD in a multiple miHA- or MHA- mismatched model via the generation of donor inducible T\textsubscript{reg} and anergic, Ag-specific CD4\textsuperscript{+} T cells\textsuperscript{130}. Moreover, depletion of CD49\textsuperscript{+}CD200R3\textsuperscript{+} cells before alloHSCT enhanced the progression of cGVHD\textsuperscript{130}.

**Cell therapies that target DC in vivo**

(i) Mesenchymal stem cells (MSC)

MSC are rare, heterogenous, pluripotent non-hematopoietic progenitors present in normal BM and adipose tissue that induce immune tolerance via effects on multiple immune cells, in particular DC. Human MSCs (hMSCs) impair DC maturation and induce T cell hyporesponsiveness in a dose- and contact-dependent manner. The effect can be partly reversed by DC maturation and by blocking IL-10 or IL-6\textsuperscript{131}. TolDC generated by co-culture of DC with human MSCs (MSC-DC) induce Ag-specific T\textsubscript{reg} via activation of the Notch pathway, but they have not been studied in vivo\textsuperscript{132,133}.

MSC have shown promise in the prevention and treatment of GVHD. As reviewed by Baron et al\textsuperscript{134} while various mouse models have generated conflicting results, they suggest the importance of MSC dose, timing, and activation status. Phase I-II human studies have demonstrated safety and possible efficacy, and multicenter randomized blinded trials on currently underway\textsuperscript{134}. Interestingly, the combination of rapamycin and MSC following experimental cardiac transplantation led to long-term graft survival with significantly increased splenic T\textsubscript{reg} and tolDC\textsuperscript{135}. This also highlights the capacity of synergistic therapies in the promotion of tolerance\textsuperscript{135}. 
(ii) Myeloid-derived suppressor cells (MDSC)

MDSC are heterogeneous hematopoietic precursor cells with immunosuppressive properties, first noted to aid tumor evasion in mice and humans. As reviewed by Lees et al, MDSC modulate both innate and adaptive immunity. While many of their functions are attributed to direct effects on T cells, MDSC additionally inhibit the differentiation and maturation of DC. In mice, MDSC generated from BM cells in G-CSF, GM-CSF, and IL-13 (MDSC-IL-13) were more potent inhibitors of MHC-mismatched GVHD than conventional MDSC. This inhibition was dependent on the L-arginine depleting enzyme arginase. Importantly, MDSC-IL-13 do not impair the GVL effect in vivo.

(iii) \(T_{\text{reg}}\)

As a bidirectional tolerogenic feedback loop exists between \(T_{\text{reg}}\) and tolDC, \(T_{\text{reg}}\) therapy supports tolerance through effects on DC. DC also control the number and function of \(T_{\text{reg}}\). Host APC alloAg expression is necessary and sufficient for \(T_{\text{reg}}\) function in both miHA- and MHC-mismatched mouse models of GVHD, independent of APC IL-10 or IDO expression. In addition, human \(T_{\text{reg}}\) (generated via CD127 [IL-7R\(\alpha\)] negative selection) and \(T_{\text{reg}}\)-conditioned DC can abrogate xenogeneic GVHD via induction of immunosuppressive PD-L1 expression on conditioned DC and on effector T cells in vivo. Furthermore, Ag-specific \(T_{\text{reg}}\) can be induced and expanded by DC, as demonstrated by human monocyte-derived DC in an IDO-dependent manner.

Adoptive transfer of \(T_{\text{reg}}\) is highly effective in the prevention of experimental GVHD, thus phase I trials are underway with initial studies demonstrating safety and some efficacy. A major impediment to \(T_{\text{reg}}\) therapy has been the generation of sufficient cell numbers, particularly for natural \(T_{\text{reg}}\) (n\(T_{\text{reg}}\)). Interestingly, the addition of rapamycin (for restimulation of n\(T_{\text{reg}}\) or for the generation of iT\(T_{\text{reg}}\) with TGF-\(\beta\)) increases \(T_{\text{reg}}\) yields, which may allow completion of dose escalation trials.

Active clinical trials utilizing interventions that target/impact dendritic cells (summarized in Table 5)
There are numerous open clinical trials for the prevention or treatment of GVHD currently studying pharmacologic or biologic interventions and cellular therapies that target or impact DC (Table 5). While not listed in Table 5, there are also many ongoing trials assessing the impact of conventional GVHD therapies (e.g. corticosteroids, CNI, rapamycin) used in new combinations and via different routes (e.g. topical, intra-hepatic, etc). Cellular therapy remains particularly intriguing, with the majority of active studies utilizing MSC. A single trial has been underway testing autologous DC in the setting of relapsed hematologic malignancy; although the DC are not being used for the prevention or treatment of GVHD, GVHD is a primary outcome measure of the study and the trial will hopefully demonstrate safety and feasibility of DC therapy in the SCT setting.

Conclusions

Despite therapies that broadly target effector T cells or globally suppress immunity, GVHD remains a significant cause of post-HSCT morbidity and mortality. Given the tolerogenic potential of some DC subsets and the critical role of others in the pathogenesis of GVHD, differences in DC characteristics may be used to predict outcome while targeting DC is an innovative treatment approach. Likewise, DC may be targeted directly in vivo through molecular pathways or cell surface expression of maturation markers or costimulatory molecules, or tolDC may be generated in vitro and given in the peri-transplant period (summarized in Figure 4). Other cellular therapies, including Treg, mediate dominant immunosuppressive effects by restraining DC stimulatory functions. Given the importance of the GVL effect, any therapy targeting or utilizing DC must conserve this process.

Further understanding of the precise immunoregulatory properties of DC and the development of DC-based therapies for GVHD will expand HSCT use beyond treatment of malignant disease and allow its use in patients lacking MHC-matched donors. Early work by Shlomchik et al\textsuperscript{2,46} elucidated the critical role of miHA expression by host hematopoietic APC for CD8\textsuperscript{+} T cell-driven GVHD; thus, therapies that orchestrate the successful and timely suppression and/or ablation of host DC are expected to be particularly beneficial to
patients following MHC-matched HSCT. Very recent findings suggest that recipient non-hematopoietic APC in
target organs may be central to promoting indirect CD4+ T cell-mediated aGVHD. In these studies, host
CD11c+ DC suppressed GVHD development. These and other data discussed above underscore the importance
of developing a clear understanding of DC involvement in the complex immunopathology of GVHD. Likewise,
infused recipient Ag-pulsed donor tolDC or recipient tolDC presenting alloAg and miHA have the potential to
prevent the pathological alloresponses of donor T cells and benefit HSCT patients given either MHC-matched
or mismatched transplants.

Finally, given the role of both DC and T cells in the pathogenesis of GVHD, synergistic therapies or those that
target both cell types in vivo may be more effective. Cellular therapies, specifically tolDC and Treg, are
intriguing in their ability to modulate one another in vivo. Importantly, cellular therapies have begun in
humans. Human tolDC have been generated and characterized in vitro using clinical grade reagents. Recently, a DC-based vaccine for the treatment of prostate cancer was approved by the FDA, and the first report has appeared of a phase I safety study of tolDC in patients with type 1 (autoimmune) diabetes. As other forms of innovative cell therapy, including testing of Treg, are underway for the prevention of GVHD, there would appear to be adequate justification for phase I studies of tolDC alone and in combination with Treg in HSCT.

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(MYM). We thank Brian Rosborough for critical reading of the manuscript.

Authorship contribution

EOS and AWT wrote the manuscript, HRT assisted in writing the manuscript and generation of the figures,
MYM assisted in editing the manuscript. The authors have no conflicts of interest.
REFERENCES


<table>
<thead>
<tr>
<th>Organ</th>
<th>Phenotype</th>
<th>Location</th>
<th>Function &amp; Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoid-tissue resident DC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>CD8α⁺CD205⁺</td>
<td>T cell area &amp; marginal zone</td>
<td>Uptake &amp; cross-presentation of Ag from apoptotic cells on MHCII</td>
</tr>
<tr>
<td></td>
<td>CD8α⁻33D1⁺</td>
<td>Red pulp &amp; bridging channels</td>
<td>Predominant population; uptake &amp; presentation of Ag on MHCII</td>
</tr>
<tr>
<td>Thymus</td>
<td>CD8α⁺</td>
<td>Medulla</td>
<td>Predominant population; cross presentation of Ag; self-tolerance</td>
</tr>
<tr>
<td></td>
<td>CD8α⁻</td>
<td>Cortex, medulla, corticomedullary junction</td>
<td>Self-tolerance</td>
</tr>
<tr>
<td>Lymph node</td>
<td>CD8α⁺</td>
<td>Cortex</td>
<td>Viral &amp; self Ag immunity; cross presentation of Ag</td>
</tr>
<tr>
<td></td>
<td>CD8α⁻</td>
<td>Cortex</td>
<td>Unknown</td>
</tr>
<tr>
<td>Migratory DC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>Langerin⁺ LC</td>
<td>Epidermis</td>
<td>Self renew in situ, radiation resistant</td>
</tr>
<tr>
<td></td>
<td>CD103⁺CD11b⁺Langerin⁺</td>
<td>Dermis</td>
<td>Viral &amp; self Ag immunity; cross presentation of Ag</td>
</tr>
<tr>
<td></td>
<td>CD103⁺CD11b⁺Langerin⁻</td>
<td>Dermis</td>
<td>Unknown</td>
</tr>
<tr>
<td>Intestine</td>
<td>CD103⁺CD11b⁺</td>
<td>Peyer's patches</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>CD103⁺CD11b⁻</td>
<td>Lamina propria</td>
<td>Transfer pathogenic bacteria from gut to mesenteric LN</td>
</tr>
<tr>
<td></td>
<td>CD103⁺CD11b⁺</td>
<td>Lamina propria</td>
<td>Transport Ag to mesenteric LN from gut lumen</td>
</tr>
<tr>
<td>Lymph node</td>
<td>CD11c⁺⁺⁺CD40⁺⁺⁺MHCII⁺⁺⁺CCR7⁺⁺⁺</td>
<td>Cortex</td>
<td>Transport Ag to LN from periphery</td>
</tr>
</tbody>
</table>

DC indicates dendritic cell; Ag, antigen; MHC, major histocompatibility complex; LC, Langerhans cell; LN, lymph node.
<table>
<thead>
<tr>
<th>Patient Population</th>
<th>DC measurement</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allo-HSCT, n=49</td>
<td>Total PB DC count at engraftment &lt;4.97 cells/µL</td>
<td>Survival ↓</td>
<td></td>
</tr>
<tr>
<td>92% PBSCT; 73% MAC</td>
<td>Relapse &amp; aGVHD ↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low PB mDC (CD11c+) at engraftment</td>
<td>Survival ↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low PB pDC (CD123+) at engraftment</td>
<td>aGVHD ↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Graft DC or DC count pre-transplant</td>
<td>No association with death/relapse</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allo-HSCT, n=30</td>
<td>Higher PB pDC (BDCA-2+) &gt;d+100 (donor)</td>
<td>cGVHD ↑</td>
<td>83</td>
</tr>
<tr>
<td>63% PBSCT; 83% MAC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allo-HSCT, n=24</td>
<td>Higher total PB host DC d+100</td>
<td>Survival ↓</td>
<td>84</td>
</tr>
<tr>
<td>100% BM; 87% RIC</td>
<td>aGVHD &amp; cGVHD (gr II-IV)↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allo-HSCT, n=40</td>
<td>Low total PB DC, mDC (CD11c+), &amp; pDC (CD123+) counts</td>
<td>GVHD severity ↑</td>
<td>85</td>
</tr>
<tr>
<td>90% PBSCT; 52% RIC</td>
<td>&gt;7.9% CMRF-44+CD11c+ DC</td>
<td>aGVHD ↑ (sens. 87.5%, spec. 79.2%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CD83+/CD86+ CD11c+ DC</td>
<td>No association with aGVHD</td>
<td></td>
</tr>
<tr>
<td>Allo-HSCT, n=69</td>
<td>Graft pDC (CD123+) &gt;2.3x10⁶/kg</td>
<td>Relapse ↑</td>
<td>83</td>
</tr>
<tr>
<td>100% PBSCT; 54% MAC</td>
<td>OS &amp; EFS ↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allo-HSCT, n=54</td>
<td>Lower PB pDC (lin-/CD11c+/ILT3+) 3 months post HSCT (median 92 d)</td>
<td>aGVHD (gr II-IV)↑</td>
<td>88</td>
</tr>
<tr>
<td>93% PBSCT; 100% RIC</td>
<td>OS &amp; NRM ↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Late infections ↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Death ↑</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PBSCT indicates peripheral blood stem cell transplant; MAC, myeloablative conditioning; PB, peripheral blood; BM, bone marrow; RIC, reduced-intensity conditioning; sens., sensitivity; spec., specificity; OS, overall survival; EFS, event-free survival; ILT3, immunoglobulin-like transcript 3; NRM, non-relapse mortality
<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Therapy</th>
<th>GVHD model; treatment</th>
<th>Effect on DC*</th>
<th>Effect on GVHD</th>
<th>Reference</th>
</tr>
</thead>
</table>
| HDAC inhibition        | SAHA or ITF2357    | MHC mismatched BALB/c- >B6; Host type DC pre-treated with HDAC inhibitor infused days -1, 0, 2 | TNFα, IL-12, & IL-6 secretion ↓  
CD40/80/86 expression ↓  
Allogeneic T cell proliferation ↓ (in vitro/vivo) | Survival ↑  
Clinical score ↓  
Serum TNFα ↓  
Preserved GVL | 104       |
| Pro teaseome inhibition| Bortezomib         | MHC mismatched BALB/c- >B6; Early (d0-2) versus late (d6-8) treatment | Response to maturation signals ↓  
MHC II, CD40/80/83/86 expression ↓  
Apoptosis ↑  
Allogeneic T cell proliferation ↓ | Survival ↑ with early treatment  
Survival ↓ with late treatment  
Preserved GVL | 108       |
| NF-κβ inhibition       | Relb−/− BM chimera recipients | MHC mismatched BALB/c - >B6 & B6->B6D2F1 | CD11chi DC (cDC) ↓  
CD40/80/86 expression unchanged  
IL-12, IL-6, TNFα secretion ↓ (following CD40L)  
CD4+ T cell proliferation (in vitro/vivo); cytokine secretion ↓ (in vitro)  
No difference PDCA-1+ DC (pDC), Treg (in vivo) | Survival ↑  
Clinical score ↓ | 109       |
|                        | Relb−/− BM chimera donor | MHC mismatched | Late (>3 wks) survival ↑ & clinical score ↓ |                                                                                   |           |
| Anti-CD83              | polyclonal Ab      | Hu SCID; Day 0 | Survival ↑  
Preserved engraftment and GVL |                                                                                   | 110       |

HDAC indicates histone deacetylase; SAHA, suberoylanilide hydroxamic acid; TNFα, tumor necrosis factor-alpha; NF-κβ, nuclear factor-kappa beta; Hu SCID, human severe-combined immunodeficiency.

*In vitro unless otherwise indicated
### Table 4: Use of tolDC therapy to inhibit GVHD in mice

<table>
<thead>
<tr>
<th>TolDC</th>
<th>GVHD model; treatment</th>
<th>Effect on DC (in vitro)</th>
<th>Effect on GVHD</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DC conditioning</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rapamycin</td>
<td>MHC mismatched (B6-&gt; BALB/c); Host-type DC d0</td>
<td>MHC II, CD80/86 expression ↓</td>
<td>Survival ↑</td>
<td>117</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intact in vivo trafficking</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intact CCR5, CCR7, and CD62L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIP</td>
<td>MHC mismatched (B6-&gt; BALB/c, BALB/c -&gt;B6) and miHA mismatched (B6-&gt;F1); Host-type DC d+2 and/or d+5</td>
<td>Donor CD4+ T cell Ag-specific response ↓</td>
<td>Survival ↑†(miHA&gt;MHA; early&gt;late for MHA)</td>
<td>118, 146</td>
</tr>
<tr>
<td>SAHA (HDAC inhibitor)</td>
<td>MHC mismatched (BALB/c -&gt;B6); Host-type DC d-1, 0, +2</td>
<td>CD40/80 expression ↓</td>
<td>Survival ↑</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TNFα ↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>T cell proliferation ↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGFβ, IL-10 + LPS (DC&lt;sub&gt;reg&lt;/sub&gt;)*</td>
<td>MHC matched, miHA mismatched cGVHD (B10.D2-&gt; BALB/c)</td>
<td>Induce anergy of Ag-specific T cells</td>
<td>Cutaneous GVHD ↓</td>
<td>129</td>
</tr>
<tr>
<td></td>
<td>Host-type DC d+2, +9, &amp; +16 or d+18, +25, &amp; +32 vs short course rapamycin</td>
<td>TNFα, IL-12p70, and IFNγ ↓</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Induce T&lt;sub&gt;reg&lt;/sub&gt;</td>
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<tr>
<td><strong>Subset (no DC conditioning)</strong></td>
<td></td>
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<tr>
<td>CD8α&lt;sup&gt;+&lt;/sup&gt; DC</td>
<td>MHC matched; miHA mismatched (C3H.SW-&gt;B6) mismatched; Host-type DC d-8, -5 to -3, and -1</td>
<td>T cell proliferation ↓</td>
<td>Survival ↑</td>
<td>124</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IFNγ &amp; TNFα ↓</td>
<td>Histopathology ↓</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>IL-10 ↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCR9&lt;sup&gt;+&lt;/sup&gt; pDC</td>
<td>MHC mismatched (BALB/c -&gt;B6); Host-type DC d0 (mobilized with Flt3L)</td>
<td>CD40/80/86 expression ↓</td>
<td>Survival ↑</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intermediate expression of MHC II</td>
<td></td>
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<td></td>
<td></td>
<td>Induce T&lt;sub&gt;reg&lt;/sub&gt;</td>
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<tr>
<td></td>
<td></td>
<td>IL-12 producing effector T cells ↓</td>
<td></td>
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<tr>
<td>CD49&lt;sup&gt;+&lt;/sup&gt;CD200R3&lt;sup&gt;+&lt;/sup&gt; DC (naturally-occurring DC&lt;sub&gt;reg&lt;/sub&gt;)</td>
<td>MHC matched, miHA mismatched cGVHD (B10.D2-&gt; BALB/c); Host-type DC d+2, +9, &amp; +16</td>
<td>Anergy of Ag-specific T cells</td>
<td>Cutaneous GVHD ↓</td>
<td>130</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TNFα, IL-12p70, and IFNγ ↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Induce T&lt;sub&gt;reg&lt;/sub&gt;</td>
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<td></td>
</tr>
</tbody>
</table>

VIP indicates vasoactive intestinal peptide; SAHA, suberoylanilide hydroxamic acid; TGFβ, transforming growth factor beta; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-alpha; IFNγ, interferon-gamma; Flt3L, FMS-like tyrosine kinase 3 ligand; ip, intraperitoneal
* Depleted of CD40<sup>+</sup>CD80<sup>+</sup>CD86<sup>+</sup> cells
<table>
<thead>
<tr>
<th>ID</th>
<th>Condition</th>
<th>Intervention</th>
<th>Phase</th>
<th>Study type</th>
<th>Sponsor</th>
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</thead>
<tbody>
<tr>
<td>NCT00810602</td>
<td>aGVHD</td>
<td>Vorinostat plus tacrolimus &amp; mycophenolate following RIC related donor allogeneic transplant; prevention</td>
<td>II</td>
<td>Single agent, open label, non-randomized safety/efficacy study</td>
<td>University of Michigan Cancer Center</td>
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<tr>
<td>NCT01111526</td>
<td>aGVHD</td>
<td>Panobinostat (LBH589) plus corticosteroids; initial treatment</td>
<td>I/II</td>
<td>Non-randomized, open label, safety/efficacy study</td>
<td>H. Lee Moffitt Cancer Center and Research Institute</td>
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<tr>
<td>NCT01158105</td>
<td>Steroid-refractory cGVHD</td>
<td>Bortezomib; treatment</td>
<td>II</td>
<td>Single agent, open label safety/efficacy study</td>
<td>Baylor Research Institute</td>
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<tr>
<td>NCT00670423</td>
<td>GVHD</td>
<td>Bortezomib plus tacrolimus and sirolimus following allogeneic PBSC transplant; prevention</td>
<td>I</td>
<td>Single agent, open label safety study</td>
<td>Indiana University School of Medicine</td>
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<td>NCT01323920</td>
<td>aGVHD</td>
<td>Bortezomib plus tacrolimus and methotrexate following myeloablative allogeneic SCT without HLA-matched related donor; prevention</td>
<td>II</td>
<td>Single agent, open label safety/efficacy study</td>
<td>Dana-Farber Cancer Institute</td>
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<td>NCT01163786</td>
<td>Bronchiolitis obliterans (cGVHD)</td>
<td>Bortezomib; treatment</td>
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<td>NCT01012492</td>
<td>aGVHD</td>
<td>Abatacept (CTLA4-Ig) plus cyclosporine and methotrexate following unrelated donor HSCT; prevention</td>
<td>II</td>
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<td>NCT00603330</td>
<td>gr II-IV steroid-refractory aGVHD</td>
<td>MSC; treatment</td>
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<td>NCT00827398</td>
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<td>NCT01045382</td>
<td>GVHD</td>
<td>MSC versus placebo in HLA-mismatched allogeneic hematopoietic cells after nonmyeloablative conditioning; prevention</td>
<td>II</td>
<td>Randomized, double-blind safety/efficacy study</td>
<td>University Hospital of Liege</td>
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<td>NCT01222039</td>
<td>cGVHD</td>
<td>Conventional therapy versus conventional therapy plus MSC derived from adipose tissue; treatment</td>
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<td>NCT00957931</td>
<td>GVHD</td>
<td>Haploidentical MSC in MUD HCT in patients with high risk non-malignant RBC disorders following RIC; prevention</td>
<td>II</td>
<td>Non-randomized, open label efficacy study</td>
<td>Stanford University</td>
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<td>NCT01050764</td>
<td>GVHD</td>
<td>Allogeneic Treg plus allogeneic conventional T cells following allogeneic MAC HCT with haploidentical related donor for patients with hematologic malignancies; prevention</td>
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<td>Non-randomized, open label safety/efficacy study</td>
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<td>NCT00935597</td>
<td>GVHD</td>
<td>Host DC infusion following allogeneic SCT for prevention or treatment of relapsed disease in patients with advanced hematologic malignancies</td>
<td>I</td>
<td>Non-randomized, open label safety/efficacy study</td>
<td>Mount Sinai School of Medicine</td>
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aGVHD indicates acute graft-versus-host disease; RIC, reduced-intensity conditioning; cGVHD, chronic graft-versus-host disease; PBSC, peripheral blood stem cell; SCT, stem cell transplant; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplant; MSC, mesenchymal stem cells; MUD, matched-unrelated donor; RBC, red blood cell; MAC, myeloablative conditioning; DC, dendritic cell.
FIGURE LEGENDS

Figure 1

**DC hematopoiesis and subsets.** (A) All identified DC subsets can be generated from either a common myeloid progenitor (CMP) or common lymphoid progenitor (CLP) depending on the cytokines and growth factors present. DC can be broadly categorized as conventional DC (cDC) or precursor DC. Plasmacytoid DC (pDC) are understood to be a subset of precursor DC that have plasma cell morphology, an immature phenotype, and secrete type I IFN following activation. Monocyte-derived DC or ‘inflammatory DC’ are similar to cDC in form and function and correlate with in vitro GM-CSF generated DC. cDC can be categorized as lymphoid tissue resident and migratory DC. DC were categorized previously as lymphoid or myeloid (mDC) based on the hypothesis that each had separate progenitors, a convention that has persisted in the experimental and clinical evaluation of DC subsets. (B) In mice, pDC are identified as CD11c^lo^CD11b^sialic acid binding immunoglobulin-like lectin H (Siglec-H)^^PDCA-1^+, while in humans, they are lin^MHCIIm^IDC11c^CD123^BDCA2(CD303)^+. Mouse mDC are identified as CD11c^CD11b^B220^ (CD45R^-^NK1.1^-, whereas human mDC are lin^MHCII^CD11c^CD123^BDCA1(CD1b/c)^+. Other phenotypic differences between mouse and human DC precursors are also listed in the table.

HSC indicates hematopoietic stem cell; MPP, multipotent progenitor; CMP, common myeloid progenitor; CLP, common lymphoid progenitor; Flt3L, fms-like tyrosine kinase 3 ligand; GM-CSF, granulocyte macrophage colony-stimulating factor; LN, lymph node; LC, Langerhans cells; LP, lamina propria.

Figure 2

**Role of DC in the pathogenesis of GVHD.** (A) Recipient pre-transplant conditioning results in target organ tissue damage, leading to the so-called ‘cytokine storm’ a progressive amplification of pro-inflammatory cytokine production and immune activation as inflammatory cytokines feed forward unabated. IL-1β, IL-6, and TNFα are particularly implicated in this process. In addition to pro-inflammatory cytokines, conditioning-released damage-associated molecular patterns (DAMPS) and translocation of LPS in the intestine also lead to
the activation of host and subsequently donor DC, including epidermal Langerhans cells and dermal DC in the skin. Mature DC upregulate MHC, co-stimulatory, and intercellular adhesion molecule expression. (B) DC present host allo-Ag to donor T cells. Host DC resistant to conditioning present alloAg via the direct pathway, while transplanted donor DC present processed alloAg peptides on MHC syngeneic with donor T cells via the indirect pathway. Donor T cell activation requires Ag presentation via MHC molecules to the T cell Ag receptor (TCR), as well as stimulation via various co-stimulatory molecules. This interaction results in T cell activation, proliferation, differentiation (Th1, Th2), migration to GVHD target organs, and secretion of various chemokines and cytokines, importantly IFNγ and IL-2. (C) Cellular and inflammatory effectors lead to target organ tissue damage. Cytotoxic T lymphocytes (CTL) mediate target cell apoptosis via interactions between tumor-necrosis factor (TNF) and TNF receptors, TRAIL (TNF-related apoptosis-inducing ligand)/TRAIL-R and Fas (CD95)/FasL interactions and release of cytotoxic mediators (perforin and granzyme). Recruited macrophages release TNFα, IL-1 and NO which also damages target cells.

RT indicates radiation therapy; ICAM-1, inter-cellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; ICOS, inducible costimulator; NO, nitric oxide.

**Figure 3**

**Potential DC-based therapies for GVHD.** Tolerogenic (tol)DC can be used as a negative cellular vaccine following in vitro generation via their pharmacologic manipulation, cell sorting (subsets), or expansion following interaction with other immune regulatory cell populations. In vivo, DC can be targeted by the inhibition of molecular pathways (HDAC; NFκβ) or the expression of maturation markers or co-stimulatory molecules (e.g. CD80/86; CD83). Other cellular therapies, such as MSC, MDSC, and Treg, mediate immunosuppressive effects through DC.

VIP indicates vasoactive intestinal peptide; SAHA, suberoylanilide hydroxamic acid; MSC, mesenchymal stem cells; NF-κβ, nuclear factor-kappa beta; MDSC, myeloid-derived suppressor cells.
HSC → MPP

CMP

Flt3L → pDC

Flt3L → GM-CSF → CMP

Monocyte precursor → Monocyte

Monocyte-derived DC
- "Inflammatory DC"
- Similar form/function to DC
- Equivalent to DC generated in vitro in GM-CSF

GM-CSF → CLP

Flt3L → pDC

Precursor DC → cDC

Lymphoid tissue-resident DC
- Spleen, thymus, LN
- Immature phenotype
- Take up and process Ag

Migratory DC
- Mature phenotype (in LN)

CD8α+ → Epidermal LC

Dermal DC
- Lung, liver, kidney, intestine

CD103+ → CD103+

CD103- → CD103-

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Fig. 1A
**Figure 1B: Phenotypic Differences Between Mouse and Human DC and Their Precursors**

<table>
<thead>
<tr>
<th></th>
<th>Mouse</th>
<th>Human</th>
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<tbody>
<tr>
<td><strong>HSC</strong></td>
<td>Lin-Sca-1⁺c-Kit⁺CD150⁺Flt3⁻CD34⁻</td>
<td>Lin⁺CD34⁺CD38⁻</td>
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<tr>
<td><strong>MPP</strong></td>
<td>Lin-Sca-1⁺c-Kit⁺Flt3⁺</td>
<td>CD34⁺CD38⁻Thy-1⁺CD45RA⁻</td>
</tr>
<tr>
<td><strong>CMP</strong></td>
<td>IL-7Rα⁺FcγRlκ⁺CD34⁺</td>
<td>CD34⁺CD38⁺CD123(IL-3Rα)⁺CD45RA⁻</td>
</tr>
<tr>
<td><strong>CLP</strong></td>
<td>IL-7Rα⁺F1t3⁺</td>
<td>Lin⁺CD34⁺CD10⁺CD24⁺</td>
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<tr>
<td><strong>pDC</strong></td>
<td>CD11c⁺CD11b⁺Siglec-H⁺PDCA-1⁺</td>
<td>Lin⁺MHCIİ⁺CD11c⁺CD123⁺BDCA2(CD303)⁺</td>
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<tr>
<td><strong>mDC</strong></td>
<td>CD11c⁺CD11b⁺B220⁺(CD45⁻)NK1.1⁻</td>
<td>Lin⁺MHCIİ⁺CD11c⁺CD123⁺BDCA1(CD1b/c)⁺</td>
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</table>

HSC indicates hematopoietic stem cells; MPP, multipotent progenitor; CMP, common myeloid progenitor; CLP, common lymphoid progenitor; pDC, plasmacytoid DC; mDC, myeloid DC.
A  Recipient conditioning (chemotherapy, RT, infections)

Tissue damage (intestine, liver, skin)

DAMPs
LPS/other microbial products (intestine)

Macrophage

“Cytokine storm”
IL-6, IL-1β, TNFα

↑MHC, co-stimulatory molecules,
ICAM-1, VCAM-1
“Cytokine storm”
IL-6, IL-1β, TNFα

Immature DC

Mature DC

Fig. 2A
**B**

Direct Ag presentation

Indirect Ag presentation

Host DC

Donor DC

Donor T cell

Host allo Ag

Ag Processing

Activation

Proliferation

Differentiation

Migration

Cytokine/chemokine secretion

**C**

CTL

Target cell apoptosis

Perforin/granzymes

Macrophage

TNFα

IL-1β

NO

Fig. 2B, C
In vitro generated tolDC
- Pharmacologic manipulation (RAPA, VIP, SAHA, dexamethasone)
- Cell sorting (subsets)
- Expansion via other cellular therapies (MSC-DC, T<sub>reg</sub>-conditioned DC)

In vivo targeting of DC
- HDAC inhibition (SAHA)
- NFκβ inhibition
- Proteasome inhibition (bortezomib)
- Ab therapy targeting maturation markers and/or costimulatory molecules (anti-CD83, CD80/86)

Cellular therapies targeting DC in vivo
- MSC
- MDSC
- Treg

Fig. 3
Dendritic cells and regulation of graft-versus-host disease and graft-versus-leukemia activity

Elizabeth O. Stenger, Heth R. Turnquist, Markus Y. Mapara and Angus W. Thomson