Plasmacytoid dendritic cells in allogeneic hematopoietic cell transplantation: benefit or burden?

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Abstract

Plasmacytoid dendritic cells (pDCs) bridge innate and adaptive immune responses and have important roles in hematopoietic engraftment, GvHD and graft-versus-leukemia responses following allogeneic hematopoietic cell transplantation (HCT). In addition, pDCs mediate antiviral immunity, particularly as they are the body’s primary cellular source of type I interferon. Given their pleiotropic roles, pDCs have emerged as cells that critically impact transplant outcomes, including overall survival. In this article, we will review the pre-clinical and clinical literature, supporting the crucial roles that pDCs assume as key immune effector cells during HCT.

INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) is the definitive cure for many hematologic malignant diseases. However, GvHD, malignant disease relapse and infection remain the primary causes of death following allogeneic HCT.1 Mechanistic understanding of immune cells and associated soluble factors underlying aberrant immune responses is
needed to effectively prevent and treat these complications. In this regard, dendritic cells (DCs) have critical roles during allogeneic HCT.\textsuperscript{2} Specifically, plasmacytoid DCs (pDC) are a distinct subset of DCs that affect innate and adaptive immune responses. This manuscript will review the pre-clinical and clinical literature, supporting the importance that pDCs assume as key immune effector cells during HCT.

**OVERVIEW OF DCS: FOCUS ON PDC**

Key features of innate immunity include microbial pattern recognition, induction of antimicrobial and immunomodulatory cytokines and chemokines, and instruction of adaptive immunity. DCs have overlapping immune functions as potent APCs for naive T cells, initiation of innate immune response and instruction of subsequent adaptive immune response.\textsuperscript{3}

DC classification has changed over the years, reflecting advances in understanding their ontogeny and function. DCs can be broadly categorized into conventional DCs (cDCs) and pDCs\textsuperscript{4} (Table 1), both of which are derived from precursor DCs (preDCs) that originate from a common DC precursor cell arising from the hematopoietic stem cell (HSC) (Figure 1). Specifically, pDC development requires the transcription factor, E2-2, and the hematopoietic cytokine, fms-like tyrosine kinase 3 ligand (FL).\textsuperscript{5,6} As absence of FL markedly reduces pDC content in the hematolymphoid tissues\textsuperscript{7} as does granulocyte-macrophage colony-stimulating factor (GM-CSF)-induced expression of inhibitor of DNA binding 2, a repressor of E2-2.\textsuperscript{8}

DC activation occurs after recognition of pathogen-associated and danger-associated molecular patterns through pattern recognition receptors known as Toll-like receptors (TLRs). TLRs belong to the TIR (Toll/interleukin-1 receptor) superfamily, which uses a conserved TIR domain in the cytosolic region to activate common signaling pathways.\textsuperscript{9} The majority of TLRs utilize myeloid differentiation primary response protein 88 as signal adaptor proteins to activate interleukin (IL)-1 R-associated kinases and TNF receptor-associated factor 6, which ultimately activate nuclear factor κB and mitogen-activated protein kinases to initiate synthesis of inflammatory cytokines like IL-6 and TNFα.\textsuperscript{10} Plasticity and redundancy of cytokine responses directly reflect DC TLR expression.\textsuperscript{11}

Upon activation, cDCs upregulate surface expression of adhesion and costimulatory molecules and change function from Ag-capturing and processing cells to potent APCs that migrate to secondary lymphoid organs and stimulate naive T cells.\textsuperscript{12} In addition to their roles as APCs, mature cDCs produce cytokines and chemokines, which regulate subsequent innate and adaptive immune responses. For example, cDCs produce IL-12p70, which regulates interferon gamma production in natural killer (NK) cells,\textsuperscript{13} directs pro-inflammatory T-helper responses\textsuperscript{14} and enhances DC-NK cell cross-talk.\textsuperscript{15}

Human pDCs are the principal type I interferon (IFNα/β)-producing cells following infectious challenge.\textsuperscript{16} Type I IFNs have pleiotropic effects including activating and enhancing NK cytotoxicity and interferon gamma production,\textsuperscript{17,18} promoting activation, survival and differentiation of Th1 cells;\textsuperscript{19,20} mediating immune tolerance;\textsuperscript{21} and...
potentiating pDC activation itself\(^\text{22}\) (Figure 2). These effects underlie the critical role that pDC have in supporting antiviral immunity. During the acute phase of RNA (TLR7, ssRNA) and DNA (TLR9, CpG DNA) viral challenge, human pDCs become activated to produce type I IFN, which enhances dendritic, B, T and NK cell function, resulting in viral clearance and generation of memory response. However, pDC type I IFN production can also mediate detrimental effects, including inhibiting viral clearance during chronic infection by modulating APC function to produce IL-10 and to express inhibitory ligands (for example, programmed cell death 1 ligand), which collectively suppress antiviral T-cell function. In addition, type I IFN can increase epithelial cytotoxicity in the host, by enhancing inflammatory monocyte function. Therefore, regulation of type I IFN signaling within pDCs is needed to maintain host homeostasis.\(^\text{23}\)

Besides their endosomal TLR7 and 9 expression, human pDCs are identified by the following surface immunophenotype: absent CD11c (CD11c\(^-\)); low-level CD4 (CD4lo\(^+\)); and high-level CD123\(^\text{bright}\) (IL-3 R\(_\alpha\)) and blood DC Ags (BDCA), BDCA-2 (CD303) and BDCA-4 (CD304)\(^\text{24}\) (Figure 2). Along with these surface proteins, TLR-activated pDCs also upregulate surface co-stimulatory (CD40, CD80 and CD86) receptors that further facilitate their Ag presentation. Activated pDCs also produce cytokines (IL-6, IL-12 and TNF\(\alpha\)) that mediate direct effects on pathogens as well as activate immune cells, including macrophages, B-, NK- and T cells. At last, activated pDCs produce chemokines that recruit additional immune cells, further promoting local and systemic immune responses. pDCs also confer tolerogenic effects through cellular and cytokine induction both as activated and inactivated cells.\(^\text{25}\) For example, pDC have been shown to induce IL-10-producing T-regulatory (Treg) cells, by expressing inducible co-stimulator ligand, which negatively regulates T-cell activation and preferentially drives Treg induction. In addition, pDCs can produce indoleamine 2,3-dioxygenase (IDO), which also inhibits pro-inflammatory T-cell activation and cytokine induction and can also induce Treg cells\(^\text{26}\) (Figure 2).

In summary, pDC influence the function of innate and adaptive immune cells and can evoke contrasting responses in the host. Plasticity in pDC function likely reflects both the activation state of pDCs themselves as well as the influence that the micro-environment has in modulating pDC function. Such licensing by the microenvironment may enable pDCs to function differently in complex microenvironments, particularly those associated with allogeneic HCT (Figure 3). This review will summarize the emerging literature describing the multifaceted effects that pDCs have during allogeneic HCT, providing the rationale to pursue novel strategies to expand in number or to modify in function pDCs in order to confer clinical benefit.

**EFFECTS OF PDC DURING ALLOGENEIC HCT**

pDCs have important roles in the setting of allogeneic HCT, including facilitating HSC engraftment, mediating GvL activity and antiviral immunity, and inducing tolerance to attenuate GvHD (Figure 2). The following sections review pre-clinical and clinical studies defining the roles that pDC assume in mediating or affecting post-transplant outcomes, including GvHD, immune reconstitution and infection risk, malignant disease relapse, and patient survival.
HSC AND PDC MOBILIZATION AND ENGRAFTMENT

The HSC niche is a complex microenvironment within the bone marrow comprised of hematopoietic (macrophages) and non-hematopoietic cells (endothelial and perivascular stromal cells, osteoblasts and osteoclasts, CXCL12-abundant reticular cells), sympathetic innervation, and chemical and oxygen gradients. HSC mobilization requires cytokines like G-CSF (G) or G in combination with chemotherapy to down regulate CXCL12 expression on stromal cells and to initiate HSC division and emigration from the endosteal to perivascular niche, ultimately leading to increased numbers of HSC and progenitors in the circulation. Specifically, the upregulation of proteolytic enzymes that interfere with CXCR4/CXCL12 or stromal cell-derived factor 1α interactions are critical to enable HSC emigration from the bone marrow (BM). In addition to HSC mobilization, hematopoietic cytokines have significant effects on immune cell content within the HSC allograft itself (Table 2). With respect to differences in immune cell graft content between G-CSF- and plerixafor (P)-mobilized grafts, the reader is directed to recent excellent reviews. A focused review on pDC and HSC mobilization and graft content in allogeneic HCT is provided.

G has been shown to mobilize and to expand pDC in the peripheral blood (PB) such that G-mobilized PB stem cell grafts have more pDC (lin−HLA-DR+CD123+CD11c−) than BM grafts, which may contribute to the comparable incidence of acute GvHD (aGVHD) between G-PB and BM grafts. GM-CSF also mobilizes DC, but preferentially expands myeloid DCs (mDCs) versus pDCs as evidenced by GM+G-mobilized HLA-matched related donor (MRD) PB allografts having fewer pDCs (lin−HLA-DR+CD11c−CD123+) and T cells with higher Th1 content than G-mobilized allografts. Furthermore, in HLA-matched unrelated donors (MUD) BM allografts and G-mobilized MRD PB allografts, myeloid and plasmacytoid DC content correlate with CD34 dose, but not DC recovery following allogeneic transplant.

P alone or in combination with G also mobilizes pDC in PB. However, unlike G-CSF, P does not polarize DC and T-cell function or expand myeloid cells in the graft. In addition to inducing pDCs, P significantly increases T effector memory (Tem) and Treg cells, both of which have been shown to attenuate aGvHD without compromising GvL.

FL significantly enhances pDC mobilization and expansion in mice and in humans. Experience using FL in the allogeneic HCT setting is largely limited to pre-clinical studies in which FL has been shown to modulate GvHD and to augment thymopoiesis and T-cell reconstitution to confer protective antiviral immunity in mice and to enhance donor HSC engraftment in dogs. Of interest, comparing PB murine LSK (Lin−Sca-1+ c-kit+) HSC mobilization following administration of G alone, FL alone, P alone, FL+P and G+P, He et al. recently showed that FL+P mobilized the most LSK cells, which produced more colony-forming units and expanded the greatest numbers of PB NK, Treg and conventional and plasmacytoid DCs. In addition, FL+P grafts significantly enhanced engraftment in both syngeneic and allogeneic PBSC transplant-recipient mice, as animals receiving FL+P grafts had the highest long-term survival rates among transplant recipient mice receiving PBSC mobilized using alternative cytokines. Results from these pre-clinical studies suggest that FL will have novel effects in the clinical allogeneic transplant setting for both donor-derived
hematopoiesis and transplant recipient outcomes including GvHD, infection and malignant disease relapse, especially given that endogenous FL has been shown to correlate with DC reconstitution following allogeneic HCT.

FL alone or in combination with G or GM-CSF induces generation of tolerogenic DC and Th2-cells in mice and humans, which may further contribute to the regulatory effects of pDCs. In addition, G-CSF modulates donor T-cell function by promoting Th2 differentiation through myeloid cytokine (IL-4 and IL-10) and pDC induction. Similarly, P induces Treg cells, but without altering T-cell phenotype and cytokine gene expression like G-CSF.

Recombinant human FL alone or in combination with perixafor is emerging as an alternative HSC mobilization regimen to G following chemotherapy. Given its NK and DC induction, recombinant human FL may offer a novel means to enhance both HSC and pDC expansion and to augment innate immune recovery and associated antiviral and antitumor response. Future results from a current clinical trial using CDX-301 (recombinant human FL) alone or in combination with P to mobilize MRD HSCs may substantiate this intended benefit in allotransplant recipients (NCT02200380).

Like mobilization, HSC homing, localization and re-entry of donor hematopoietic progenitors back into the BM (engraftment) is dependent upon soluble factors like CXCL12/stromal cell-derived factor 1 and very late Ag 4 and cellular factors, including Treg cells. Along with Treg cells, a small CD8+TCR-CD11b+CD11c+B220+ BM population in mice has been identified as engraftment facilitating cells (FCs). Specifically, plasmacytoid precursor DCs (p-preDCs) comprise the main FC population and share properties with pDCs themselves, including type I IFN and TNFα production, activation by TLR9 ligand (CpG) and FL-induced expansion. Mechanistically, p-preDCs and FCs have been shown to promote HSC engraftment following murine allogeneic bone marrow transplantation (BMT) through Treg induction, which may also explain the reduction in aGvHD severity seen in HSC+FC recipient mice. FCs may also promote HSC survival via TNFα production; as FCs from TNFα-deficient mice fail to promote engraftment or HSC survival.

Expanding p-preDCs via cytokine mobilization could potentially facilitate or enhance HSC engraftment in the allogeneic HCT setting. In this regard, P administered to healthy donors and lymphoma patients preferentially mobilized the CD34dimCD45RA+CD123+ subset, which was further characterized as p-preDC. Interestingly, the CD34dimCD45RA+ population significantly over-expressed CXCR4 and late Ag 4. Whether the human plasmacytoid progenitor population functions like its murine p-preDC counterpart with respect to facilitating engraftment and enhancing tolerance remains undefined.

**ACUTE AND CHRONIC GVHD**

Host DC are critical APCs-initiating aGvHD via direct host Ag presentation, whereas donor APCs present host Ag to donor T cells via indirect host Ag presentation. pDCs can initiate GvHD through radiation-induced MHC II expression, thereby maturing pDCs for T-
In this model, MHC-expressing pDCs were infused into MHC-deficient mice after receiving radiation. MHC II expression was required as it enabled cognate pDC-T-cell interaction, resulting in alloreactive T-cell activation. In contrast, depletion of host cDCs and pDC does not attenuate GvHD, suggesting that host APC-directed approaches alone for preventing GvHD alone will not be successful and inferring that other APC types and/or Ag presentation itself will require targeting. Furthermore, although host-type pDC can initiate GvHD under appropriate inflammatory stimuli, it is expected that myeloablative (MA) conditioning regimens will typically deplete host pDC prior to the infusion of donor T cells.

Interestingly, patients with gastrointestinal aGvHD had higher levels of Th17 cells and more RORγt expression in their intestinal mucosa than patients without gastrointestinal aGvHD. Furthermore, pDCs were increased in the aGvHD patient’s intestinal mucosa, and their numbers correlated with severity of histologic grading. Together, these findings suggest that pDCs are recruited into GvHD target tissues, wherein they can promote Th17 cytokine production or differentiation or promote Th17 response through IFNα production. How pDCs actually traffic to gastrointestinal sites of GvHD-associated injury is likely mediated by chemokine receptor expression. Using a model of cholera toxin-induced gut inflammation, Wendland et al. showed that CCR9 expression was critical for pDC mobilization to sites of inflammation-induced intestinal injury. In the context of aGvHD, CCR9-expressing cells migrated from secondary lymph tissues to the gut via the CCR9 ligand, CCL25, where they attenuated GvHD. Further characterization revealed that these CCR9+ cells were immature pDCs that inhibited T-cell proliferation and cytokine production and induced Treg cells.

pDCs induce Tregs from naive T cells both within primary and secondary lymph tissues, which attenuate and prevent aGvHD in the pre-clinical and clinical settings. Therefore, pDC content would seemingly modulate GvHD incidence and severity (Table 3). Several pre-clinical studies have demonstrated the protective effect of donor-derived pDC in attenuating GvHD in transplant recipients. Banovic et al. showed that pDC depletion from BM grafts exacerbated GvHD and that reconstituting donor precursor pDCs (CD11c<low>/PDCA-1+ or CD11c<low>/120G8+) suppressed allogeneic T cells. Like Banovic et al., Waller and colleagues demonstrated that donor-derived, p-preDCs (lin−CD11b−CD11c+B220+PDCA-1+) diminished GvHD in recipient mice. Such a protective effect of donor and graft pDCs has also been noted in the clinical transplant setting. Increased pDC cells in MRD BM allografts associated with less-chronic GvHD (cGvHD) in HLA-matched sibling BM recipients. Similarly, recipients receiving MUD BM allografts with higher pDC content had higher overall survival (OS) due to fewer deaths from GvHD and graft rejection.

In addition to the protective roles of pDCs in the donor graft in attenuating GvHD, post-transplantation reconstitution of pDCs is predictive for subsequent GvHD risk. Following MA pediatric allogeneic SCT, Horvath et al. noted that patients with aGvHD had significantly lower numbers of circulating myeloid and plasmacytoid DCs than non-GvHD patients. Furthermore, the decrease in PB DC numbers preceded the onset of clinical symptoms by at least 24 h and was independent of steroid administration. Low numbers of post-transplant myeloid and plasmacytoid DC in the blood were also associated with the
development of aGvHD in another pediatric HCT population receiving MA and either matched sibling donor or MUD allografts. Rajasekar et al.\textsuperscript{97} found that D28 PB pDC predicted incidence of acute and cGvHD following MRD PBSCT. Interestingly, DC chimerism as a predictor of GvHD has also been studied. Pihusch et al.\textsuperscript{99} found no significant correlation between GvHD and PB DC chimerism, though patients with aGvHD had more mixed pDC chimerism than patients without aGvHD. Similarly, Chan et al.\textsuperscript{100} noted that persistence of host-type DCs at D100 correlated with the development of severe acute and cGvHD. In contrast, Clark et al.\textsuperscript{101} found that patients with cGvHD had elevated donor PB pDC versus those recipients without cGvHD. Disparities between these studies regarding pDC origin and content post-transplant may be explained by the analysis of transplant patients receiving predominantly reduced-intensity conditioning (RIC) in the Chan study, whereas patients received MA in the Clark study.

Given their influential roles in mobilizing DCs and T cells and in modulating their expression and function, cytokines used for HSC mobilization may affect GvHD induction and severity. For example, G-CSF modulates donor T-cell function by promoting Th2 differentiation through myeloid cell cytokine (IL-4 and IL-10)\textsuperscript{59,60} and pDC induction.\textsuperscript{32,41} In contrast to G-CSF, P induces Treg cells,\textsuperscript{43} but without altering T-cell phenotype and cytokine gene expression.\textsuperscript{61} Based upon general DC expansion, FL would seemingly have a complex influence on aGvHD risk. In this regard, Blazar et al.\textsuperscript{51} found that FL therapy expanded splenocyte myeloid and plasmacytoid DCs and reduced T cells in donors, but did not modify subsequent aGvHD induction when donor splenocytes were administered to transplant recipients. In contrast, transplant mice receiving post-transplant FL succumbed from augmented aGvHD, suggesting that FL had no effect on T-cell phenotype or alloreactivity in donor and upregulated inflammatory cytokines (TNFα) in transplant recipients.\textsuperscript{51} However, Teshima et al.\textsuperscript{50} found that FL administered to transplant recipients prior to BMT reduces aGvHD by attenuating early donor T-cell expansion and activation to host alloantigens. In ex vivo experiments, the authors determined that FL expanded ex vivo CD8α+ DCs, which were poor stimulators of allogeneic T cells. Progenipoietin-1 (ProGP-1), a synthetic G-CSF/FL agonist, has also been studied for its effects on aGvHD following murine allogeneic BMT. Comparing effects between donor ProGP-1 and G-CSF pre-treatment and subsequent GvHD in recipient mice, MacDonald et al.\textsuperscript{102} found that ProGP-1 or G-expanded donor splenocytes co-infused with BM cells into recipient mice resulted in 90% versus 50% survival, respectively. Enhanced survival associated with reduced TNFα induction and gastrointestinal aGvHD in recipients. The ProGP-1 effects were not mediated by donor pDCs, but rather its effects on donor T-cell Ag response and cytokine production. Further interrogation into ProGP-1 effects on donor APCs (that is, its effect on indirect host Ag presentation) revealed that ProGP-1 expanded granulocyte/monocyte precursors, which prevented GvHD through an IL-10-mediated process, whereas myeloid and plasmacytoid DCs augmented GvHD.\textsuperscript{103}

**SEPARATING GVL FROM GVHD**

Like other DC subtypes, pDCs influence GvL activity following allogeneic HCT.\textsuperscript{104} First, pDCs produce type I IFN, which has direct effects on tumor cells as well as modulates antitumor immunity.\textsuperscript{105} Second, in contrast to mDC cells, pDC (lin−HLA-
DR<sup>+</sup>CD11c<sup>-</sup>CD123<sup>+</sup>) induce Tregs,<sup>106</sup> which can attenuate GvHD and preserve GvL activity. Third, precursor p-preDCs promote Th1/Th2-type 1 cytotoxic lymphocyte differentiation, enhancing GvL activity without increasing GvHD.<sup>91,92,107</sup> Finally, hematopoietic cytokines like FL synergize with DC survival and activation cytokines like CD40 ligand to augment antitumor response.<sup>108</sup>

Given these properties, increasing pDC allograft content could potentially impact the separation of GvHD from GvL (Table 4). In this regard, type I IFN has recently been shown to impact both GvHD and GvL response following murine allogeneic BMT.<sup>109</sup> Specifically, type I IFN protected against CD4-dependent GvHD, as it suppressed both <i>ex vivo</i> and <i>in vivo</i> CD4<sup>+</sup> T-cell proliferation and differentiation when administered to recipient mice during conditioning. In contrast, type I IFN paradoxically increased CD8<sup>+</sup>-mediated aGvHD without modulating CD8<sup>+</sup> T-cell function and post-BMT cytokine administration eradicated low-level tumor burden in recipients. As type I IFN signal through STAT1,<sup>110</sup> Capitini et al.<sup>111</sup> utilized STAT1-deficient mice as BM donors to determine the effects of STAT1 deficiency on subsequent GvHD and GvL. Recipients of STAT1KO BM had decreased GvHD, preserved GvL activity and enhanced OS compared with transplant recipients receiving WT BM. Furthermore, STAT1KO recipients had increased pDCs, which when depleted post BMT, reversed the protective effect associated with STAT1 deficiency in modulating GvHD. STAT1-deficient, expanded pDCs produced less IFN and more IL-10 via STAT3 induction.

The pDC content in MRD BM,<sup>94,98</sup> but not MUD PB or BM,<sup>95</sup> allografts has been shown to increase the risk of disease relapse following allogeneic HCT. Given these observations, the Waller laboratory has investigated the effect of manipulating donor APC content as a novel strategy to enhance GvL without exacerbating GvHD during experimental allogeneic BMT. Initial experiments incorporating CD11b depletion from donor BM resulted in enhancement in GvL without increasing GvHD.<sup>112</sup> Specifically, recipients of CD11b-depleted BM had significant expansion in splenic donor CD4<sup>+</sup> memory T cells compared with recipients of unmanipulated BM, which was proportional to the number of CD11b<sup>-</sup> DCs in the BM graft and associated with increased levels of interferon gamma. Subsequent experiments focused on identifying the cell source within the donor CD11b<sup>-</sup> APC population mediating these effects and defining its underlying mechanism. Donor CD11b<sup>-</sup> APCs were identified as pDC progenitors (lin<sup>-</sup>CD11b<sup>-</sup>CD11c<sup>-</sup>PDCA-1/CD317<sup>-</sup>) that upregulated CD80/86 and IL-12 during alloAg presentation in contrast to CD11b<sup>+</sup> APCs that upregulated programmed death ligands-1 and 2 after activation.<sup>91</sup> Transplanting FACS-purified donor pDC progenitors with purified HSCs and congenic T cells induced Th1 donor cytotoxic lymphocyte polarization, enhancing GvL without increasing GvHD in both MHC and minor histocompatibility Ag-mismatched models. In contrast, transplantation using CD11b<sup>+</sup> APCs led to donor Th2 type 2 cytotoxic lymphocyte polarization. Sorted donor p-preDC (lin<sup>-</sup>CD11c<sup>+</sup>B220<sup>+</sup>PDCA-1<sup>-</sup>) added to purified HSCs and T cells augmented GvL activity while attenuating GvHD via bidirectional signaling between donor T cells and donor pDCs with interferon gamma produced by donor T cells, inducing indoleamine 2,3-dioxygenase synthesis by donor pDCs, which limited GvHD.<sup>92</sup> As cell sorting to enrich for small cell populations like pDCs and p-preDCs is impractical in the clinical setting, Waller et al.<sup>107</sup> utilized CD11b selection of BM grafts to enrich for CD11b<sup>-</sup> pDC. Mice receiving mDC-depleted BM grafts had enhanced
donor T-cell expansion and GvL activity versus mice receiving mDC-replete BM grafts without exacerbating GvHD. Using CD11b–depleted BM from IL-12p40KO mice, investigators showed that enhanced donor T-cell engraftment and proliferation was abrogated, demonstrating that the enhanced GvL activity seen in recipients of donor pDCs was IL-12-dependent.

Together, these pre-clinical experiments demonstrate the potential ways that pDCs and their associated type I IFN production might be exploited to attenuate GvHD and preserve, or even enhance, GvL activity. Furthermore, hematopoietic cytokines like P,44 which can increase pDC as well as modify T-cell (increase numbers of Tem and Treg) allograft content, could be used to modify subsequent risk for aGvHD in the transplant recipient while preserving GvL activity.

IMMUNE RECONSTITUTION AND INFECTION

In general, donor-derived pDC reconstitution occurs rapidly around the time of myeloid engraftment following allogeneic HCT, regardless of intensity of conditioning regimen (MA vs RIC)113 and HSC graft (BM vs PB).38 In addition, endogenous levels of FL correlate with PB myeloid and plasmacytoid DC recovery.56 In some studies, PB CD11c+CD123low (mDC) cells were noted to recover faster than PB CD11c+CD123+ (pDC)56,114,115 while other investigators did not measure a different kinetic of recovery in DC subtypes.96,97,116

CD34 and DC content within HSC graft have been studied to determine their influence on subsequent DC reconstitution in the transplant recipient (Table 3). Comparing donor CD34 and DC content between matched sibling donor G-primed PBSC and MUD BM allografts and its effect on subsequent DC reconstitution, Urbini et al.38 noted that higher CD34 dose in matched sibling donor PBSC and MUD BM grafts correlated with mDC and pDC graft content, respectively, but did not affect mDC and pDC reconstitution, respectively. In the setting of RIC, CD34+ cell dose infused with the allograft did not affect pDC recovery.117 Similarly, Clark et al.101 found no relationship between stem cell source or pDC graft content and subsequent PB pDC elevation in patients with cGvHD. In contrast to these studies, Porta et al.114 found that pDC (lin−CD11c−CD123+) graft content was higher in patients receiving PBSC (n = 11) versus BM (n = 8) allografts, and levels of circulating pDC in these patients were increased on D30 and D100 relative to BM allograft recipients.

Few studies have analyzed the function of pDC reconstituted in the blood after allogeneic HCT. Notably, Giraud et al.118 retrospectively assessed pDC function in 25 adult BMT recipients (conditioning and GvHD prophylaxis not specified, 19 with aGvHD, 10 with cGvHD, 22 were receiving immunosuppression), by counting PB CD123hiCD4− cells and measuring ex vivo HSV-1-inducible type I IFN production from peripheral blood mononuclear cells, and compared these with healthy donor cells. pDCs from transplant recipients were initially lower in number and reached near control levels by 14 months post transplant. In addition, transplant recipients had less HSV-1-inducible IFNα, which was not correlated to pDC. Furthermore, immunosuppressive drugs (steroids and cyclosporine inhibited inducible type I IFN when added to culture of healthy donor pDC. Together, these results
suggest that susceptibility to viral infection post-allogeneic BMT may partly be due to
dysfunction in reconstituted pDCs.

Like pDC function, pDC recovery and its effects on post-transplant infection risk have not
been extensively studied. In 54 patients receiving RIC matched sibling donor transplant,
Mohty et al.\textsuperscript{117} studied the impact of circulating levels of pDCs on transplant outcomes,
including GvHD, malignant disease progression or relapse, infection and survival. The
investigators noted that those patients with ‘high’ pDC recovery (≥0.725/µL) at 3 months
post transplant had improved OS in contrast to patients with a ‘low’ pDC recovery profile (<
0.725/µL) who had an increased incidence of non-relapse mortality (GvHD, infection).
Furthermore, the overall incidence of late infections (viral, fungal and bacterial) was
significantly higher in the ‘low’ pDC recovery group as compared with the ‘high’ pDC
recovery group (59\% vs 19\%; \emph{P} = 0.002). In multivariate analysis, only ‘high’ pDC recovery
was significantly predictive of decreased death risk. Kitawaki et al.\textsuperscript{119} investigated the
recovery of interferon-producing cells (lin\textsuperscript{−}CD11c\textsuperscript{−}CD4\textsuperscript{+}CD123\textsuperscript{+}) in 28 patients following
MA conditioning followed by predominantly BM graft (\emph{n}=17) infusion and RIC followed by
PBSC graft (\emph{n}=11) infusion. p-preDCs recovered to near control-level values within 30 days
post transplant, but recovery was impeded by aGvHD and steroid administration. In
addition, steroid administration inhibited HSV-inducible IFN\textalpha production from ex vivo
peripheral blood mononuclear cells. At last, patients with lower numbers of p-preDCs had
more viral infections (CMV antigenemia, adenouria, herpes zoster).

Longitudinal and cross-sectional studies of the kinetics of immune reconstitution post
transplant have shown that GvHD and its associated IST are major impediments to pDC
reconstitution. Numerous clinical studies have demonstrated reduced numbers of PB pDCs
in both adult and pediatric transplant patients with aGvHD.\textsuperscript{96,97,116,119–122} In addition,
GvHD also inhibits donor pDC development in the transplant recipient, potentially
inhibiting hematopoiesis and enhancing allo-driven GvHD.\textsuperscript{90} Wikstrom and co-investigators
recently have shown that mice with aGvHD are highly susceptible to murine CMV infection
and fulminant CMV-associated hepatic necrosis due to impaired CMV-specific CD8\textsuperscript{+} T-cell
response, resulting from GvHD-associated deficiencies in CD8\textsuperscript{+}DCs, CD11b\textsuperscript{+}DCs and
pDCs, DC activation, and viral Ag presentation.\textsuperscript{123}

Immunosuppressive therapy including steroids,\textsuperscript{118–120,124} mycophenolate mofetil,\textsuperscript{118}
cyclosporine,\textsuperscript{56,118} further inhibits pDC recovery and function. For example, Tajima and co-
investigators co-cultured CD11b\textsuperscript{+}, CD11c\textsuperscript{+} and CD11c\textsuperscript{−} cells isolated from human
peripheral blood mononuclear cells with cyclosporine and found that DC subtypes had
reduced inducible CD80/86 expression and IFN\textalpha production.\textsuperscript{125} Chklovskiaia et al.\textsuperscript{56} studied
the effect of cyclosporine on \textit{ex vivo} DC differentiation from CD34\textsuperscript{+} precursors and found
that generated DCs had decreased ability to prime naive T cells.

**SURVIVAL**

pDC recovery is emerging as a key determinant of survival following allogeneic HCT (Table
3). Importantly, the kinetics of pDC recovery in transplant recipients seem to be independent
of patient-, transplant- and graft-related characteristics, suggesting that post-HCT pDCs in
the transplant recipient are robust biomarkers for predicting survival.122,126

CONCLUSION

pDCs assume influential roles directing immune response in the allogeneic HCT recipient
(Figures 2 and 3). Whether these innate effector cells can be expanded in vivo or
manipulated ex vivo to direct specific immune response in the HCT recipient is the next
frontier of scientific and translational discovery to enhance their effects and ultimately to
improve post-transplant outcomes.

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Figure 1.
Human dendritic cell development. Classical (cDC) and plasmacytoid dendritic cells (pDC) derive from a common DC precursor (CDP) cell distinct from monocyte or inflammatory dendritic cells (Mo-DC) that derive from the same common monocyte precursor that macrophages and monocytes arise. Both the common DC and monocyte precursor cells differentiate from common and myeloid progenitor cells (not shown), which arise from hematopoietic stem cells (HSC). Plasmacytoid and monocyte DC development is dependent upon the hematopoietic cytokines, fms-like tyrosine kinase 3 ligand (FL) and granulocyte-macrophage colony-stimulating factor (GM-CSF), respectively. Specifically, FL induces expression of the transcription factor E2-2, which is essential for committing CDP to the pDC lineage. In contrast, GM-CSF induces the transcription factor inhibitor of DNA binding 2 (ID2), which represses E2-2 expression, inhibiting pDC development.
Figure 2.
Common and distinct characteristics between murine and human plasmacytoid dendritic cells (pDCs) and their putative roles during allogeneic hematopoietic cell transplantation based upon affecting innate and adaptive immune responses. Ab = antibody, Ag = antigen, GvHD = graft-versus-host disease, HSC = hematopoietic stem cell, IFN = interferon, Mo/Mϕ = monocyte/macrophage, NK = natural killer, TLR = toll-like receptor, TNF = tumor necrosis factor, Treg = T-regulatory cell.
Figure 3.
Proposed pDC-mediated immunoregulation in the context of allogeneic HCT. In general, inactivated pDC attenuate T-cell activation through tolerogenic effects mediated by induction of T-regulatory (Treg) cells and production of type I interferon (IFNα/β) and indoleamine 2,3-dioxygenase (IDO). Such tolerogenic effects could be potentially helpful in attenuating acute GvHD, but detrimental in promoting malignant disease relapse and increased risk for infection. In contrast, activated pDC induce T-cell activation via Ag presentation enhanced by surface expression in CD40, CD80 and CD86 as well as production in cytokines (IL-6, IL-12 and TNFα). These immunogenic effects could potentially exacerbate GvHD, but enhance graft-versus-tumor activity and antimicrobial immunity. How pDCs respond in various contexts associated with allogeneic HCT remains undefined. We propose that plasticity in function enables pDC to act as sentinel cells, licensed by the microenvironment to respond to the milieu in which they are found or to which they migrate. In this regard, pDCs may ultimately function as a critical fulcrum, balancing immune surveillance and activation with immune tolerance and modulation.
### Table 1

Human dendritic cell classification and function

<table>
<thead>
<tr>
<th></th>
<th>Conventional DCs</th>
<th>Plasmacytoid DCs</th>
<th>Plasmacytoid precursor DC</th>
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<tbody>
<tr>
<td><strong>Former names</strong></td>
<td>Classical or myeloid DCs</td>
<td>Interferon-producing cells; lymphoid or plasmacytoid DC2 BDCA-2(CD303)+DC2, CD123+DC2 pDC2=pDC2=precursor of type II dendritic cells</td>
<td>CD8+TCR− FC,127 Plasmacytoid progenitors</td>
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<tr>
<td><strong>Subtypes</strong></td>
<td>DC1 =cDC1 =CD141(BDCA-3)+ DC2 = cDC2 = CD1c(BDCA-1)+ DC</td>
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<tr>
<td><strong>General functions</strong></td>
<td>Ag uptake, processing and presentation IL-12 production enhance CTL and allogeneic T cells</td>
<td>Steady state: tolerance Activated Immunogenicity IFNα/β production</td>
<td>Major constituents of FC population Induce Treg via IL-10, IDO and TGFβ</td>
</tr>
</tbody>
</table>

Abbreviations: BDCA = blood DC Ag; cDCs = conventional DCs; CTL = cytotoxic lymphocyte; DC = dendritic cell; DC2 = type 2 DC or Th2-inducing DC; FC = facilitating cells; IDO = indoleamine 2,3-dioxygenase; IFN = interferon, IL = interleukin; pDCs = plasmacytoid DCs; p-preDC = plasmacytoid precursor DC; TGF = transforming growth receptor; Treg=T-regulatory cell.
### Table 2

Cytokine and CXCR4 antagonist effects on pDC mobilization and allograft content

<table>
<thead>
<tr>
<th>Cytokine(s)</th>
<th>Pre-clinical experience</th>
<th>Clinical experience</th>
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<tbody>
<tr>
<td>G-CSF</td>
<td>Mobilizes pDC(^{48})</td>
<td>PBSC have more pDC (lin^−^HLA-DR^−^CD11c^−^CD123^+^) expansion/enrichment than BM(^{32,33,41})</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Preferential expansion of myeloid DCs versus pDCs(^{35,36,48})</td>
<td>MSD PBSC: fewer pDCs and T cells, higher Th1(^{17})</td>
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<tr>
<td>G+GM</td>
<td>Induces p-preDC(^{30})</td>
<td>Induces plasmacytoid progenitors (CD34^dim^CD45RA^+^CD123^+)(^{71})</td>
</tr>
<tr>
<td>Plerixafor</td>
<td></td>
<td>TCR(^{αβ})/CD19-depleted haploidentical PBSC grafts are enriched for mDC and pDC(^{42})</td>
</tr>
<tr>
<td>P+G</td>
<td>Enrichment of conventional T cells, Tregs (CD4^+^CD25(^{high})CD127(^{low})/FoxP(^{3+})) and pDCs (lin^−^CD11c^−^HLA-DR^−^CD123^+^) in PB allograft(^{43})</td>
<td>Increased pDC content in murine splenocyte grafts(^{128})</td>
</tr>
<tr>
<td>Flt3 ligand</td>
<td>Expands mDCs and pDCs(^{58,129}) and CD8(^{α+})DCs</td>
<td>Endogenous FL correlates with DC and NK cell reconstitution(^{46})</td>
</tr>
<tr>
<td>FL+P</td>
<td>Progenipoietin-1 expands CD8(^{α+})DCs,(^{48}) mDCs and pDCs(^{103})</td>
<td>Mobilized more pDCs than G alone(^{55})</td>
</tr>
</tbody>
</table>

Abbreviations: BM = bone marrow; DC = dendritic cell; FL = Flt3 ligand; G = G-CSF; GM = GM-CSF; mDCs = myeloid DCs; MSD = matched sibling donor; NA = unknown/not reported; NK = natural killer; P = plerixafor; PB = peripheral blood; p-preDC = plasmacytoid precursor DC.
# Table 3

Transplant outcomes based upon pDC graft content and immune recovery following allogeneic hematopoietic cell transplantation

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Clinical experience</th>
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<tr>
<td>Graft failure</td>
<td><strong>aGvHD</strong>&lt;br&gt;TCRαβ/CD19-depleted haploidentical PBSC grafts (P+G): 4/23 primary graft failures&lt;sup&gt;130&lt;/sup&gt;&lt;br&gt;MSD PBSC (G): low DC1+ pDC (lin&lt;sup&gt;−&lt;/sup&gt;CD123&lt;sup&gt;+&lt;/sup&gt;) (&lt; 4.97/µL) at engraftment, higher risk&lt;sup&gt;131&lt;/sup&gt;&lt;br&gt;MUD allografts: low D21 pDC, higher incidence aGvHD&lt;sup&gt;126&lt;/sup&gt;&lt;br&gt;MRD PBSC (G): low D28 PB pDC (&lt;[3.4.5]/µL) higher risk aGVHD&lt;sup&gt;132&lt;/sup&gt;&lt;br&gt;TCRαβ/CD19-depleted haploidentical PBSC grafts (P+G): 2/23 patients with skin Grade I/II only&lt;sup&gt;130&lt;/sup&gt;&lt;br&gt;Lower post-transplant pDC recovery, higher risk severe aGVHD&lt;sup&gt;97&lt;/sup&gt;&lt;br&gt;Persistence in host DC chimerism at D100 correlates with aGvHD severity&lt;sup&gt;100&lt;/sup&gt;&lt;br&gt;GvHD histologic grading correlates with pDC content in intestinal mucosa in GI GvHD patients&lt;sup&gt;79&lt;/sup&gt;</td>
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<td><strong>cGvHD</strong>&lt;br&gt;MRD BM: higher pDC cell content, lower risk&lt;sup&gt;93,94&lt;/sup&gt;&lt;br&gt;TCRαβ/CD19-depleted haploidentical PBSC grafts (P+G): No cGvHD&lt;sup&gt;130&lt;/sup&gt;&lt;br&gt;MRD PBSC (G): low D28 PB pDC (&lt;[4.5]/µL) higher risk cGVHD&lt;sup&gt;132&lt;/sup&gt;&lt;br&gt;Patients with cGvHD have elevated PB donor-derived pDCs&lt;sup&gt;100&lt;/sup&gt;</td>
</tr>
<tr>
<td>Relapse</td>
<td>MRD BM: higher pDC cell content, higher relapse risk&lt;sup&gt;94&lt;/sup&gt;&lt;br&gt;MRD PBSC (G): higher graft pDC content (&lt;2.3 M/kg), higher risk of relapse&lt;sup&gt;98&lt;/sup&gt;&lt;br&gt;MSD PBSC (G): low DC1+ pDC (lin&lt;sup&gt;−&lt;/sup&gt;CD123&lt;sup&gt;+&lt;/sup&gt;) (&lt; 4.97/µL) at engraftment, higher risk of relapse&lt;sup&gt;131&lt;/sup&gt;&lt;br&gt;Low D100 pDC level (&lt; 0.2% PBMC), higher relapse&lt;sup&gt;122&lt;/sup&gt;</td>
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<tr>
<td>Immune recovery</td>
<td>MUD BM: higher CD34 content did not correlate with pDC recovery&lt;sup&gt;38&lt;/sup&gt;&lt;br&gt;MRD PB (G+GM): lower pDC graft content, faster T-cell recovery&lt;sup&gt;77&lt;/sup&gt;&lt;br&gt;MUD allografts: low D21 pDC associated with poor prognosis reflected by higher NRM and shorter OS&lt;sup&gt;126&lt;/sup&gt;&lt;br&gt;pDC (lin&lt;sup&gt;−&lt;/sup&gt;CD11c&lt;sup&gt;−&lt;/sup&gt;CD123&lt;sup&gt;+&lt;/sup&gt;) content is higher in PBSC grafts and correlates with D30 and D100 PB pDC levels&lt;sup&gt;114&lt;/sup&gt;&lt;br&gt;Endogenous levels of FL correlated with pDC recovery&lt;sup&gt;96&lt;/sup&gt;&lt;br&gt;Lower post-transplant pDC recovery, higher risk severe aGVHD&lt;sup&gt;97&lt;/sup&gt;&lt;br&gt;GvHD impedes PB pDCs recovery&lt;sup&gt;96,97,114,116,120-122&lt;/sup&gt;&lt;br&gt;Stereoids reduce PB pDC&lt;sup&gt;118,119,124,133&lt;/sup&gt;&lt;br&gt;Stereoids&lt;sup&gt;118-120,124&lt;/sup&gt; and CSA&lt;sup&gt;56,118,125&lt;/sup&gt; impair pDC function</td>
</tr>
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<td>Survival</td>
<td>MUD BM: higher pDC content, increased OS due to reduced TRM (fewer GvHD and graft rejection deaths)&lt;sup&gt;95&lt;/sup&gt;&lt;br&gt;MRD BM: higher pDC cell content, lower EFS&lt;sup&gt;94&lt;/sup&gt;&lt;br&gt;MRD PBSC (G): higher graft pDC content (&lt;2.3 M/kg), lower OS and EFS&lt;sup&gt;98&lt;/sup&gt;&lt;br&gt;MSD PBSC (G): low DC1+ pDC (lin&lt;sup&gt;−&lt;/sup&gt;CD123&lt;sup&gt;+&lt;/sup&gt;) (&lt; 4.97/µL) at engraftment, higher death&lt;sup&gt;131&lt;/sup&gt;&lt;br&gt;MUD allografts: low D21 pDC associated with poor prognosis reflected by higher NRM and shorter OS&lt;sup&gt;126&lt;/sup&gt;&lt;br&gt;RIC MRD PBSC (G): Low D30 PB pDC, higher TRM, lower EFS and OS&lt;sup&gt;134&lt;/sup&gt;&lt;br&gt;RIC MRD PBSC (G) &amp; BM: D90 ‘High’ pDC recovery profile (&lt;0.725/µL), higher OS&lt;sup&gt;117&lt;/sup&gt;&lt;br&gt;Low D100 pDC level (&lt; 0.2% PBMC), lower OS&lt;sup&gt;122&lt;/sup&gt;</td>
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Abbreviations: aGvHD = acute GvHD; BM = bone marrow; CSA = cyclosporine; cGvHD = chronic GvHD; EFS= event-free survival FL = flt3 ligand; G = G-CSF; GM = GM-CSF; M = million; MSD = matched sibling donor; MUD = matched unrelated donor; NRM = non-relapse mortality; OS = overall survival; P = plerixafor; PB = peripheral blood; RIC = reduced-intensity conditioning; TRM = transplant-related mortality.
Table 4

Mechanistic pre-clinical studies addressing the influence of pDC on GvHD and GvL

<table>
<thead>
<tr>
<th></th>
<th>Contributory or neutral role</th>
<th>Protective role</th>
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<tbody>
<tr>
<td><strong>aGvHD</strong></td>
<td>XRT-activated recipient-type pDC prime alloreactive donor T cells and cause aGvHD(^77)</td>
<td>p-preDC induce Tregs and reduce aGvHD(^65,69)</td>
</tr>
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<td></td>
<td>ProGP-1-expanded host pDCs augment GvHD(^103)</td>
<td>BM pDC attenuate aGvHD(^85)</td>
</tr>
<tr>
<td></td>
<td>Depletion of host cDCs and pDC does not attenuate GvHD(^78)</td>
<td>p-preDC attenuate aGvHD(^90-92)</td>
</tr>
<tr>
<td></td>
<td>Increased pDC in G+P mobilized donor splenocyte grafts increased aGvHD clinical scores and intestine pathology scores in allogeneic recipient mice(^128)</td>
<td>CCR9(^+) immature pDCs attenuate GI aGvHD(^83)</td>
</tr>
<tr>
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<td></td>
<td>Pre-transplant FL given to BMT recipients reduces aGvHD via CD8(^+) DC expansion(^10)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pDC (lim-HLA-DR(^+) CD11c(^-) CD123(^+)) induce Tregs(^106)</td>
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<td>Type I IFN protects against CD4-dependent aGvHD(^109)</td>
</tr>
<tr>
<td><strong>GvL</strong></td>
<td>p-preDCs promote Th1/type 1 CTL differentiation, enhancing GvL activity without increasing GvHD(^91,92,107)</td>
<td>Recipients of STAT1KO BM had increased pDCs, decreased GvHD, preserved GvL activity and enhanced OS compared with transplant recipients receiving WT BM(^111)</td>
</tr>
</tbody>
</table>

Abbreviations: aGvHD = acute GvHD; Allo = allogeneic; BM = bone marrow; CTL = cytotoxic T lymphocyte; FL = flt3 ligand; G = G-CSF; GM = GM-CSF; M = million; p-preDC = plasmacytoid precursor DC; P = plerixafor; PB = peripheral blood; ProGP-1 = progenipoietin (FL+G); XRT = radiation.