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Graft-versus-Host Disease Prevents the Maturation of Plasmacytoid Dendritic Cells

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The role of Ag presenting cell subsets in graft-versus-host disease (GVHD) remains unclear. We have thus examined the ability of plasmacytoid dendritic cells (pDC) to modulate transplant outcome. Surprisingly, host pDC were exquisitely sensitive to total body irradiation and were depleted before transplantation, thus allowing us to focus on donor pDC. The depletion of all pDC from bone marrow grafts resulted in an acceleration of GVHD mortality while the depletion of mature pDC from G-CSF mobilized splenic grafts had no effect. Thus, donor bone marrow pDC, but not mature pDC contained within stem cell grafts attenuate acute GVHD. In the presence of GVHD, donor pDC completely failed to reconstitute although a CD11clow120G8+ precursor DC reconstituted in an exaggerated and transient manner. These cells expressed Flt-3, the macrophage colony stimulating factor receptor and, consistent with a common dendritic cell (DC) precursor, were capable of differentiation into pDC and conventional DC in vivo in the absence of GVHD. These precursors were MHC class II+ and CD80/86+ but lacked CD40, were actively presenting host Ag and inhibited GVHD and T cell proliferation in a contact-dependent fashion. These data demonstrate that GVHD prevents the maturation of pDC and instead promotes the generation of a suppressive precursor DC, further contributing to the state of immune paralysis after transplantation. The Journal of Immunology, 2009, 182: 912–920.

Abbreviations used in this paper: BMT, bone marrow transplantation; GVHD, graft-versus-host disease; DC, dendritic cell; cDC, conventional DC; MLC, mixed lymphocyte culture; pre-DC, precursor DC; pDC, plasmacytoid DC; SCT, stem cell transplantation; TBI, total body irradiation.

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is responsible for the propensity of G-CSF to induce Th2 differentiation and attenuate acute GVHD (10). The adoptive transfer of purified pDC is laborious and technically challenging in murine systems and typically requires an in vivo expansion step to generate sufficient cells to harvest from lymphoid organs. Using this approach, we have previously demonstrated that the adoptive transfer of mature pDC from cytokine mobilized animals did not attenuate and in fact exacerbated acute GVHD (11). We have now taken advantage of new, more specific reagents to address the role of pDC in allogeneic stem cell transplantation more methodically. These studies clearly demonstrate the ability of donor precursor but not mature pDC to suppress GVHD.

Materials and Methods

Mice

Female C57BL/6 (B6, H-2b, CD45.2−), B6 Pptm Ly-5a (H-2b, CD45.1−), B6, B6Ptfc Ly-5a (H-2b, CD45.1−), B6D2F1 (H-2b/d, CD45.2−), and BALB/c (H-2d, CD45.2−) mice were purchased from the Animal Resource Centre. IL-10−/− (B6, H-2b, CD45.2−) mice (12) were bred in the Herston Medical Research Centre (University of Queensland, Brisbane, Australia). Tεα transgenic mice on a B6 background were provided by Jonathan Bromberg (Mount Sinai School of Medicine, New York, NY). FoxP3.EGFP transgenic mice were supplied by A.Y. Rudensky (University of Washington, Seattle, WA). Mice were housed in sterilized microisolator cages and received normal chow and acidified water post BMT.

Bone marrow transplantation

Well-established murine transplant models were used and mice were transplanted according to a standard protocol as previously described (11, 13–15). Recipient B6D2F1 or BALB/c mice received 1100cGy-1300cGy or 900cGy respectively (split dose separated by 3 h on day 0) and were transplanted with either 5 × 10⁶ bone marrow and 2 × 10⁶ purified splenic T cells or 10⁷ G-CSF mobilized splenocytes from B6 (allogeneic) or B6D2F1 (syngeneic) donors. In some experiments non-GVHD controls received T cell depleted allogeneic bone marrow or G-CSF-mobilized splenocytes. For total T cell depletion, grafts were incubated with hybridoma supernatants containing anti-CD4 (RL172), anti-CD8 (TIB211), and Thy1.2 (HO-13-4) mAbs followed by incubation with rabbit complement (Cederlane Laboratories) as previously described (11). Resulting cell suspensions contained <1% contamination of viable CD3+ T cells. G-CSF (Amgen) was administered in some experiments to donors at 10mcg/dose/day subcutaneously on days −6 to −1. In brief, 120G8 or control Abs (MAC49) were administered at 0.3 mg or 1 mg per dose by i.p injection as detailed in results.

Assessment of GVHD

The degree of systemic GVHD was assessed by a scoring system that sums changes in five clinical parameters: weight loss, posture (hunching), activity, fur texture, and skin integrity (maximum index = 10) (16). Animals with severe clinical GVHD (scores ≥6) were sacrificed according to ethical guidelines and the day of death deemed to be the following day, as previously described (11, 13, 14, 17).

mAbs and cytokine analysis

The following mAbs were purchased from BioLegend: FITC-conjugated I-A/E (MS/114.15.2), CD45.1 (A20), and IgG2a isotype control; PE-conjugated CD4 (RM4-5), CD8a (53-6.7), NK1.1 (PK136), Sca-1 (D7), CD11b (M1/70), CD11c (N418), CD45R/B220 (RA3-6B2), CD45.2 (104), and IgG2b isotype control; PE-Cy5-conjugated CD8, CD4, and IgG2b isotype control; PE-Cy7-conjugated c-kit (2B8); Alexa647-conjugated anti-FoxP3 and isotype control; allopurinol-conjugated CD11c (N418) and IgG2b isotype control. FITC-conjugated Vβ6e (RR4-7), Ly6C (Ly6C), CD11b (M5/114.15.2), CD45.1 (A20), and IgG2a isotype control; PE-Cy7-conjugated c-kit (2B8); Alexa647-conjugated anti-FoxP3 and isotype control; allopurinol-conjugated CD11c (N418) and IgG2b isotype control. FITC-conjugated Vβ6e (RR4-7), Ly6C (Ly6C), CD11b (M5/114.15.2), CD45.1 (A20), and IgG2a isotype control; PE-Cy7-conjugated c-kit (2B8); Alexa647-conjugated anti-FoxP3 and isotype control; allopurinol-conjugated CD11c (N418) and IgG2b isotype control. FITC-conjugated Vβ6e (RR4-7), Ly6C (Ly6C), CD11b (M5/114.15.2), CD45.1 (A20), and IgG2a isotype control; PE-Cy7-conjugated c-kit (2B8); Alexa647-conjugated anti-FoxP3 and isotype control; allopurinol-conjugated CD11c (N418) and IgG2b isotype control. FITC-conjugated Vβ6e (RR4-7), Ly6C (Ly6C), CD11b (M5/114.15.2), CD45.1 (A20), and IgG2a isotype control; PE-Cy7-conjugated c-kit (2B8); Alexa647-conjugated anti-FoxP3 and isotype control; allopurinol-conjugated CD11c (N418) and IgG2b isotype control. FITC-conjugated Vβ6e (RR4-7), Ly6C (Ly6C), CD11b (M5/114.15.2), CD45.1 (A20), and IgG2a isotype control; PE-Cy7-conjugated c-kit (2B8); Alexa647-conjugated anti-FoxP3 and isotype control; allopurinol-conjugated CD11c (N418) and IgG2b isotype control. FITC-conjugated Vβ6e (RR4-7), Ly6C (Ly6C), CD11b (M5/114.15.2), CD45.1 (A20), and IgG2a isotype control; PE-Cy7-conjugated c-kit (2B8); Alexa647-conjugated anti-FoxP3 and isotype control; allopurinol-conjugated CD11c (N418) and IgG2b isotype control.
Cell preparation, culture, and depletion

DC purification was undertaken as previously described (18). In brief, low-density cells were enriched from digested spleen by Optiprep density gradient centrifugation. For culture experiments, highly purified DC populations (≥98%) were obtained from the density-gradient enriched APC fraction by fluorescence-activated cell sorting (MoFlo, DakoCytomation) of precursor DC (pre-DC) (CD11clow/PDCA-1 or CD11clow/120G8 cells). Splenic CD3+ T cells were purified to ≥90% by depleting B cells (B220, CD19), monocytes (CD11b), granulocytes (Gr-1), and erythroid cells (Ter119) using magnetic bead depletion. Naive T cells were purified from spleen for in vitro assays by sorting of the Thy1.2+CD62L+ population. Transgenic TEa cells were sort purified on the basis of Vβ2 and Vβ6 to ≥99% purity. In in vitro culture experiments using pre-DC, 10^6 sort-purified pre-DC (CD11clow/120G8) were cultured in 24-well plates supplemented with murine Flt-3L (300 ng/ml) and IL-3 (50 ng/ml) for 8–9 days as previously described (19, 20). CFSE (Molecular Probes) labeling of T cells was performed as described (21). In brief, T cells were suspended at a density of 3×10^7 cells/ml in RPMI 1640 and CFSE was added at final concentration of 2 μM. Cells were analyzed using FACS Calibur (BD Biosciences) and the data were processed with ModFit LT cell cycle analysis software (Verity Software House). For mixed lymphocyte culture (MLC) studies, naive (CD62L<sup>hi</sup>/thy1<sup>+</sup>) B6 T cells were labeled with CFSE and T cells were plated with B6D2F1 sort-purified (CD11clow<sup>+</sup>/120G8<sup>+</sup>) cDC with or without sort purified donor (B6) pre-DC from animals with GVHD in the presence of IL-3 (12.5 ng/ml). Transwell plates (Corning) were used to establish the contact-dependence of pre-DC induced suppression and proliferation was assessed after 4 or 5 days of culture. CpG stimulation and latex bead uptake was undertaken as previously described (13).

Phenotypic analysis

For analysis of cell surface molecules, cells were incubated with mAb as per the manufacturer’s instructions, or at 5–20 μg/ml in 2% FCS in PBS, for 20–60 min at 4°C. Cells were washed in 2% FCS in PBS. Biotinylated mAb were detected with the appropriate conjugated streptavidin detection reagents diluted in 2% FCS in PBS and analyzed on a FACS Calibur (Becton Dickinson). 7-AAD (1 μg/ml) was added in the final wash to identify dead cells.

Microscopy

Images of cytospin cell samples were acquired on an Olympus BX41 microscope and Olympus DP12 camera (Japan) using a ×10 ocular objective and ×100/1.25 oil objective at room temperature.

Statistics

Survival curves were plotted using Kaplan-Meier estimates and compared by log-rank analysis. The Mann-Whitney-U test was used for the statistical analysis of all other data. p < 0.05 was considered statistically significant.
Results

pDC within bone marrow grafts attenuate GVHD

We first studied the profile of bone marrow vs splenic pDC. As shown in Fig. 1A, bone marrow pDC had lower expression of CD40, CD80, CD86, and MHC class II than splenic pDC. When we analyzed splenic (and marrow, data not shown) DC before and 24 h after TBI, we were surprised that pDC were already completely depleted (Fig. 1B). In contrast, cDC still remained, albeit in reduced numbers, as previously described (15, 22). Because recipient pDC were unlikely to influence the initiation of GVHD following myeloablative TBI doses, we focused on the ability of donor derived pDC to influence alloreactivity. We used the pDC-specific Ab 120G8 to deplete pDC and study the effect of this cell population within bone marrow grafts on subsequent GVHD. As shown in Fig. 1C, administration of this Ab to donors depleted bone marrow pDC by >80%. When these depleted B6 grafts were transplanted into lethally irradiated B6D2F1 recipients they induced significantly greater levels of GVHD than grafts from non-depleted, control Ab-treated donors (Fig. 1D). Because stem cell mobilization with G-CSF has been shown to increase pDC numbers in the peripheral blood, we used G-CSF mobilized splenic grafts subsequently transplanted into lethally irradiated B6D2F1 recipients. Donor pDC were depleted throughout the mobilization and transplantation procedure, and the recipients were transplanted with normal numbers (as determined by flow cytometry) of cDC and pDC. As shown in Fig. 2A, the depletion process did not deplete other cell populations within the graft (Fig. 2B). Surprisingly the depletion of mature pDC from mobilized splenic grafts had no effect on GVHD mortality or clinical scores (Fig. 2C). These data confirm that bone marrow pDC are indeed immunomodulatory and suppress GVHD while this property is lost as these cells mature in the periphery.

Reconstitution of donor DC after bone marrow transplantation

We next studied the kinetics of donor pDC reconstitution after BMT. Surprisingly there was an almost complete failure of pDC maturation in animals with GVHD, as opposed to the normal reconstitution in non-GVHD recipients of T cell depleted grafts (Fig. 3, A–C). In contrast, cDC reconstituted in a relatively normal fashion (Fig. 3, A–C) although at later time points their numbers were reduced in conjunction with the lymphoid atrophy seen in animals with GVHD. Interestingly, in animals with GVHD, a CD11clow/PDCA-1+ or CD11cmed/120G8+ cell with plasmacytoid morphology (Fig. 3, B and C) reconstituted quickly after BMT and stem cell transplantation, peaking at day 7–14 (Fig. 3, B and C). Thereafter, they diminished rapidly and by day 28, when GVHD mortality was maximal, they were largely absent. These cells were clearly distinguishable from mature pDC seen in syngeneic animals (which had a classical

FIGURE 3. Donor pDC reconstitution after transplantation. A, Density gradient enriched cDC (CD11chigh/120G8-) and pDC (CD11cint/120G8+ and CD11cint/B220low) in animals with GVHD and non-GVHD controls receiving T cell replete or depleted G-CSF mobilized splenocytes. Note that in animals with GVHD there is a failure of pDC maturation and the presence of a CD11cint/120G8- population (pre-DC) early after transplant. Identical profiles were seen in recipients of bone marrow and T cell grafts. B, Numbers of reconstituting donor pre-DC, pDC, and cDC cells in the spleen of animals with and without GVHD (n = 3–6 per group) over time in recipients of bone marrow transplants with or without splenic T cells as shown. ND refers to the fact that mature pDC are not detectable or below scale in animals with GVHD. One of three experiments is shown and is representative of data generated in both stem cell and bone marrow transplant models. C, Morphology (×400) of pre-DC from animals with GVHD 7 days after BMT. Morphology of pDC and cDC is from non-GVHD animals seven days after BMT.
pDC phenotype (23)) on the basis of morphology and their reduced expression of CD11c, 120G8, and, most importantly, the absence of B220 expression). These cells reconstituting during GVHD are herein referred to as pre-DC.

Phenotypic and functional analysis of reconstituting donor pre-DC

Because 120G8 can be promiscuously expressed during immune responses (5), we more extensively phenotyped the reconstituting "pre-DC" in animals with GVHD. These cells had the characteristic CD11clow, MHC class IIhigh, 120G8/H11001, PDCA-1/H11001, and Ly6C/H11001 phenotype of pDC but lacked B220 (Fig. 4A) and produced large amounts of cytokines in response to CpG (Fig. 4B). They were highly phagocytic (Fig. 4C), as has been recently described for pDC (4), and only weakly stimulated responses in allogeneic T cells (Fig. 4D). Importantly, they were actively presenting host Ag because they stimulated TEa transgenic T cells (Fig. 4E). Notably, the cells did not produce IFN-α in response to CpG motifs (data not shown). Although they expressed intermediate levels of Flt-3 and M-CSFR, consistent with common DC precursors (25), a proportion also expressed CD11b suggesting they were, at least partially, differentiating down a monocytic lineage. However, after culture in IL-3 and Flt-3L, the majority of these precursor cells differentiated into CD11c+PDCA-1+ cells of clear plasmacytoid morphology that were Siglec-H+ (Fig. 5A), all characteristic of mature pDC. A smaller fraction matured into granulocytes that were Siglec-H−, suggesting heterogeneity in the precursors contained within the initial population. To study the maturation of these cells in vivo in the absence of GVHD, congenic CD11cint/120G8H11001 cells reconstituting during GVHD were sort purified 7 days after BMT and added to syngeneic bone marrow grafts so that maturation could be followed in vivo. The majority (70%) of these cells differentiated into DC with both cDC and pDC represented (Fig. 5B), confirming the original CD11clow/120G8H11001 population was indeed highly enriched for a common DC precursor.

Reconstituting DC precursors are potent suppressors of allogeneic T cell responses

We next sought to investigate the suppressive properties of reconstituting DC precursors (pre-DC) by adding them to MLC. The MLC was used as a surrogate to study the ability of pre-DC to suppress allogeneic T cell responses to host Ag. After BMT, this Ag presentation may be occurring by host APC (which can persist long term in BMT recipients within tissue; Ref. 26) or by donor APC that present exogenous host Ag, primarily within MHC class II (27). As shown in Fig. 6, A and B, reconstituting donor DC

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**FIGURE 4.** Phenotype and function of reconstituting donor pre-DC. A, Phenotype of 120G8+ pre-DC in animals with GVHD. B, Cytokine generation from sort purified 120G8+ pre-DC from animals with GVHD 7 days after BMT in response to 18 h stimulation with CpG. C, Intense phagocytosis of latex beads in overnight cultures by reconstituting donor pre-DC (×1000). D, Relative capacity of B6 (H-2b) naïve pDC and the pre-DC reconstituting in animals with GVHD to stimulate alloreactive proliferation in BALB/c (H-2b) T cells in IL-3 supplemented MLC. Proliferation was determined by [3H]thymidine incorporation and results expressed as mean ± SE of triplicate wells. One of two replicate experiments. E, B6 bone marrow and T cells were transplanted into lethally irradiated BALB/c recipients. Reconstituting donor pre-DC were sort purified and used to stimulate TEa transgenic T cells (that recognize processed I-Eα Ag in the context of I-Aβ). B6D2F1 splenocytes were used as a positive control and BALB/c and B6 splenocytes as negative controls. Proliferation determined by [3H]thymidine incorporation and results expressed as mean ± SE of triplicate wells. One of two replicate experiments shown.
precursors dramatically suppressed T cell responses to allogeneic DC, at ratios of pre-DC to T cells from 1:1 to at least 1:16 (data not shown). This inhibition was independent of IL-10, TGFβ, IFN-γ, or IDO as it was present when the pre-DC were derived from IL-10−/− donors or when TGFβ, IFN-γ, or IDO were inhibited by Ab or 1-methyl tryptophan (data not shown). Consistent with this, the inhibition could not be transferred by addition of supernatants from a "suppressed" MLC where pre-DC were present, to a second MLC containing only T cells and allogeneic DC (Fig. 6C). This suppression did not require FoxP3+ regulatory T cells since suppression by pre-DC remained when they were removed from the T cell inoculum by sort exclusion of EGFP+ cells derived from B6.FoxP3-EGFP transgenic T cells (Fig. 6D). Furthermore, the use of split well MLCs confirmed that the DC precursors required cell contact to suppress allogeneic T cell responses (Fig. 6E). Finally, when reconstituting DC precursors were depleted in vivo by administration of 120G8 after BMT, there was a marked acceleration of GVHD from day 28 forward to day 7–14 after BMT (Fig. 7A). Thus, the depletion of pre-DC reconstituting from bone marrow during GVHD further significantly accelerated GVHD over that seen when only bone marrow pDC were depleted, confirming that pre-DC themselves exert a suppressive effect in vivo. Importantly, the depletion process was specific and did not influence the reconstitution of other donor cell populations in vivo (Fig. 7B), confirming that these donor DC precursors were indeed suppressive in vivo. Additionally, donor T cells from animals in which pre-DC were depleted demonstrated enhanced proliferative and Th1 responses to host Ags (Fig. 7C).

Discussion

The importance of the contribution of APC to the induction of acute GVHD has become clearer over the last decade. It is now established that host APC are critical for the induction of CD8 dependent acute GVHD while either host or donor are sufficient to induce CD4-dependent disease (28, 29). The subsets of APC important in this process remain less clear although increasing circumstantial evidence suggests that mature conventional DC are at least one important stimulatory population (22, 30). Conversely, B cells seem to have a limited capacity to induce acute GVHD (as opposed to chronic GVHD) and instead appear to possess suppressive properties (31). The increase in circulating donor pDC during stem cell mobilization with G-CSF has been associated with Th2 differentiation and a reduction in the potential of donor T cells to induce acute GVHD on a per cell basis (10) although this link has
not been causally established in vivo. The data provided here formally establish this link and further extend this paradigm, confirming that it is bone marrow derived DC precursors that are important for attenuation of GVHD.

The ability of pDC to modulate immunity has been widely described and in the transplant setting, this effect has predominantly suppressive in nature, usually via the induction of classical or induced regulatory T cells (3, 4). Furthermore, this pathway has recently been described as IDO dependent (32) and the pDC producing high levels of IDO are a CD19\(^{+}\)/H11001 subset which in turn induce high levels of PD-L1/PD-L2 expression on conventional DC.

We were unable to demonstrate a significant population of CD19\(^{+}\)/H11001 mature pDC from the spleen or lymph nodes of our donor animals and this did not appear to be a suppressive pathway in our transplant system. Conversely, the suppressive bone marrow-derived DC precursors did not express CD19 by flow cytometry or IDO by real time PCR, nor did they themselves express or induce PD-L1/PD-L2 on conventional DC (data not shown). Thus, it appears unlikely that IDO or PD-L1/PD-L2 are the major suppressive pathways invoked by these cells. Likewise, TGF\(\beta\), IL-10, or IFN-\(\gamma\) appear redundant for this suppressive activity. Importantly, the donor DC precursors were highly phagocytic and were actively expressing processed host alloantigens, albeit in the absence of CD40. The induction of tolerance by “regulatory DC” that express Ag in the absence of CD40 in vivo is widely described (13, 33, 34) and, at present, this appears the dominant pathway of suppression (i.e., anergy) invoked by these cells. It should be noted however that the requirement for contact-dependent suppression is not possible to validate in vivo. The finding that these immature DC precursors are suppressive in nature is consistent with the described ability of immature DC to inhibit T cell responses (33, 35, 36).

The lineage of pDC has been debated considerably in the recent past and it is clear that both common myeloid and lymphoid progenitors can generate pDC. However, at steady state, pDCs appear to be predominantly myeloid in origin (37). Furthermore, a common precursor, expressing both the M-CSF receptor (c-fms) and Flt-3 has recently been described that is capable of generating both cDC and pDC (25, 38). A second macrophage, DC precursor with similar phenotype, has also been described (39) that likely represents an overlapping population (40). The bone marrow-derived suppressive precursor population identified in these studies includes large numbers of this population, as demonstrated by phenotypic analysis and the demonstration of preferential differentiation to both DC subsets in irradiated syngeneic recipients. This is consistent with the broad definition of a precursor DC as described by Shortman (41). However, it should be noted that the majority of the DC precursors defined to date are within steady state conditions, not the profoundly inflammatory setting of GVHD encountered in these studies. We and others (13, 42) have demonstrated the ability of suppressive myeloid granulocyte-monocyte precursors within the donor graft to attenuate GVHD and the IL-10 dependence of this process. Importantly, the pre-DC described in this

![FIGURE 6. Suppression of alloreactive T cell responses by reconstituting donor pre-DC.](http://www.jimmunol.org/)

A. \(10^7\) B6(H-2\(b\)) CFSE-labeled CD45.1\(^{+}\)/Thy1\(^{+}\)/CD62L\(^{+}\) naive T cells were cultured in MLCs with \(10^4\) B6D2F1 (H-2\(d\)) cDC, with or without \(10^4\) sort purified pre-DC from animals with GVHD 7 days after BMT. CFSE dilution in CD4 and CD8 T cells was determined 96 h later. One of six experiments shown. B. Proliferation indices from A as determined by Modfit analysis. Data are mean \(\pm\) SE from triplicate wells. C. MLC were undertaken with cDC and T cells as in A with or without the addition of tissue culture supernatants (TCSN) from a primary MLC containing pre-DC. One of two replicate experiments shown. D. Pre-DC were added to MLC in which T cells contained both effector T cells and FoxP3\(^{+}\) regulatory T cells or effector T cells only (by sort exclusion of EGFP\(^{+}\) cells derived from B6.FoxP3-EGFP transgenic T cells). Similar data was also obtained by (FACS) removal of CD4\(^{+}\)/CD25\(^{high}\) T cells. E. MLC were undertaken as in A, with pre-DC present in the upper chamber (UC) or lower chamber (LC) of transwell plates. One of two replicate experiments shown.
study are not conventional myeloid suppressor cells based on size, morphology, their MHC class II<sup>high</sup>/CD11<sup>c</sup> phenotype, the fact that they are CD31<sup>low</sup> (data not shown) and their ability to generate pDC in vivo. In addition, this phenotype and function is not characteristic of inflammatory monocytes (41, 43) and instead is most consistent with a pre-DC corrupted in maturation by the GVHD milieu.

To our knowledge this is the first description of a specific failure of pDC maturation during GVHD (as opposed to the global lymphoid atrophy more characteristic of GVHD). This suggests that GVHD either provides potent inhibitory signals to maturation, or alternatively, generates a cytokine milieu that is lacking a critical pDC growth factor. The best described growth factor for both DC subsets is Flt-3L and to a lesser extent, GM-CSF and M-CSF (25, 44). Certainly the provision of Flt-3L in vitro (and removal from the GVHD environment) did allow the generation of Siglec-H<sup>+</sup> plasmacytoid cells while in vivo full cDC and pDC maturation occurred. Thus, it appears that the additional signals required to allow the full differentiation of DC precursors are absent or perhaps more likely, additional inflammatory stimuli corrupt normal cDC and pDC differentiation.

The promotion of tolerance remains the ultimate therapeutic goal in transplantation. These studies highlight and delineate mar-
cDC and pDC differentiation.

Importantly, the impairment of cDC and pDC maturation by GVHD may be responsible for the severe immune paralysis associated with this disease and delineation of the pathways involved may allow therapeutic intervention to circumvent the severe infective mortality and morbidity associated with GVHD.

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**Disclosures**

The authors have no financial conflict of interest.

**References**


![FIGURE 7. Depletion of reconstituting pre-DC from bone marrow exacerbates acute GVHD. A, Survival by Kaplan Meier analysis following transplantation of B6 bone marrow and T cells into lethally irradiated (1300 cGy) B6D2F1 recipients (n = 12 per group). 120G8 or control Ab was administered to donors at day -1 and then to recipients on alternate days to day +14, beginning at day +1. Data combined from two replicate experiments. P < 0.001, 120G8 pre and post transplant vs control Ab and p < 0.02 vs 120G8 donor only. P, Phenotype of splenic cell lineages 7 days after BMT in recipients receiving 120G8 Ab (n = 4–12 per group) from two experiments. Data presented as mean ± SE. *p < 0.002, for splenic pDC (PDCA-1<sup>+</sup>/CD11c<sup>+</sup>) in 120G8 pre and post transplant vs 120G8 Ab pre and control Ab post transplant and p < 0.001 vs control Ab pre- and post transplant. B, Donor B6 CD4<sup>+</sup> T cells were sort purified from allograft recipients (B6D2F1) receiving 120G8 or control Ab (n = 4–6 per group) and restimulated with host-type splenic cDC. Proliferation and cytokine levels were determined in culture supernatants 48 h later. One of two representative experiments shown.](http://www.jimmunol.org/Downloadedfrom)
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