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Profound Depletion of Host Conventional Dendritic Cells, Plasmacytoid Dendritic Cells, and B Cells Does Not Prevent Graft-versus-Host Disease Induction

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The efficacy of allogeneic hematopoietic stem cell transplantation can be a lifesaving therapy for hematologic malignancies and acquired or inherited nonmalignant disorders of blood cells. Mature donor T cells in allografts play important roles in allogeneic hematopoietic stem cell transplantation. They contribute to T cell reconstitution in the recipient, promote donor hematopoietic engraftment, and mediate an antitumor effect called graft-versus-leukemia. Unfortunately, donor T cells can broadly target host tissues, causing graft-versus-host disease (GVHD) (1). Because of GVHD, all patients receive some type of prophylactic immunosuppression, either by depleting T cells from the allograft or, more commonly, with pharmacologic agents that inhibit T cell function. However, even with pharmacologic immunosuppression, GVHD remains a major cause of morbidity and mortality. Novel approaches are clearly needed.

GVHD is initiated by APCs that prime alloreactive donor T cells (1–6). Recipient APCs that survive conditioning are essential for GVHD in MHC-mismatched transplants and in CD8-mediated GVHD across only minor histocompatibility Ags (mHAs) (1–4, 7, 8). They also have an important and nonredundant role in CD4-mediated GVHD across mHAs (6). Thus, recipient APCs would be a logical target for suppressing GVHD.

APCs, which include dendritic cells (DCs), B cells, macrophages, and basophils, are diverse cells that have in common the ability to prime T cells. Among APCs, DCs are perhaps the most efficacious in priming naive T cells (9), which are potent inducers of GVHD (1). Consistent with this, in an MHC-mismatched allogeneic bone marrow transplant (alloBMT) model, infusing wild type recipient conventional DCs (cDCs) or plasmacytoid DCs (pDCs) can partially restore GVHD in mice with MHC-deficient hematopoietic cells (3, 4).

The importance of cDCs in adaptive T cell responses has been highlighted by data from experiments studying immune responses in mice in which cDCs can be inducibly depleted or in mice with a constitutive absence of cDCs. Both antiviral and antibacterial T cell responses were greatly diminished after induced depletion of CD11c+ cells (10–17). cDC depletion also blunted T cell responses in allergic asthma (18) and in a model of antitumor immunity (19). Mice that constitutively lack cDCs with a variable depletion of pDCs have impaired antiviral clearance (20) and diminished T cell responses in a mouse lupus model (21).

Taken together, these data made DCs attractive cells to target to prevent GVHD. We therefore studied the role of host DCs in two
models of GVHD in which host hematopoietic APCs are absolutely required for GVHD induction (2, 8, 22). Contrary to expectations, neither induced nor constitutive profound depletion of CD11c+ cDCs mitigated clinical or histopathologic GVHD. The addition of Ab-based depletion of pDCs and B cells and partial depletion of additional CD11b+ cells also did not prevent GVHD. These data highlight the unique aspects of T cell activation in GVHD and suggest that there are redundant populations of APCs capable of priming allogeneic T cells and/or that few residual host APCs are sufficient. These results make it unlikely that reagent-based host DC depletion will be effective in preventing GVHD in the clinic.

Materials and Methods

Mice

C57BL/6 (B6) CD45.1 mice were purchased from Taconic/NCI Frederick (Frederick, MD). C3H.SW and B6 bm12 mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and bred at Yale. C3H.SW β2M−/− mice (5) were bred at Yale. CD11c-diphtheria toxin receptor (DTR) (10) and CD11b-DTR mice (23) were purchased from The Jackson Laboratory and bred at Yale. These mice were crossed to create mice heterozygous for both transgenes and typed by PCR as described previously (10, 23). CD11c-cre mice were a gift of B. Reizis (Columbia University, New York, NY) (24). Rosa26-eGFP-diphtheria toxin (DT) B6 mice were provided by J.P. Martinez-Barbera (University College, London, U.K.) (25). The Rosa26-eGFP-DT locus contains the gene for the diphtheria toxin a-chain (DTA) that is preceded by a loxP-flanked STOP cassette so that expression of the toxin is restricted to those cells expressing Cre recombinase. These mice were intercrossed to obtain constitutively DC-deficient CD11c-cre+ Rosa26-eGFP-DT+ mice (CD11c-DTA).

Cell purifications

CD8 cells were purified via depletion from lymph node (LN) using biotin-conjugated Abs, streptavidin microbeads, and an AutoMACs cell separator (Miltenyi Biotec, Auburn, CA) as described previously (5). CD8 cells were >90% pure with CD4+ T cell contamination of <3%. All donor bone marrow (BM) was depleted of T cells using anti-Thy1.2 microbeads and the AutoMACs (Miltenyi Biotec).

BM transplantation

All transplants were performed according to protocols approved by the Yale University Institutional Animal Care and Use Committee. Mice were irradiated (500 cGy × 2) and reconstituted with 10⁶ T cell-depleted donor BM cells to create BM chimeras. Chimeras were reirradiated (450 cGy × 2) and reconstituted with T cell-depleted donor BM with or without T cells to induce GVHD. In the C3H.SW→B6 system, GVHD was induced by 2 × 10⁶ CD8 cells. In the B6 bm12→B6 model, GVHD was induced by splenocytes containing 10⁶ CD4+ cells. Mice were weighed two to three times per week; weights from mice that died, were sacrificed due to predefined measures of morbidity, or were censored when experiments of different lengths were included in averages for subsequent time points at the last value recorded. Deaths before day 11 in the C3H.SW→B6 model were scored as not being GVHD-related based on our extensive experience in this model, and these mice were censored (indicated by ticks on survival curves). Skin disease was scored when mice were weighed; minimal criteria for clinical skin disease was fur loss in an area >1 cm².

Assessment of APC engraftment in BM chimeras

Spleens and LNs were digested with collagenase D as described previously (8). To distinguish residual recipient (CD45.1+) and donor-derived (CD45.2+) DCs in initial BM chimeras, we stained cells with Abs against CD45.1 or CD45.2 (clones A20 and 104; BD Pharmingen), CD11c (allogycocycin; clone HL3; BD Pharmingen), a mixture of biotin-conjugated Abs against Gr-1 (RB6-8C5; BD Pharmingen), CD11c (allogycocycin; clone HL3; BD Pharmingen), a mixture of biotin-conjugated Abs against Gr-1 (RB6-8C5; BD Pharmingen), CD19 (1D3; BD Pharmingen) or CD45RB (B220); Ly76 (TER119; BD Pharmingen), and Thy1.2 (30H12; laboratory prepared). Cells were stained with streptavidin-PerCP (BD Pharmingen). DCs were identified as CD11c+ lineage−. A cohort of chimeras was sacrificed for analysis before each second GVHD-inducing transplant. CD11c+ DCs were at least >96% donor derived and in most mice >98% donor derived.

Measurement of serum cytokines

Serum cytokines were measured using the Bioplex kit Th1/Th2 panel (Bio-Rad, Hercules, CA) and a Luminex 100 system (Luminex; Austin, TX) as previously described (26).

DT treatment and B cell and pDC depletion

DT (Sigma) was resuspended in sterile water. Females, which were <25 g, were treated with 100 ng DT, whereas males, which were mostly >25 g,
were weighed and treated with 4 ng/g. The BST2 hybridoma (27) was a gift from Marco Colonna (Washington University School of Medicine, St. Louis, MO) and was laboratory prepared. Anti-mouse CD20 (an IgG2a derivative of clone 18B12) (28) was provided by Marilyn Kehry (Biogen/IDEC, San Diego, CA).

**Scoring of pathology**

In the C3H.SW→B6 strain pairing, some CD8 recipients reached endpoints for extent of skin disease and morbidity that required earlier sacrifice, and pathology was harvested at that time. Otherwise, pathology was harvested at the conclusion of each experiment. Skin and gastrointestinal pathology were scored by J.M. and A.J.D., respectively, as previously described (5, 22).

**Statistics**

Significance for differences in weight loss was calculated by an unpaired t test. p values for differences in survival and the incidence of skin disease were calculated by a log-rank test. p values for histology scores and serum cytokine levels were calculated by Mann–Whitney. Error bars for weights represent SE measurements (GraphPad Prism).

**Results**

**DT treatment depletes CD11c+ cells in CD11c-DTR→B6 radiation chimeras**

To deplete cDCs, we exploited mice transgenic for a construct that targets the simian DTR to CD11c+ cells (CD11c-DTR). DT treatment of these mice rapidly and profoundly depletes cDCs (10, 29). Because repeated DT treatment is lethal due to promiscuous transgene expression (29), we used as hosts in alloBMT experiments B6 CD11c-DTR (H-2b; CD45.2)→B6 (CD45.1) BM chimeras in which 96% of LN and splenic CD11c+ cells were donor derived (29, data not shown). To confirm that DT treatment depletes splenic and LN DCs, we treated CD11c-DTR (CD45.2, heterozygous for the CD11c-DTR transgene)→B6 CD45.1 radiation chimeras with a single dose of DT and cohorts were sacrificed daily for 4 d to quantitate the number of CD45.2+CD11c+ cells. Consistent with prior reports, within 24 h of DT treatment there was a profound depletion of CD11c-DTR CD11c+ cells, which gradually recovered over the following 72 h (Fig. 1A, 1B). We next determined whether DT treatment augmented DC depletion beyond what is induced by radiation. CD11c-DTR→B6 (CD45.1) and control B6→B6 (CD45.1) chimeras received DT followed several hours later by split-dose radiation. Splenic DCs were quantitated 24 and 48 h later. DT treatment further diminished splenic DC numbers both 24 and 48 h after radiation (Fig. 1C, 1D). In a separate experiment, B6 CD11c-DTR mice were irradiated and reconstituted with C3H.SW BM and 2×10^6 CD8 cells. On days −1 and +1, mice were treated with 4 ng/g (100 ng/mouse) of DT or PBS. Cohorts were sacrificed on days +3 and +6 to quantitate host DCs. In transplanted mice, at day +3, there was a nearly 200-fold reduction in splenic DC numbers and >100-fold reduction in LN DCs in DT-treated mice (Supplemental Fig. 1A, representative flow cytometry; Supplemental Fig. 1B, quantitation). The absolute DC numbers were quite small in DT-treated mice: ~200/spleen and 50 in pooled cervical, subscapular, mesenteric, axillary, andinguinal LNs. At day +6, we could detect no host DCs in DT-treated mice and <200/spleen in non-DT treated mice (Supplemental Fig. 1B). We could detect no host DCs in LNs of untreated or DT-treated mice at day +6 (Supplemental Fig. 1B).

**FIGURE 2.** Depletion of host CD11c+ cells does not diminish CD8-mediated GVHD in the C3H.SW→B6 strain pairing. CD11c-DTR→B6 (CD45.1) chimeras (“CD11c+”) or control WT→WT chimeras (“CD11c−”) mice were injected with DT (100 ng/mouse i.p.) on days −1, 0, +1, +3, +5, and +7. On day 0, chimeras were reirradiated (450 cGy×2) and reconstituted with 10^7 T cell-depleted C3H.SW BM cells, with or without 2×10^6 C3H.SW CD8 cells. An additional group of CD11c+ chimeras was transplanted but not injected with DT. (A) Weight loss. p < 0.02 comparing any CD8 recipient group with its BM alone control beginning on day +25; p > 0.13 comparing any CD8 recipient group with any other CD8 recipient group beginning on day +11. (B) Incidence of skin disease. p < 0.04 comparing any CD8 recipient group with its BM alone control; p > 0.93 comparing any CD8 recipient group with any other CD8 recipient group. (C) Organ pathology scores. p ≥ 0.05 comparing any CD8 recipient group with its BM alone control except skin and ear scores in the CD11c+ without DT treatment group (p = 0.14 for both) and skin scores in the CD11c+ with DT treatment group (p = 0.10), p > 0.29 comparing any CD8 recipient group with any other CD8 recipient group. Data are representative of three independent experiments.
Host cDC depletion does not prevent GVHD in the C3H, SW→B6 GVHD model

In an initial experiment, we DT-treated CD11c-DTR→B6 (CD45.1) chimeras on day –1 before transplantation with C3H, SW (H-2b) BM with no T cells or purified C3H.SW CD8 cells. Despite profound cDC depletion, neither clinical nor histopathologic GVHD was reduced as compared with non–DT-treated CD11c-DTR→B6 chimeras or DT-treated WT→WT chimeras (data not shown). We next considered the possibility that prolonged DT treatment would be required because there could be radiation-resistant CD11c+ host cDC progenitors that are not killed by a single DT treatment, but that later mature into functional cDCs (30). We therefore repeated the prior experiment (in the C3H.SW→B6 model), except DT treatment was on days –1, 0, +1, +3, +5, and +7. Again, neither clinical nor histopathologic GVHD was reduced (Fig. 2).

Although we previously found that C3H.SW→B6 and β2M–/–→B6 BM chimeras are resistant to GVHD when retransplanted with C3H.SW BM and CD8 cells (8), we considered the possibility that with DT treatment, donor APCs were sufficient. We therefore repeated the experiment described in Fig. 2, except we used C3H.SW β2M–/– BM donors, thereby preventing Ag presentation by donor APCs (5). All recipients were treated with anti-NK1.1 to prevent rejection of donor β2M–/– cells. DT-treated CD11c-DTR→B6 chimeric CD8 recipients developed similar weight loss and histopathologic GVHD of liver as did the control CD8 recipient groups (Fig. 3). There was reduced colon histopathology in DT-treated CD11c+ CD8 recipients as compared with DT-treated CD11c– CD8 recipients, but not as compared with non-DT-treated CD11c+ CD8 recipients (Fig. 3). No CD8 recipient group developed significant clinical or histopathologic skin GVHD, as we previously found when donor BM was β2M–/– in this model (5, data not shown). These data indicate that the failure of host cDC depletion to prevent GVHD was not due to Ag presentation by donor APCs.

cDC depletion does not prevent GVHD in the B6m12→(B6→B6m12) GVHD model

We next considered the possibility that residual host nontransgenic cDCs in the CD11c-DTR→B6 chimeras were initiating GVHD. We thought this was unlikely given the quality of donor engraftment and the aforementioned resistance of C3H.SW→B6 and β2M–/–→B6 chimeras to GVHD induction (8) and graft-versus-leukemia (31). To formally exclude priming by residual host nontransgenic APCs, we used the MHC class II-mismatched B6m12→B6 model in which allogeneic host hematopoietic cells are suffi-

FIGURE 3. Failure of cDC depletion to decrease GVHD is not due to donor T cell activation by donor APCs. Mice were transplanted as in Fig. 2 except donor BM was C3H.SW β2M–/–. All mice were treated with 0.2 mg anti-NK1.1 (PK136) on days –2, –1, and +7 as described previously (5, 8). (A) Weight loss. p < 0.03 comparing CD11c+ and CD11c– DT-treated CD8 recipient groups with their BM alone controls beginning on day +27; p < 0.044 comparing the CD11c+ no DT group with its BM alone control beginning on day +35. (B) Organ pathology scores. Skin and ear GVHD were not significant, consistent with the reduced cutaneous GVHD we previously observed when donor BM is β2M–/– (5), p < 0.013 comparing any CD8 recipient group with its BM alone control; p > 0.23 comparing any CD8 recipient group with any other CD8 recipient group except for p = 0.035 comparing colon scores in CD11c+ and CD11c– DT-treated CD8 recipients. Data are from one experiment.

FIGURE 4. Depletion of host CD11c+ cells does not diminish CD4-mediated GVHD in the B6m12→(B6→B6m12) model. CD11c-DTR→B6m12 chimeras (CD11c+) or control WT→B6m12 chimeras (CD11c–) mice were injected with DT (4 ng/gm i.p.) on days –1, 0, +1, +3, +5, and +7. On day 0, chimeras were reirradiated (450 cGy × 2) and reconstituted with T cell-depleted B6m12 BM with or without B6m12 splenocytes containing 10^6 CD4 cells. An additional CD11c+ group was transplanted without DT injection. (A) Weight loss. p ≤ 0.0003 comparing any CD4 recipient group with its BM alone control on days +7 and +9; p > 0.06 comparing any CD4 recipient group with any other CD4 recipient group at all days except day +26. (B) Serum IFN-γ levels measured by Luminox at day +7 (three BM alone and seven spleen cell recipients for each group). p = 0.0083 comparing any CD4 recipient group with its BM alone control; p > 0.7 comparing any CD4 recipient group with any other CD4 recipient group. (C) Liver pathology scores from one of two experiments. p ≤ 0.0029 comparing any CD4 recipient group with its BM alone control; p < 0.027 comparing DT-treated CD11c+ and CD11c– CD4 recipients and DT-treated and untreated CD11c+ CD4 recipients.
cient for GVHD (2, 22). To prevent allogeneic T cell priming by residual host nontransgenic APCs, we made B6 CD11c-DTR→B6bm12 chimeras and retransplanted them with B6bm12 BM and spleen cells. In such chimeras, any residual B6bm12 hematopoietic APCs and all nonhematopoietic cells are syngeneic to donor B6bm12 GVHD-inducing CD4 cells. Further, because B6 and B6bm12 mice differ only by a few amino acids in I-A (32), there are essentially no mHAs to be presented by donor APCs. Therefore, allogeneic stimulation of donor B6bm12 CD4 cells in these chimeras is exclusively by B6 background hematopoietic APCs. Host DT treatment reduced neither weight loss nor serum IFN-γ levels (characteristic of GVHD in this model) (22, 33) as compared with non-DT–treated CD11c-DTR→B6bm12 and DT-treated transgene→B6bm12 CD4 recipients (Fig. 4A, 4B). Liver pathology was present in all CD4 recipient groups in one of two repetitions, and it was modestly reduced in DT-treated CD11c+ CD4 recipients (Fig. 4C). Therefore, when Ag presentation by residual host type APCs is excluded and the only functional APCs are derived from CD11c-DTR transgene→BM, DT-mediated depletion of cDCs was insufficient to prevent GVHD, although in one experiment there was reduced liver histopathology in cDC-depleted CD4 recipients.

Additional depletion of pDCs, B cells, and partial depletion of CD11b+ cells do not mitigate GVHD

To broaden the scope of APC depletion, we added Ab-mediated pDC (27) and B cell depletion (28). B cells were depleted as we previously reported (28). pDCs were depleted by injecting the anti-BM stromal–derived Ag Ab BST2 on days −2 and −1 before BMT (Supplemental Fig. 2A). In the C3H.SW→(CD11c-DTR→B6) model, additional depletion of B cells and pDCs did not affect clinical or histologic GVHD (data not shown). We next crossed the CD11c-DTR mice to CD11b-DTR mice (CD11c/b-DTR) (23). DT treatment of CD11b-DTR mice depletes tissue macrophages and induces a modest reduction in subsets of CD11b+ cells in spleen and LN (23, 34–40) (Supplemental Fig. 2). We created CD11c/b-DTR→B6 (CD45.1; referred to as Tg+) and WT→B6 (CD45.1; WT) BM chimeras and used them as recipients in the C3H.SW→B6 model. All chimeras, Tg+ or WT, were DT-treated every other day from days −5 to +5. All Tg+ chimeras received anti-CBD20 2 wk before transplant. We further added pDC depletion to one group of Tg+ chimeras. DT-treated and B cell-depleted Tg+ chimeras developed similar clinical GVHD as did WT chimeras as measured by weight loss and incidence of clinical skin disease, whether pDCs were additionally depleted (Fig. 5A, 5B). Survival was worse in DT-treated Tg+ DT recipients (Fig. 5C) as compared with DT-treated WT CD8 recipients. Histopathologic GVHD of the skin and ear were similar in all CD8 recipient groups (Fig. 5D). Colon and liver GVHD were reduced in DT-treated Tg+ CD8 recipients (data not shown). However, this difference is difficult to interpret because some of the most severely affected Tg+ CD8 recipients died before tissue harvest or had their tissues collected at earlier times when sacrificed secondary to the extent of skin disease.

FIGURE 5. DT treatment of CD11c/b-DTR→B6 (Tg+) chimeras with B cell depletion, with or without pDC depletion, does not decrease GVHD. All BM chimeric hosts (Tg+ and WT) were DT treated (4 ng/gm i.p.) every other day from days −5 to +5. All Tg+ chimeras received 0.25 mg anti-CBD20 2 wk before transplantation as previously described (28). One group of Tg+ chimeras also received 0.5 mg BST2 (27) i.p. on days −2 and −1 to deplete pDCs (Supplemental Fig. 2A). Mice were otherwise transplanted as in Fig. 3. DT treatment and B cell and pDC depletion did not diminish weight loss (A) or incidence of clinical skin disease (B). Tg+ chimeras received 0.25 mg anti-CBD20 2 wk before transplantation as previously described (28). One group of Tg+ chimeras also received 0.5 mg BST2 (27) i.p. on days −2 and −1 to deplete pDCs (Supplemental Fig. 2A). Mice were otherwise transplanted as in Fig. 3. DT treatment and B cell and pDC depletion did not diminish weight loss (A) or incidence of clinical skin disease (B). (A) Tg+ BM alone + BST2 (n=10) vs Tg+ BM alone (n=10). (B) Tg+ BM alone + BST2 (n=5) vs Tg+ BM alone (n=10). (C) WT BM alone + BST2 (n=10) vs WT BM alone (n=10). (D) Histopathology scores. Tg+ BM alone + BST2 vs WT BM alone (p=0.09 comparing WT CD8 recipients and BM alone controls). Survival was inferior in Tg+ CD8 recipients, with or without pDC depletion, as compared with WT CD8 recipients (C, p<0.027). Histopathology scores. p<0.05 comparing skin or ear scores in any CD8 recipient group versus its BM control. p=0.014 and p=0.083 comparing skin and ear scores (respectively) of Tg+ + BST2 to WT CD8 recipients. Data combined from two experiments, terminated on days +29 and +39; the Tg+ BM alone group was only present in the experiment that ended on day +29.
We also tested this approach in the B6bm12 model. We created CD11c/b-DTR→B6bm12 (Tg+) and WT→B6bm12 chimeras. All mice were DT treated every other day from days 2 to +5, and Tg+ chimeras additionally were B cell- and pDC-depleted. These chimeras were retransplanted with B6 bm12 BM and spleen cells. Weight loss and serum IFN-γ levels were similar in DT-treated chimeras.

**FIGURE 6.** DT treatment of CD11c/b-DTR→B6bm12 (Tg+) chimeras with B cell depletion and pDC depletion does not decrease GVHD. CD11c/b-DTR→B6bm12 chimeras (Tg+) or control WT→B6bm12 chimeras mice were injected with DT (4 ng/g i.p.) every other day from days −5 to +5. All Tg+ mice received 0.25 mg anti-CD20 on day −14 and with 0.5 mg BST2 i.p. on days −2 and −1 to deplete B cells and pDCs, respectively. On day 0, chimeras were reirradiated (450 cGy × 2) and reconstituted with T cell-depleted B6 bm12 BM with or without B6bm12 splenocytes containing 10^6 CD4 cells. (A) Weight change. p < 0.03 comparing weights of Tg+ or WT→B6bm12 (WT) spleen cell recipients versus their BM alone controls from days +5 to +27. p > 0.05 comparing Tg+ and WT spleen recipients on days +5, +7, +12, and +14. p < 0.0001 comparing serum IFN-γ levels (B) at day +7 in Tg+ or WT spleen cell recipients versus BM alone controls (10 Tg+ and WT BM alone controls; 10 Tg+ and WT spleen cell recipients). (C) Liver histopathology scores. p = 0.353 comparing spleen cell recipient groups. p = 0.14 and 0.04 comparing Tg+ and WT spleen cell recipients with their BM alone controls, respectively. p = 0.03 comparing Tg+ and WT spleen cell recipients. p = 0.095 comparing Tg+ and WT BM alone groups. Data combined from two experiments.

**FIGURE 7.** GVHD is not reduced in CD11c:DTA recipients. (A) Shown are staining of representative Tg+ and CD11c:DTA splenocytes for CD11c+ MHCII+ (left panel) and BST2+B220+ pDCs (right panel). (B–D) CD11c:DTA or control CD11c-cre or ROSA26stop/flox/stop DT mice were irradiated (475 cGy × 2) and reconstituted with 10^7 T cell-depleted C57.B16 SW BM with no T cells or with 2 × 10^6 C3H. SW CD8 cells. CD11c:DTA mice were also depleted of B cells and pDCs as described in Fig. 5. (B) Weight change. p < 0.0006 comparing CD11c:DTA or control CD8 recipients with their BM alone controls from day +22 onward. (C) Survival. p < 0.0001 comparing CD8 recipient groups. (D) Histopathology scores. p ≤ 0.008 comparing skin, ear, and colon scores in CD11c:DTA CD8 recipients as compared with the CD11c:DTA BM alone controls. p < 0.0003 comparing skin and colon scores and p = 0.053 comparing ear scores in control CD8 recipients and BM alone controls. p ≥ 0.15 comparing all histopathology scores in CD11c:DTA and control CD8 recipients. Liver GVHD could not be evaluated because of a high baseline level of inflammation in the CD11c:DT BM alone group (not shown). Data combined from two experiments with similar results.
and B cell/pDC-depleted Tg+ chimeras and control DT-treated WT chimeras (Fig. 6A, 6B). Liver GVHD, which developed in one of two repetitions, was significant in both spleen cell recipient groups, and scores were lower in Tg+ spleen cell recipients (Fig. 6C). However, scores in the WT BM alone group were greater than in the Tg+B6 BM alone group; thus, the difference in spleen cell recipient groups could have been caused by inflammation unrelated to GVHD.

Lastly, we used as hosts in the C3H.SW→B6 system CD11c-cre transgenic mice (24) crossed to mice that have a floxed STOP cassette flanking the DT-a-chain gene, inserted into the ROSA26 locus (CD11c-DTA) (25). CD11c-DTA mice have a constitutive absence of CD11c+ cDCs, Langerhans cells (LCs), and most pDCs (20, 21) (Fig. 7A). We further added Ab-mediated B cell depletion and pDC depletion. Both CD8 recipient groups lost weight relative to their BM alone controls (Fig. 7B), and there were more deaths in CD11c-DTA CD8 recipients (Fig. 7C). Histopathologic skin, ear, and colon GVHD were similar in both CD8 groups (Fig. 7D).

Discussion

Contrary to expectations, depletion of host cDCs failed to significantly mitigate GVHD in two models in which host hematopoietic APCs are necessary and sufficient for GVHD induction. Importantly, we excluded priming by donor or residual Tg+ host APCs. We cannot completely exclude that GVHD was induced by residual Tg+ cDCs not killed by DT treatment or by expression of DTA. However, cDC ablation was profound in DT-treated CD11c-DTR and CD11c-b-DTR BM chimeras, and in CD11c-DTA mice, and this degree of cDC ablation was sufficient to block T cell responses in many other model systems (20, 21, 29, 41). From these data we conclude that host cDCs are not required for GVHD induction or that a very small number of cDCs, insufficient to initiate substantial T cell response in other systems, are adequate in GVHD. Either way, targeting host cDCs will be unlikely to prevent GVHD in the clinic. Additional partial depletion of host CD11b+ cells along with B cells and pDCs did not meaningfully mitigate GVHD in either model system or reduce serum IFN-γ levels in the B6bm12 model.

Although overall cDC depletion did not reduce GVHD, liver GVHD was reduced in DT-treated mice in one experiment in the B6bm12 model (Fig. 4) and colon GVHD relative to DT-treated Tg+ CD8 recipients in one experiment in the C3H.SW→B6 system (but not relative to PBS-treated Tg+ CD8 recipients; Fig. 3). It is possible that these reductions were a true consequence of cDC depletion.

Our results raise the question of what other APCs could be priming donor T cells. We can exclude LCs because they are neither sufficient nor required for GVHD in the C3H.SW→B6 model (8, 42) and are depleted in CD11c-DTA mice (21). In B6→B6bm12 chimeras, LCs remained Beαβ+ in origin (42) and therefore would not prime syngeneic Beαβ+ donor CD4 cells. Although we used state-of-the-art methods to deplete APCs, all host CD11b+ cells were not eliminated in DT-treated CD11b-DTR mice, and we were unable to use higher doses of DT that may have improved depletions because of lethality in Tg+ and Tg− mice (data not shown). Future studies testing the importance of these cells will require the development of less toxic depletions.

Could nonhematopoietic host cells be priming donor T cells? This is unlikely in the C3H.SW→B6 system wherein others and we have previously shown host hematopoietic cells to be essential APCs (8, 31). Host hematopoietic cells were also required for lethal GVHD in the BALB/c (H-2b)→B6 (H-2b) MHC-mismatched strain pairing (7). In the B6bm12→(B6→B6bm12) model, nonhematopoietic cells were Beαβ+ and would not have primed syngeneic Beαβ+ CD4 cells.

As to why APC requirements differ in GVHD than in other models of T cell immunity, we hypothesize this relates to differences in the availability and distribution of target Ags. In most models of T cell immunity wherein CD11c+ APCs have been shown to be critical, APCs are directly infected or more commonly must acquire, process, and present exogenous Ags. cDCs and other specialized APCs have receptors and Ag-processing mechanisms that facilitate this (43–46). Further, because in infections only a minority of APCs acquire pathogen-derived Ags, such specialized properties of APCs and their activation by pattern recognition receptors may be pivotal for the efficient attraction, priming, and programming of rare Ag-reactive T cells (47, 48). In contrast, in GVHD, all host cells express and directly present alloantigens, a situation akin to a viral infection of every cell. Therefore, the specialized properties of “professional APCs” that evolved to respond to pathogens may not be critical for alloreactive T cell activation. Consistent with this, GVHD induction in the same models presented in this study do not require that APCs be stimulated through TLRs or the IL-1R in response to IL-1 generated by inﬂammasome activation (28). In summary, these studies demonstrate that the APC requirements for activation of T cells in alloBMT are unique and clearly distinct from those in other models. Host APC-directed approaches for preventing GVHD in the clinic cannot focus only on cDCs, pDCs, and B cells, and will need to additionally target other APCs classically considered to be less potent or alternatively interfere with Ag-processing mechanisms found in all cells.

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Disclosures

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