Host-Derived CD8⁺ Dendritic Cells Protect Against Acute Graft-versus-Host Disease after Experimental Allogeneic Bone Marrow Transplantation

Michael Weber¹, Berenice Rudolph², Pamela Stein¹, Nir Yoge³, Markus Bosmann⁴,⁵, Hansjörg Schild¹, Markus P. Radsak⁴,*

¹Institute of Immunology, Johannes Gutenberg-University Medical Center, Mainz, Germany
²Department of Dermatology, Johannes Gutenberg-University Medical Center, Mainz, Germany
³Institute of Molecular Medicine, Johannes Gutenberg-University Medical Center, Mainz, Germany
⁴IIIrd Department of Medicine, Johannes Gutenberg-University Medical Center, Mainz, Germany
⁵Center for Thrombosis and Hemostasis, Johannes Gutenberg-University Medical Center, Mainz, Germany

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ABSTRACT
Graft-versus-host disease (GVHD) is a frequent life-threatening complication after allogeneic hematopoietic stem cell transplantation (HSCT) and induced by donor-derived T cells that become activated by host antigen-presenting cells. To address the relevance of host dendritic cell (DC) populations in this disease, we used mouse strains deficient in CD11c⁺ or CD8α⁺ DC populations in a model of acute GVHD where bone marrow and T cells from BALB/c donors were transplanted into C57BL/6 hosts. Surprisingly, a strong increase in GVHD-related mortality was observed in the absence of CD11c⁺ cells. Likewise, Batf3-deficient (Batf3⁻/⁻) mice that lack CD8α⁺ DCs also displayed a strongly increased GVHD-related mortality. In the absence of CD8α⁺ DCs, we detected an increased activation of the remaining DC populations after HSCT, leading to an enhanced priming of allogeneic T cells. Importantly, this was associated with reduced numbers of regulatory T cells and transforming growth factor-β levels, indicating an aggravated failure of peripheral tolerance mechanisms after HSCT in the absence of CD8α⁺ DCs. In summary, our results indicate a critical role of CD8α⁺ DCs as important inducers of regulatory T cell-mediated tolerance to control DC activation and T cell priming in the initiation phase of GVHD.

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INTRODUCTION
For patients with high-risk hematological malignancies, allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative treatment option. The therapeutic efficacy is based on the emergence of curative immune responses against residual malignant cells in the host induced by donor lymphocytes [1]. Although effective for many patients, undesired immune responses against otherwise healthy tissues frequently occur after HSCT and cause graft-versus-host disease (GVHD). Complications related to GVHD are the most important contributors to the high-treatment-related morbidity and mortality rates post-HSCT [2]. Therefore, a deeper understanding of the immunological mechanisms that initiate and maintain GVHD is necessary to improve the feasibility and allow broader application of this otherwise elegant immunological treatment approach.

Acute GVHD is primarily caused by donor-derived T cells within the allogeneic stem cell graft that become activated after contact with host-derived antigen-presenting cells (APCs) [3]. These primed allogeneic T cells successively assault healthy tissues (eg, in the liver, gut, and skin), creating GVHD [4]. Although dendritic cells (DCs) are highly potent in T cell priming in general [5] and also important in the context of GVHD, there is considerable debate on the precise role of DCs in the regulation of GVHD. On one hand, host-derived DCs are sufficient for the initiation of GVHD [6], but on the other, donor-derived DCs may also contribute to the priming of allogeneic T cells [7]. Beyond this, host-derived non-hematopoietic cells may likewise be sufficient to induce GVHD [8,9]. Interestingly, depletion of specific DC subsets, such as Langerhans cells [10] and conventional or plasmacytoid DC populations, does not prevent GVHD, illustrating the complexity of T cell activation in this setting.

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*Correspondence and reprint requests: PD Dr. med. Markus P. Radsak, IIIrd Department of Medicine, University Medical Center, Johannes Gutenberg-University, Langenbeckstr. 1, D-55131 Mainz, Germany.
E-mail address: radsak@uni-mainz.de (M.P. Radsak).

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Moreover, DCs are also involved in the maintenance of central and peripheral tolerance [11], mostly regulated by their activation state [12] and contact with regulatory T (Treg) cells [13]. In particular, CD8α− DCs are critical for CD8+ T cell responses initiated by cross-priming [14] but, conversely, may be also involved in the induction of tolerance [15]. With respect to GVHD, vaccination with host-type CD8α− DCs reduces GVHD [16], demonstrating the relevance of CD8α− DCs in the regulation of immune responses after HSCT. Moreover, CD8α+ DCs play a role for the induction of graft-versus-tumor (GVT) responses, as shown in a minor histocompatibility antigen (miHA) mismatched HSCT model [2,17]. Nevertheless, the direct role of CD8α+ DC in induction of GVHD has only been incompletely defined.

To elucidate the role of host-derived DCs in the initiation of GVHD, we used 2 transgenic mouse strains deficient of CD11c+ DC populations in a mouse model of acute GVHD and surprisingly found a strong increase in GVHD-related mortality in the absence of CD11c+ cells. Because Batf3−/− mice are devoid of the CD8α− DCs, mice from A. Waisman (Mainz) and bred as described [21] were from A. Waisman (Mainz) and bred as described previously [22,23]. We also established a minor histocompatibility antigen (miHA) mismatched HSCT model [24]. Additionally, we found a strong increase in GVHD-related mortality in the absence of CD11c+ cells. Because Batf3−/− mice are devoid of the CD8α− DCs, mice from A. Waisman (Mainz) and bred as described previously [21] were from A. Waisman (Mainz) and bred as described previously [22,23].

For mixed lymphocyte reaction (MLR), spleens and mesenteric lymph nodes (MLNs) were digested in Dnase I (100 μg/ml; Sigma) and collagenase-type 2 (1 mg/ml; Worthington Biochemical Corporation, Lakewood, NJ, USA) followed by density centrifugation as described previously [12].

Clinical symptoms of GVHD were monitored daily by assessing weight loss, posture, activity, fur texture, and skin integrity, adding up to a clinical score [24]. Animals with severe GVHD defined by clinical scores >6 were killed as required by the institutional animal ethics guidelines and the day subsequent to death determined as the following day.

Samples of large intestine, liver, and skin were taken on day 10 and stained with H & E. The sections were reviewed and scored by one of the authors (B.R.) who was blinded to the experimental groups according to a previously published histopathology scoring system [11,25].

Flow Cytometry Staining and Analyses

All analyses were performed with an LSR II flow cytometer and FACS Diva (Becton Dickinson, Heidelberg, Germany) or FlowJo (Tree Star Inc, Ashland, OR, USA) software. The following mAbs were used: CD3 (clone 145-2C11), CD4 (clone GK1.5), CD8 (clone 53-6.7), CD11c (clone N418), MHC class II (clone M5/114.15.2), CD45.2 (clone 104), CD80 (clone 16-10A1), CD86 (clone GL1), CD90.2 (clone 53-2.1), CD229.1 (clone 30CT7), PD-1 (clone 10F9.G2), PD-L2 (clone 122), and FoxP3 (clone FJK-16S) (all antibodies were purchased from Biologend, San Diego, CA, USA or eBioScience, Frankfurt, Germany). Viability was determined by propidium iodide (Sigma-Aldrich). Total cell counts in the spleen were determined by flow cytometry using counting beads (Beckmann Coulter, Krefeld, Germany) according to the manufacturer's instructions.

Cell Purification and Culture

Spleen DCs were purified by digestion using DNase I (100 μg/ml; Sigma-Aldrich) and collagenase-type 2 (1 mg/ml; Worthington Biochemical Corporation, Lakewood, NJ, USA) followed by density centrifugation as described previously [12].

mRNA Detection

RNA was isolated using TRIzol (Invitrogen, Darmstadt, Germany) and cDNA was synthesized with RevertAid M-MuLV reverse transcriptase following the recommendations of the supplier (Fermentas, Thermoscientific, Schwerte, Germany). Quantitative real-time (qRT)-PCR was performed using the following oligonucleotides: murine IL-10 forward 5′-GAC GCT CAT CTC TT-3′; murine IL-10 reverse 5′-GGG TCT CAC AAC GAA AGG TA-3′; β2-microglobulin forward 5′-TTT GTC CTC GGT GCC CTC ACC G-3′; β2-microglobulin reverse 5′-GCC ATG TAT CCG TCC CCA C-3′; murine IL-12p35 forward 5′-GTC ATC GAC CCT ACC TCC-3′; murine IL-12p35 reverse 5′-CTG CAC TCA TCG AGG C-3′; murine IL-2 reverse 5′-GTG GCA TTC ACC TCC-3′. qRT-PCR analyses were performed in triplicates on an iCycler (BioRad, Munich, Germany) using the SYBR GreenER qPCR Supermix (Invitrogen). After normalization of the data according to the expression of β2-microglobulin mRNA, the relative expression level of Ifng, Il-10, and Il-12p35 mRNA was calculated.

Detection of IL-2 and TGF-β

Mice were killed on day 7 post-HSCT by CO2 asphyxiation and peripheral blood taken by retro-orbital bleeding and centrifuged. Cell free serum samples were frozen at −20 °C until required. IL-2 was detected by a specific ELISA using anti-IL-2 JES6-1A12 and biotinylated anti-IL-2 JES6-5H4, both from BD Biosciences, Heidelberg, Germany) as previously described [26]. TGF-β ELISA (from R&D Systems, Wiesbaden, Germany) was used according to the manufacturer's instructions.

Statistical Analysis

Analyses were performed by a 2-tailed Student's t-test for comparison between 2 groups as indicated. Multiple groups were compared by 1-way ANOVA with Bonferroni's post-test. Survival analysis was performed by the Mantel-Cox test. For all analyses, P < 0.05 was considered significant. All
RESULTS

Host-Derived Conventional DCs Are Dispensable for the Induction of Acute GVHD

To analyze the general role of conventional DCs in GVHD induction, we used our previously described diphtheria toxin A (Rosa-DTA) model [22] in which DCs are continuously ablated when crossed to the CD11c-Cre line 21. We used a well-established mouse model for HSCT, where C57BL/6 (B6) or DC-deficient CD11c-DTA recipients received a lethal dose of TBI (11 Gy) and were transplanted with T cell–depleted bone marrow and purified T cells from MHC and miHA mismatched BALB/c donors. Wild-type B6 animals developed clinical signs of acute GVHD as evidenced by weight loss, decreased activity, and loss of fur (not shown), resulting in a lethal course of disease in all animals with a median survival of 28 days (Figure 1A). Unexpectedly, DC-deficient CD11c-DTA mice developed more severe signs of GVHD as compared with controls, resulting in death of all transplanted CD11c-DTA recipients within a significantly accelerated timeframe (median survival of 11 days, P < .001 by Mantel Cox test).

Because the continuous ablation of DCs resulted in an autoimmune disorder phenotype because of the lack of control by Treg cells [27], we next used another model of transient DC ablation in vivo. For this we used our previously described iDTR model crossed to the CD11c-Cre line (CD11c-iDTR) [22]. Depletion of DCs in these mice was achieved by the injection of diphtheria toxin. As shown in Figure 1B, when transplanting CD11c-iDTR recipients with or without additional DC depletion by diphtheria toxin treatment, we observed all mice that had received diphtheria toxin rapidly died (median survival, 8 days), similar to the course of GVHD in CD11c-DTA mice (Figure 1A). In contrast, CD11c-iDTR recipients not receiving diphtheria toxin displayed a prolonged survival (median survival, 23 days, P < .001 by Mantel Cox test), comparable with the course of GVHD in B6 recipients.

DCs are a heterogeneous population of cells, and distinct DC subsets may exert diverse effects on the immune responses post-HSCT. Therefore, we decided to focus on 1 particular subset, namely CD8α+ DCs, that are a defined lineage depending on the AP1 transcription factor Batf3. Hence, Batf3−/− mice constitutively lack the CD8α+ DC subpopulation [14]. As depicted in Figure 1C, the transplanted wild-type B6 animals developed acute signs of illness, and nearly all mice succumbed to GVHD (94%; median survival, 43 days), whereas Batf3−/− mice displayed a strongly aggravated course of GVHD as illustrated by the 100% lethal outcome and a median survival of 8 days (P < .001 by Mantel Cox test). When comparing transplanted wild-type B6 and Batf3−/− mice for histological evidence of GVHD (Figure 1D), we detected increased signs of GVHD in the colon (P < .05 by Student’s t-test) and in the liver, the latter not reaching statistical significance (P = .15 by Student’s t-test). In contrast, we found decreased signs of GVHD in the skin as another typical GVHD target organ (P < .05 by Student’s t-test), suggesting a distinct role of CD8α+ DCs during GVHD in different target organs.

Taken together, these results indicate in 3 distinct models that host-derived conventional DCs are protective against the adverse outcomes. Apparently, the organ-specific distribution of DC subsets contributes to the pace and histopathology phenotype of GVHD.

Rapid Depletion and Concurrent Activation of Host DCs after HSCT

Allogeneic T cell priming occurs on contact of transplanted donor T cells with host APCs [28]. Because host-derived DCs were not required for the induction of GVHD and the TBI conditioning regimen rapidly depletes various hematopoietic cell types, inducing apoptosis, including DCs [29], we were interested in the depletion kinetics of

![Figure 1](image-url). Enhanced GVHD-related mortality in the absence of conventional DCs. (A-C) Recipient mice (B6, CD11c-DTA, CD11c-iDTR or Batf3−/− as indicated) were lethally irradiated (11 Gy in split dose) and transplanted allogeneic T cell–depleted bone marrow cells (5 × 10^6 cells) and CD90.2+ T cells (5 × 10^6 cells) from BALB/c donor mice. Kaplan-Meier survival analysis of GVHD-related mortality of the indicated treatment groups is shown. In (B) the indicated group of CD11c-iDTR mice received diphtheria toxin (25 ng/g, on days −2 and 1) for DC depletion. The results in (A) show a single experiment. The analyses in (B, C) are cumulated from 2 independent experiments with 5 to 8 mice per group. (D) HSCT was performed as in (C) except mice were killed on day 10. Samples from the intestine, liver, and skin were taken and sections were stained with H & E and histopathological scores (inflammation, apoptosis) determined (n = 7 per group). *Statistical significance (P < .05) by Mantel-Cox test. **Statistical significance (P < .05) by Student’s t-test.
host-derived DC and the repopulation by donor-derived DCs. Therefore, we quantified the number of viable DCs (gating on viable propidium iodide– MHC II+ CD11c+ cells) in the spleen of transplanted B6 mice (Figure 2A). As expected, the number of DCs daily decreased by a factor of 2 until day 4 post-HSCT. In line with previous reports [30], the remaining viable DCs displayed an activated phenotype (Figure 2B).

Because Batf3-/− mice have residual CD8α+ DCs due to Batf-dependent compensatory mechanisms that may expand under inflammatory conditions [18], we examined the fate of donor CD8α+ DCs after TBI in wild-type and Batf3-/− mice. Under steady-state conditions, we detected a reduced frequency of CD8α+ DCs in the spleen and MLNs that was strongly reduced 24 hour after TBI (Figure 2C), suggesting these compensatory mechanisms are unable to account the aggravated course of GVHD we observed in Batf3-/− mice since Tussiwand et al. [18] observed an expansion of CD8α+ DCs in Batf3-/− mice in various inflammation models.

To further investigate whether the DCs shown in Figure 2A were of host or donor origin, we used the congenic surface marker CD229.1 (Ly9.1) expressed only in hematopoietic cells from BALB mice [31] and assessed the DC chimerism in the spleen over time by flow cytometry. Most DCs were of host origin until day 3 post-HSCT (Figure 2D). Although the absolute numbers of DCs were very low by day 4, it appears possible and consistent with previous data [30] that a sufficient number of host- or donor-derived DCs are present during the initial phase of GVHD to influence the course of GVHD.

**CD8α+ DCs Suppress the TBI-Induced Activation of the Remaining DC Subsets**

Because we observed an aggravated course of GVHD in the absence of host conventional DCs and conversely in the absence of CD8α+ DCs, we were interested in the activation phenotype of CD8α+ versus CD8α− DC subsets after HSCT.

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**Figure 2.** Host DCs are activated but rapidly depleted after HSCT. Recipient B6 mice were lethally irradiated (11 Gy in split dose) and transplanted allogeneic T cell–depleted bone marrow cells (5 × 10⁶ cells) and CD90.2+ T cells (5 × 10⁵ cells) from BALB/c donor mice as described before. Splenocytes were harvested at the indicated time points and stained for viable DCs (identified by propidium iodide– CD11c– MHC II– cells). (A) The absolute number of DCs was determined using calibrated counting beads. The cumulated results from 2 independent experiments are depicted (n = 6 per time point). (B) Representative flow cytometry overlay histograms of splenic DCs (gated on propidium iodide– CD11c– MHC II– cells) for surface expression of MHC II, CD80, and CD86, respectively, of untreated mice (red) or 24 hours after HSCT (blue) are depicted. (C) Mice were left untreated (B6 or Batf3-/−) or lethally irradiated (11 Gy in split dose) and spleens (left) and MLNs were harvested 24 hours later and analyzed by flow cytometry for CD8α+ DCs (gating on propidium iodide– CD3– CD19– F4/80– and CD11c– MHC II– cells). A cumulative analysis of 2 independent experiments with 6 mice per group is shown. (D) Representative flow cytometry histograms of splenic DCs (gated on propidium iodide– CD11c– MHC II– cells) and shown after staining for the donor (BALB) specific marker CD229.1 at the indicated time points post HSCT. The percentage of donor-derived DCs is indicated. *Significant difference (P < .05) to the untreated control group by 1-way ANOVA and Bonferroni’s posttest comparison. **Significant difference (P < .05) between the indicated groups by Student’s t-test.
Therefore, we analyzed DC populations (gating on viable propidium iodide− MHC II+ CD11c+ cells) in the spleen and MLNs for the activation phenotype in wild-type B6 or Batf3−/− mice after TBI. In line with others [17,18], we detected no CD8α+ DCs in Batf3−/− mice under steady-state conditions. In contrast to splenic DCs (data not shown), in the MLNs the surface expression patterns of MHC class II and the costimulatory molecules CD80 and CD86 were comparable in CD8α− and CD8α− DC populations in untreated wild-type (B6) and Batf3−/− mice (Figure 3A). Consistent with the previous work by Zhang et al. [30] and Lin et al. [13], we observed a strong up-regulation of MHC class II and costimulatory molecules on DCs after TBI. Interestingly, this TBI-induced activation phenotype was restricted to the CD8α− DC populations and significantly attenuated in the CD8α+ DC population in the wild-type B6 animals. Moreover, although we did not observe any differences in the steady-state DC phenotype of Batf3−/− mice, the expression levels of MHC class II as well as CD86 and the related B7 family molecules PD-L1 and PD-L2 were even increased in DCs from Batf3−/− mice, suggesting a hyperactivated state in these animals post-TBI. Further corroborating this finding, we detected increased levels of Il-12p35, Il-10, and Ifng mRNA in the MLNs of Batf3−/− mice after TBI compared with the respective wild-type B6 controls (Figure 3B). Collectively, these data indicate that CD8α− DCs contribute to the suppression of DC activation post-TBI and suggest the aggravated course of GVHD in Batf3−/− mice may be mediated by the enhanced allogeneic T cell activation in the absence of CD8α+ DCs.

**CD8α+ DCs Regulate Allogeneic MLR after TBI-Conditioning Treatment**

So far, our results described a more activated DC phenotype in the MLNs after TBI conditioning in Batf3−/− mice but did not clarify whether this is related to the adverse outcome of these animals. We hypothesized that this enhanced DC activation more effectively triggers allogeneic T cell activation, resulting in an aggravated course of GVHD and early death of Batf3−/− mice post-HSCT. To directly address this question, we harvested MLNs from Batf3−/− mice or the respective wild-type B6 control animals after TBI and used these cells as stimulator APCs for naive allogeneic T cells from BALB/c donors in an allogeneic MLR. As shown in Figure 4A using MLN cells from untreated mice as stimulators, we only observed a minor increase in T cell proliferation in the presence of Batf3−/− cells compared with the wild-type B6 controls (P < .05 by paired Student’s t-test). In contrast, when using MLN cells from mice 24 hours post-TBI conditioning as stimulators, we observed a reduction of T cell proliferation by about 50% compared to the wild-type B6 controls (Figure 4B), most likely due to a TBI-induced reduction of APC numbers.

![Figure 3](image-url) Enhanced activation of conventional DCs in the absence of CD8α− DCs after TBI. Mice were left untreated (B6 or Batf3−/−) or lethally irradiated (11 Gy in split dose as before; B6 post TBI or Batf3−/− post TBI) and MLNs were harvested 24 hours after TBI. (A) The cells were stained for viable DCs (identified by propidium iodide− CD11c− MHC II− cells, indicated as “all”), DCs from B6 mice were further subdivided by the surface expression of CD8α (indicated as CD8α− or CD8α+) and analyzed by flow cytometry. The expression levels MHC II and the activation markers CD80/CD86 are quantified in the respective DC subsets. The expression levels of the inhibitory B7 family molecules PD-L1 and PD-L2 were quantified on DCs (gating on propidium iodide− CD11c+ MHC II+ cells). The depicted data are mean and SD from 1 representative of 3 independent experiments performed with 3 mice per group. (B) Total RNA was extracted from MLN cells of untreated mice or 24 hours post-TBI (B6 or Batf3−/−); mRNA levels of Il12p35, Il10 and Ifng (mean plus SD) were assessed by real-time PCR and normalized to levels of untreated mice. B2m was used as housekeeping gene. Data shown are from 1 representative (n = 3 mice per group) of 2 independent experiments. *Significant difference (P < .05) between the indicated groups by 1-way ANOVA and Bonferroni’s post-test comparison. **Statistical significance (P < .05) by Student’s t-test. *Statistical significance (P < .05) by Student’s t-test compared with the untreated control (B6 or Batf3−/−).
On the other hand, we detected a strongly enhanced T cell response induced by Batf3−/− MLN cells after TBI. Thus, the absence of CD8α+ DCs not only leads to a more activated DC phenotype after TBI but also induces an enhanced allogeneic T cell response compatible with the aggravated course of GVHD and increased GVHD-related mortality observed in Batf3−/− mice upon HSCT.

CD8α+ DCs Suppress Allogeneic T Cell Responses by the Induction of Treg Cells

Because CD8α+ DCs can induce FoxP3+ Treg cells in the presence of TGF-β [32], we hypothesized that this might also be a relevant mechanism in the context of allogeneic HSCT because Treg cells are well known for their ability to suppress GVHD [33]. First, we analyzed the number of donor T cell recovery in spleen and the CD4/CD8 T cell ratio to characterize the T cell response post-HSCT. Although the absolute number of donor T cells appeared lower in Batf3−/− compared with wild-type B6 hosts, there was no significant difference in the number of CD90+ T cells, suggesting a comparable T cell recovery in Batf3−/− and wild-type B6 in the spleen (Figure 5A, left). Likewise, we observed low CD4/CD8 ratios indicative of CD8 skewed T cell responses without any significant differences in Batf3−/− or wild-type B6 hosts (Figure 5A, middle). All donor T cells had an activated phenotype (positive for CD25, not shown) compatible with a strong allogeneic effector T cell response in GVHD. Notably, we found a significantly lower number of CD4+ CD25+ FoxP3+ Treg cells and serum levels of TGF-β are decreased in the absence of CD8α+ DCs after HSCT. Recipient mice (B6 or Batf3−/−) were lethally irradiated (11 Gy in split dose) and transplanted allogeneic T cell–depleted bone marrow cells (5 × 10⁶ cells) and CD90.2− T cells (5 × 10⁵ cells) from BALB/c donor mice as described before. (A) Splenocytes were harvested at day 7 post-HSCT and stained for viable donor-derived H2-Kb CD90.2 T cells, CD4/CD8 ratio (CD90.2+ CD4+ cells), T reg cells (identified by propidium iodide− CD3− CD4− FoxP3+ cells), respectively. The absolute number of T cells and Treg cells (mean and SD) was determined using calibrated counting beads. (B) Serum samples were collected and analyzed for IL-2 and TGF-β concentrations (mean ± SD) by ELISA. The depicted results are pooled from 2 independent experiments (n = 6 per group). *Statistical significance (P < .05) by Student’s t-test, n. s. indicates no significant difference by Student’s t-test (P > .05).
Treg cells in transplanted Batf3−/− mice compared with the wild-type B6 controls after allogeneic HSCT (Figure 5A, right). In addition, we analyzed sera of these transplanted animals for IL-2 to further characterize the T cell response und TGF-β, because induction of Treg cells by CD8a+ DCs can be driven by TGF-β [32]. IL-2 levels were strongly elevated (Figure 5B, left), whereas TGF-β levels were clearly reduced in the transplanted Batf3−/− hosts compared with the wild-type B6 controls (Figure 5B, right). Taken together, these results suggest that Batf3−/− mice experience an aggravated course of GVHD accompanied by increased IL-2 release suggestive of an enhanced activation of allogeneic T cells and reduced TGF-β levels, possibly related to the induction of Treg cells by CD8a+ DCs.

**DISCUSSION**

Although nonhematopoietic cells can induce GVHD [8], host-derived hematopoietic APCs have been shown to be the essential for the induction of GVHD [3,34]. Although host-derived DCs are sufficient to mediate GVHD [6], we used 2 models of conventional DC depletion to clearly show that host-derived CD11c+ DCs are not necessary, which is well in line with a previous report by Li et al. [11]. Although they had to use bone marrow chimeras of CD11c-DTR mice for HSCT [35] because repeated diphtheria toxin treatments are lethal in these animals due to promiscuous expression of the transgene [36], this problem does not occur in the CD11c-DTR strain used [22]. Therefore, our results are important because they add a distinct model system (CD11c-iDTR strain) and independently confirm the previous data [11]. From our own previous work, where we have extensively characterized the residual DCs in the CD11c-iDTR and CD11c-DTA strains, we know that less than 5% of the normal DC numbers are left in the host [22]. Therefore, we cannot formally exclude that these remaining DCs are sufficient to induce GVHD. However, this degree of DC depletion is sufficient to ablate immune responses in other models [21,35,37].

Although it remains controversial to what extent DCs are required for the induction of GVHD, our results allow the conclusion that host-derived DCs contribute to the amelioration of GVHD, raising the question what DC subpopulation is involved. Toubai et al. [36] previously demonstrated that the vaccination with host-type CD8a+ DCs suppresses GVHD. Hence, a suppressive effect of this DC subset on the course of GVHD can be anticipated. Consistent with this notion, we observed an aggravated course of GVHD in Batf3−/− mice where CD8a+ DCs are lacking because the development of CD8a+ DCs depends on the AP1 transcription factor Batf3 [14]. Our results nicely complement previous observations by Teshima [38] detecting an expansion of CD8a+ DCs and a decrease of GVHD-related mortality upon treatment of the HSCT recipient with Flt3 ligand. The augmented GVHD-related mortality in Batf3−/− mice and the enhanced MLR using Batf3−/− APCs as stimulators are also compatible with an enhanced allogeneic T cell priming induced by more activated residual DCs in the absence of CD8a+ DCs [39]. This supports our ex vivo data showing increased levels of Ilf4 mRNA in the DCs from the MLNs on day 2 and increased IL-2 levels in the serum on day 7 post-HSCT in Batf3−/− recipients compared with the wild-type controls. Notably, we were unable to detect any significant differences in the recovery of donor T cells or CD4/CD8 ratios post-HSCT of Batf3−/− or wild-type recipients (Figure 5A). However, this may still be compatible with the enhanced activation of allogeneic T cells in Batf3−/− recipients because most effector cells may have been recruited to GVHD target organs (ie, gut and liver) as suggested by our histopathology findings (Figure 1D). Interestingly, we found significantly increased histopathology signs of GVHD in the gut as opposed to the skin where the signs of GVHD were diminished. Although we did not follow up on this observation, this can be suspected to be related to differences in the distribution of CD8a+ DCs and the related nonlymphoid CD103+ DCs in the intestine versus the skin [40,41].

As shown in Figure 2, host DCs were rapidly depleted after TBI, leaving only a narrow time frame of 3 to 4 days for priming of allogeneic T cells, which is in line with previous reports [13,30]. Regarding the role of CD8a+ DCs in the priming phase of GVHD, our data obtained with Batf3−/− hosts suggest suppressive effects. Although Batf3−/− mice on B6 background are devoid of CD8a+ DCs under steady state, they may acquire CD8a+ DCs under inflammatory conditions after 3 to 14 days depending on the infection model [18]. This is mediated by compensatory mechanisms via Batf. Therefore, it needs to be considered for our data that CD8a+ DCs may be induced in Batf3−/− hosts upon TBI that we were unable to detect. However, given the rapid depletion of host DCs after lethal TBI (Figure 2) and the enhanced GVHD-related mortality in Batf3−/− hosts (Figure 1), these Batf-dependent mechanisms are apparently insufficient to fully compensate the primary lack of CD8a+ DCs in Batf3−/− hosts. Therefore, our results allow the conclusion that host CD8a+ DCs are important counter-regulators in the initiation phase of GVHD.

In contrast to our results, a study using a murine miHA mismatch model showed no role for Batf3-dependent CD8a+ DCs in GVHD induction but a requirement of this cell population to induce an optimal GVT reaction [17]. However, an obvious difference to our study is the MHC-matched HSCT model and the use of purified CD8+ T cells in the graft, where the ability of CD8a+ DC for the cross-presentation of tumor-related miHA is clearly important. It appears unlikely that a lack of cross-presentation of miHA can explain this difference, because miHA are ubiquitously and directly presented by all host APCs in contrast to tumor antigens for the GVT effect, which have to be taken up and presented by host APCs. However, another major difference between both models is that GVHD in the C3H.SW→B6 model is more CD8+ T cell dependent, whereas our BALB/c→B6 model mainly requires CD4+ T cells and CD8+ T cells to a lesser extent [42]. Nevertheless, in the model of Toubai et al. [17] no CD4+ T cells were transplanted, which may contribute to the severity and kinetic of GVHD and explain the differences in outcome. However, when considering the role of CD8a+ DCs for GVT-specific CTL responses [17] in context of our results demonstrating suppressive effects on priming of GVH-specific T cell responses, it is tempting to speculate that targeting of CD8a+ DCs may be important gate keepers to separate GVH from GVT responses to concurrently improve the efficacy (GVT) and reduce toxicity (GVH) of HSCT. Although clinical evidence in humans is currently missing, the previous work by Teshima [38] using Flt3 ligand to expand CD8a+ DCs in a similar MHC mismatched model of GVHD (B6→B6D2F1) suggests a potential way to go.

The importance of the interaction of APCs with CD4+ T cells is not only evident for priming of T cell responses in the context of GVHD but also for the induction or maintenance of tolerance, as Treg cells are of well-known significance in controlling GVHD by various mechanisms
and PD-L2 on DCs from wild-type as well as for GVHD, we detected an increased expression of PD-L1 for a study. However, a similar mechanism involving ing to enhanced T cell priming and the exacerbation of GVHD, CD8 along with an enhanced MLR response in the absence of activated DC phenotype after the TBI conditioning regimen is the key regulators of allogeneic T cell priming in this context. Taken together, we confirmed that conventional DCs are not required for the induction of acute GVHD but also demonstrated an important role of host-derived CD8+ DCs in counter-regulating the early inflammatory response after HSCT. This is relevant for the severity and mortality of acute GVHD. Our results enhance our current understanding of how GVHD is initiated and may provide the basis for novel concepts for an improved control of GVHD and better feasibility of HSCT in the future.

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