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

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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.

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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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*-deficient (*Batf3*$^{-/-}$) mice are devoid of the CD8α^+ DC subpopulation under steady state conditions [14,18], we transplanted *Batf3*$^{-/-}$ mice with bone marrow and T cells from MHC and miHA mismatched BALB/c to directly address the role of CD8α^+ DCs in the initiation of GVHD, again resulting in a strongly increased GVHD-related mortality. Interestingly, the lack of this DC subset was accompanied with an increased activation of the remaining DC populations post-HSCT, leading to an enhanced priming of allogeneic T cells. Moreover, we found reduced numbers of Treg cells and transforming growth factor (TGF)-β levels, suggesting an aggravated failure of peripheral tolerance mechanisms after HSCT in the absence of CD8α^+ DCs. In summary, our results indicate a role of CD8α^+ DCs as important inducers of Treg cell–mediated tolerance that control DC activation and T cell priming in GVHD.

METHODS

Reagents

Anti-NK1.1 mAb (clone PK136) was purified from hybridoma supernatants according to standard protocols. If required, mAbs were affinity purified using protein G-Sepharose columns (GE Healthcare, Munich, Germany).

Mice

C57BL/6 (B6) and BALB/c were obtained from Charles River Laboratories (Sulzfeld, Germany). *Batf3* deficient (B6.129S(C)-Batf3$^{tm1.1Kmm}$/J; *Batf3*$^{-/-}$) mice were from The Jackson Laboratory (Sulzfeld, Germany). *Batf3*$^{-/-}$ mice were backcrossed for 10 generations with B6 and at least 1 filial generation upon purchase. Filial breedings were continued at our center. All mice were bred in a specific pathogen-free colony in the animal facility of the Johannes Gutenberg-University. CD11c-Cre [19], DTA [20], and inducible diphtheria toxin receptor (iDTR) [21] mice were from A. Waisman (Mainz) and bred as previously described [22]. All animal procedures were performed in accordance with the institutional guidelines and approved by the responsible national authority (National Investigation Office Rheinland-Pfalz, approval ID AZ 23 177-07/G11-1-034).

Bone Marrow Transplantation Model and Histopathology Scoring

Mice were transplanted following a standard protocol as previously described [23]. B6 recipients were natural killer cell depleted with an anti-NK1.1 specific antibody (clone PK136, 500 μg i.p. per mouse on day −2) and received allogeneic T cell–depleted bone marrow cells (10^7 cells per animal) and 5×10^6 CD90.2$^+$ T cells from BALB/c donors by i.v. transfer after total body irradiation (TBI; 11 Gy split into 2 doses of 5.5 Gy on days −2 and −1) from a ^{137}Cs source (OB58-BA, Buchler, Braunschweig, Germany). The animals were maintained under specific pathogen-free conditions and received antibiotics (sulfadoxine-trimethoprim 1 g/mL in drinking water) post-transplantation. For in vivo DC depletion, CD11c-iDTR mice were injected i.p. with 25 ng/g body weight diphtheria toxin (Sigma-Aldrich, Taufkirchen, Germany) on days −2 and 1.

Clinical symptoms of GVHD were monitored daily by assessing weight loss, posture, activity, fur texture, and skin integrity, adding up to a clinical GVHD score as previously described [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 LSRII flow cytometer and FACSDiva (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 30C7), PD-L1 (clone 10F.9G2), PD-L2 (clone 122), and FoxP3 (clone FJK-16S) (all antibodies were purchased from Biolegend, 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

Splenic 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 [12,23] and further enriched by using CD11c specific magnetic microbeads according to the manufacturer's protocol (Miltenyi Biotech, Bergisch Gladbach, Germany). These cells were typically >95% CD11c$^+$ as determined by flow cytometry.

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) and dissected with needles, erythrocytes were lysed, and cells subsequently treated with mitomycin C (60 μg/mL; Sigma) for 30 minutes to inhibit further cell division. Responder T cells from BALB/c splenocytes were purified by anti-CD90.2–conjugated magnetic beads (Miltenyi Biotech). Purified T cells were generally >95% positive for CD90 and CD3 as determined by flow cytometry. Stimulator and responder cells were cultured for 3 days, pulsed with ^{3}H-thymidine (0.5 μCi/mL; Perkin Elmer, Rodgau, Germany), and harvested the following day. ^{3}H-thymidine incorporation was assessed with a 1205 betaplate reader (LKB Wallac, Turku, Finland).

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, Thermo-Scientific, Schwerte, Germany). Quantitative real-time (qRT)-PCRs were performed using the following oligonucleotides: murine IL-10 forward 5′-GAG AGC GCT CAT CTC GAT TT-3′; murine IL-10 reverse 5′-GGG TCT CCC AAG GAA AGG TA-3′; β_2-microglobulin forward 5′-TTT CTG GTG CTT GTC TCA CTG ACC G-3′; β_2-microglobulin reverse 5′-GCA GTT CAG TAT GTT CGG CTT CCC A-3′; murine IL-12p35 forward 5′-GTC AAT CAC GCT ACC TCC TC-3′; murine IL-12p35 reverse 5′-CTG CAC AGC TCA TCG ATG GC-3′; murine Ifng forward 5‘-GAT GCA TTC ACC AGG T-3‘; murine IFN-γ reverse 5′-GTG GAC CAC TGA GCT C-3′. qRT-PCR analyses were performed in triplicates on an iCycler (Bio-Rad, Munich, Germany) using the SYBR GreenER qPCR Supermix (Invitrogen). After normalization of the data according to the expression of β_2-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 CO_2 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-mIL-2 (JES6-1A12) and biotinylated anti-mIL-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, $P < .05$ was considered significant. All