Conditional ablation of CD205$^+$ conventional dendritic cells impacts the regulation of T-cell immunity and homeostasis in vivo

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Dendritic cells (DCs) are composed of multiple subsets that play a dual role in inducing immunity and tolerance. However, it is unclear how CD205$^+$ conventional DCs (cDCs) control immune responses in vivo. Here we generated knock-in mice with the selective conditional ablation of CD205$^+$ cDCs. CD205$^+$ cDCs contributed to antigen-specific priming of CD4$^+$ T cells under steady-state conditions, whereas they were dispensable for antigen-specific CD4$^+$ T-cell responses under inflammatory conditions. In contrast, CD205$^+$ cDCs were required for antigen-specific priming of CD8$^+$ T cells to generate cytotoxic T lymphocytes (CTLs) mediated through cross-presentation. Although CD205$^+$ cDCs were involved in the thymic generation of CD4$^+$ regulatory T cells (Tregs), they maintained the homeostasis of CD4$^+$ Tregs and CD4$^+$ effector T cells in peripheral and mucosal tissues. On the other hand, CD205$^+$ cDCs were involved in the inflammation triggered by Toll-like receptor ligand as well as bacterial and viral infections. Upon microbial infections, CD205$^+$ cDCs contributed to the cross-priming of CD8$^+$ T cells for generating antimicrobial CTLs to efficiently eliminate pathogens, whereas they suppressed antimicrobial CD4$^+$ T-cell responses. Thus, these findings reveal a critical role for CD205$^+$ cDCs in the regulation of T-cell immunity and homeostasis in vivo.

knock-in mouse | innate immunity | adaptive immunity

Dendritic cells (DCs) are essential antigen-presenting cells (APCs) that consist of heterogeneous subsets, mainly classified as conventional DCs (cDCs) and plasmacytoid DCs (pDCs) (1). DCs serve as sentinels, recognizing the presence of invading pathogens or virus-infected cells through various pattern-recognition receptors, including Toll-like receptors (TLRs) (1). DCs process such exogenous antigens intracellularly and present them to CD4$^+$ T cells via MHC class II (MHC II) for induction of CD4$^+$ effector T (Teff) cells (1–3). DCs also show an unusual specialization in their MHC class I (MHC I) presentation pathway to prime CD8$^+$ T cells. Although most cells use MHC I molecules to present peptides derived from endogenously synthesized proteins, DCs have the capacity to deliver exogenous antigens to the MHC I pathway, a phenomenon known as cross-presentation, that underlies the generation of cytotoxic T lymphocyte (CTL) immunity (1–3). DCs thereby play a critical role in the link between innate and adaptive immunity. Conversely, DCs are also crucial for the induction of immunological tolerance under steady-state conditions, and the mechanisms involved include recessive tolerance mediated by deletion and anergy, and dominant tolerance by maintaining the homeostasis of self-reactive CD4$^+$Foxp3$^+$ naturally occurring regulatory T cells (nTregs) and de novo generation of antigen-specific CD4$^+$Foxp3$^+$ inducible Tregs (iTregs) (4–7).

Mouse cDCs in lymphoid organs are comprised of two major subsets, classified as CD8α$^+$ cDCs and CD8α$^-$ cDCs. CD8α$^+$ cDCs mainly reside in the T-cell zone, and CD8α$^-$ cDCs reside in the red pulp and marginal zone (2, 8). Series of in vitro and ex vivo studies reported that CD8α$^+$ cDCs compared with CD8α$^-$ cDCs strongly generate T-helper cell type 1 (Th1) cells because of the potential high-level production of IL-12 (9). In addition, CD8α$^+$ cDCs are more efficient in the phagocytic uptake of dead cells and in the cross-presentation of cell-bound or soluble antigens on MHC I to generate CTLs than other DC subsets (9).

CD205, an endocytic type I C-type lectin-like molecule that belongs to the mannose receptor family, is mainly expressed on CD8α$^+$ cDCs and cortical thymic epithelium, as well as interdigitating DCs in cutaneous lymph nodes (LNs) derived from dermal DCs and epidermal Langerhans cells (LCs), usually at a higher level than seen on macrophages and B cells (10–13). CD205 may function as an endocytic receptor involved in the uptake of extracellular antigens. Although an endogenous ligand for CD205 has not been identified, an antigen-conjugated mAb specific for CD205 was internalized, processed in the endosomal compartment, and presented to both MHC II and MHC I for cross-presentation with high efficiency (10). Although these observations based on analyses in vitro and ex vivo provide the functions of CD205$^+$ cDCs, their role in the immune system under physiological conditions remains unclear because of the lack of a system that selectively eliminates this cell subset in vivo.

To precisely evaluate the contribution of CD205$^+$ cDCs to the immune system, we engineered knock-in mice that express the diphtheria toxin (DT) receptor (DTR) (12, 14, 15) under the control of the *Cd205* gene, which allows the selective conditional ablation of CD205$^+$ cDCs in vivo. Using these mice, we demonstrated the unique role of CD205$^+$ cDCs in the control of T-cell responses and homeostasis in vivo.

Results

Inducible Ablation of CD205$^+$ cDCs in Mice. To allow the specific elimination of CD205$^+$ cDCs in lymphoid tissues in vivo, we created *Cd205*$^{dtr/dtr}$ mice harboring cDNA encoding the human DTR fused to enhanced GFP (EGFP) and an internal ribosome entry site (12, 15) into the 3′ UTR of the *Cd205* gene (Fig. S1). *Cd205*$^{dtr/dtr}$ mice were born at normal Mendelian frequencies and healthy. We observed that a single injection with DT at 0.5∼1 μg per mouse (about 25∼50 ng/g body weight) resulted in the death of *Cd205*$^{dtr/dtr}$ mice in 10 d. To exclude the possible role of CD205$^+$ cortical thymic epithelium (10) and radioresistant CD8α$^-$CD205$^+$ LCs (12), we generated bone marrow (BM) chimeric mice by reconstitution with BM from *Cd205*$^{dtr/dtr}$ mice into lethally irradiated recipient WT mice and used them for all of our experiments.

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The authors declare no conflict of interest.

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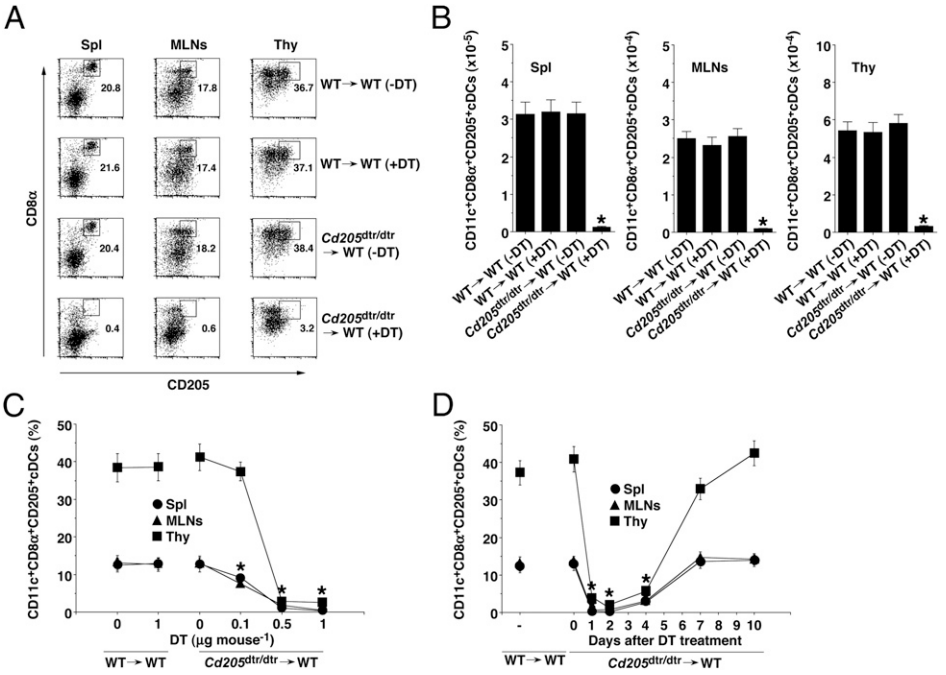

Fig. 1. Conditional ablation of CD205$^+$ cDCs in *Cd205*$^{dtr/dtr}$ mice. (*A* and *B*) WT→WT chimeras (n = 6) and *Cd205*$^{dtr/dtr}$→WT chimeras (n = 6) were injected with DT (0.5 μg/mouse), and Spl, MLNs, and thymus (Thy) were obtained 2 d after the injection. (*A*) The frequency of CD11c$^+$ CD8α$^+$CD205$^+$ cDCs was analyzed by flow cytometry. Data are represented by a dot plot, and numbers represent the proportion of CD8α$^+$CD205$^+$ cDCs among CD11c$^+$ cells in each quadrant. (*B*) The absolute number of CD11c$^+$CD8α$^+$CD205$^+$ cDCs was analyzed by flow cytometry. (*C*) WT→WT chimeras (n = 6) and *Cd205*$^{dtr/dtr}$→WT chimeras (n = 6) were injected with various doses of DT, and Spl, MLNs, and Thy were obtained 2 d after the injection. The frequency of CD11c$^+$CD8α$^+$CD205$^+$ cDCs was analyzed by flow cytometry. Data are the percentage of positive cells among CD11c$^+$ cells. (*D*) WT→WT chimeras (n = 6) and *Cd205*$^{dtr/dtr}$→WT chimeras (n = 6) were injected with DT (0.5 μg/mouse), and Spl, MLNs, and Thy were obtained at the indicated days after the injection. The frequency of CD11c$^+$CD8α$^+$CD205$^+$ cDCs was analyzed by flow cytometry. Data are the percentage of positive cells among CD11c$^+$ cells. *P < 0.01 compared with WT→WT chimeras. Data are the mean ± SD, and the results are representative of six independent experiments.

To validate the *Cd205*$^{dtr/dtr}$→WT chimeras in terms of DT-induced elimination of CD205$^+$ cDCs, *Cd205*$^{dtr/dtr}$→WT chimeras received a single injection of DT, and we monitored 2 d later the subsequent frequency among CD11c$^+$ DCs and the absolute number of CD205$^+$ cDCs. Treatment with DT at 0.5 μg per mouse almost completely ablated CD8α$^+$CD205$^+$ cDCs and CD8α$^-$CD205$^+$ cDCs among CD11c$^+$ DCs in various lymphoid tissues in *Cd205*$^{dtr/dtr}$→WT chimeras, whereas this treatment had no effect on their frequency and absolute number in WT→WT chimeras (Fig. 1 *A* and *B* and Fig. S2). The DT-mediated ablation of CD205$^+$ cDCs occurred in a dose-dependent manner (Fig. 1*C*), and near-complete elimination persisted for 2 d, after which

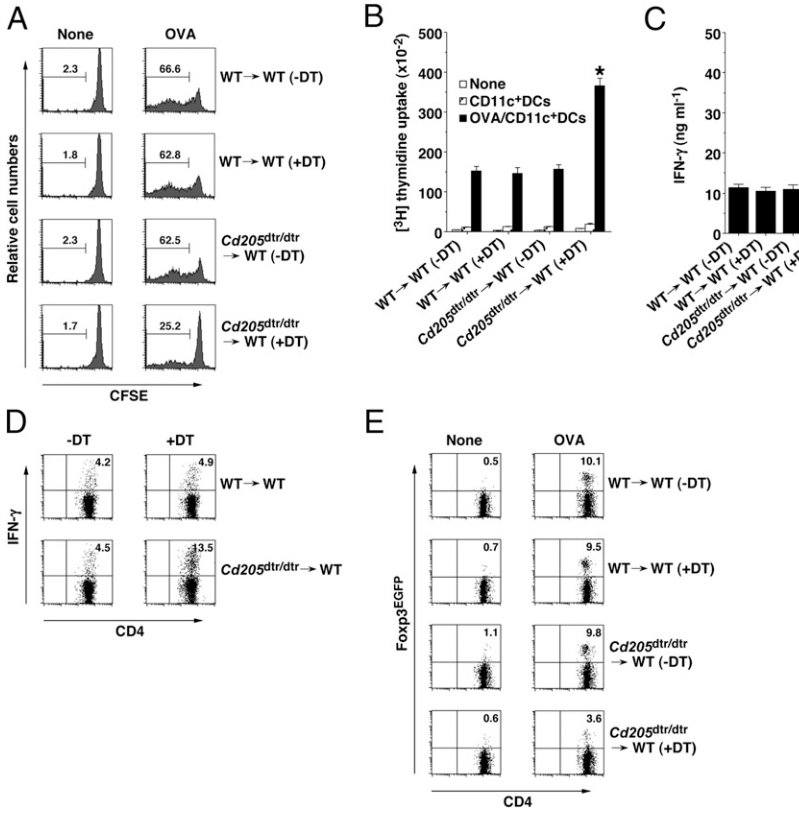

Fig. 2. Ablation of CD205$^+$ cDCs influences antigen-specific CD4$^+$ T-cell responses in vivo. (*A*) CD45.1$^+$OT-II CD4$^+$ T cells were transferred into WT→WT chimeras (n = 6) and *Cd205*$^{dtr/dtr}$→WT chimeras (n = 6) that had been treated with DT (0.5 μg/mouse), and then the mice were immunized with or without OVA protein. Antigen-specific division of CD45.1$^+$OT-II CD4$^+$ T cells was analyzed at 3 d after the immunization by flow cytometry. Data are represented by a histogram, and numbers represent the proportion of CFSE dilution among gated CD45.1$^+$OT-II CD4$^+$ T cells in each quadrant. (*B–D*) WT→WT chimeras (n = 6) and *Cd205*$^{dtr/dtr}$→WT chimeras (n = 6) that had been treated with DT (0.5 μg/mouse) were immunized with CFA plus OVA protein. At 14 d after the immunization, Spl CD4$^+$ T cells were cultured with WT CD11c$^+$ DCs in the presence or absence of OVA protein for the measurements of proliferative response by [^{3}H]thymidine incorporation (*B*) and production of IFN-γ by ELISA (*C*). *P < 0.01 compared with WT→WT chimeras. Data are the mean ± SD. (*D*) Intracellular expression of IFN-γ in the cultured CD4$^+$ T cells was analyzed by flow cytometry. Data are represented by a dot plot, and numbers represent the proportion of IFN-γ$^+$ cells among gated CD4$^+$ T cells in each quadrant. (*E*) CD45.1$^+$OT-II CD4$^+$*Foxp3*$^{EGFP-}$ T cells were transferred into WT→WT chimeras (n = 6) and *Cd205*$^{dtr/dtr}$→WT chimeras (n = 6) that had been treated with DT (0.5 μg/mouse), and then the mice were immunized with or without OVA protein. Expression of *Foxp3*^{EGFP} among gated CD45.1$^+$OT-II CD4$^+$ T cells in Spl was analyzed at 8 d after the immunization by flow cytometry. Data are represented by a dot plot, and numbers represent the proportion of *Foxp3*$^{EGFP+}$ cells among gated CD45.1 OT-II CD4$^+$ T cells in each quadrant. The results are representative of three independent experiments.

their proportions were gradually restored by 7 d (Fig. 1*D*). On the other hand, *Cd205*$^{dtr/dtr}$→WT chimeras exhibited increased proportions of CD11c^{+}CD8α^{-} cDCs and CD11c^{+}BST2^{+} pDCs in spleen (Spl) (Figs. S3*A* and S4), but not in mesenteric LNs (MLNs) (Fig. S5), until 4 d after the injection with DT, but their cell numbers were returned to the basal levels by day 7 (Fig. S3*B*).

We also addressed whether other leukocyte populations are affected by the DT treatment in *Cd205*$^{dtr/dtr}$→WT chimeras. There was no change in the frequency of CD4^{+} T cells, CD8^{+} T cells CD3^{+}CD49b^{+} NKT cells, CD3^{-}CD49b^{+} NK cells, and CD11b^{+} CD11c^{-} macrophages in Spl (Fig. S4) and MLNs (Fig. S5) or in the content of thymocytes (Fig. S6) in DT-treated *Cd205*$^{dtr/dtr}$→WT chimeras. In addition, analysis of T-cell receptor (TCR) Vβ use revealed that there were no major differences in the formation of the TCR repertoire of thymic and peripheral CD4^{+} T cells and CD8^{+} T cells between WT→WT chimeras and DT-treated *Cd205*$^{dtr/dtr}$→WT chimeras (Fig. S7). However, DT-induced ablation of CD205^{+} cDCs led to a slight reduction in the frequency of splenic B220^{+} B cells (Fig. S4), although not MLN B220^{+} B cells (Fig. S5). We also observed that DT had no effect on the viability of splenic B220^{+} B cells in vitro, indicating that this reduction might not be because of the direct cytotoxicity of DT.

Taken together, *Cd205*$^{dtr/dtr}$→WT chimeras provide a unique means of specifically probing the impact of CD205^{+} cDCs on innate and adaptive immune responses.

Ablation of CD205^{+} cDCs Has Distinct Effects on Antigen-Specific CD4^{+} T-Cell Responses in Vivo. To address the role of CD205^{+} cDCs in the antigen-specific priming of CD4^{+} T cells in vivo, we adoptively transferred carboxyfluorescein diacetate-succinimidyl ester (CFSE)-labeled ovalbumin (OVA)-specific TCR transgenic OT-II CD4^{+} T cells (15) into chimeric mice, and analyzed their division in Spl 3 d after the systemic injection of soluble OVA protein under steady-state conditions (Fig. 2*A*). Although the immunization of WT→WT chimeras with OVA protein led to a potent antigen-specific division of OT-II CD4^{+} T cells, DT-treated *Cd205*$^{dtr/dtr}$→WT chimeras showed imapired antigen-specific responses.

To clarify the influence of the ablation of CD205^{+} cDCs on antigen-specific CD4^{+} T-cell responses in vivo under inflammatory conditions, we examined the antigen-specific recalled response of splenic CD4^{+} T cells from chimeric mice 14 d after subcutaneous immunization with OVA protein emulsified in complete Freund's adjuvant (CFA). CD4^{+} T cells from DT-treated *Cd205*$^{dtr/dtr}$→WT chimeras showed a more vigorous proliferation on restimulation with OVA protein than did CD4^{+} T cells from WT→WT chimeras (Fig. 2*B*). Furthermore, CD4^{+} T cells from DT-treated *Cd205*$^{dtr/dtr}$→WT chimeras markedly enhanced the production of IFN-γ as well as the frequency of IFN-γ–producing cells (Th1 cells) compared with the cells from WT→WT chimeras (Fig. 2 *C* and *D*, and Fig. S8 *A* and *B*).

We also investigated the role of CD205^{+} cDCs in the antigen-specific differentiation of CD4^{+}Foxp3^{-} T cells into CD4^{+}Foxp3^{+} iTregs in peripheral tissues. Chimeric mice were adoptively transferred with OT-II CD4^{+}*Foxp3*$^{EGFP-}$ T cells, and we monitored the generation of OT-II CD4^{+}*Foxp3*$^{EGFP+}$ iTregs in Spl 8 d after systemic immunization with OVA protein (Fig. 2*E*). An injection with OVA protein efficiently generated OT-II CD4^{+}*Foxp3*$^{EGFP+}$ iTregs in WT→WT chimeras under steady-state conditions. However, such antigen-specific peripheral generation of OT-II CD4^{+}*Foxp3*$^{EGFP+}$ iTregs was severely diminished in DT-treated *Cd205*$^{dtr/dtr}$→WT chimeras.

Ablation of CD205^{+} cDCs Impairs Antigen-Specific CD8^{+} T-Cell Responses in Vivo. To address the impact of the absence of CD205^{+} cDCs on the cross-presentation of soluble antigen for the priming of CD8^{+} T cells in vivo, chimeric mice that had been

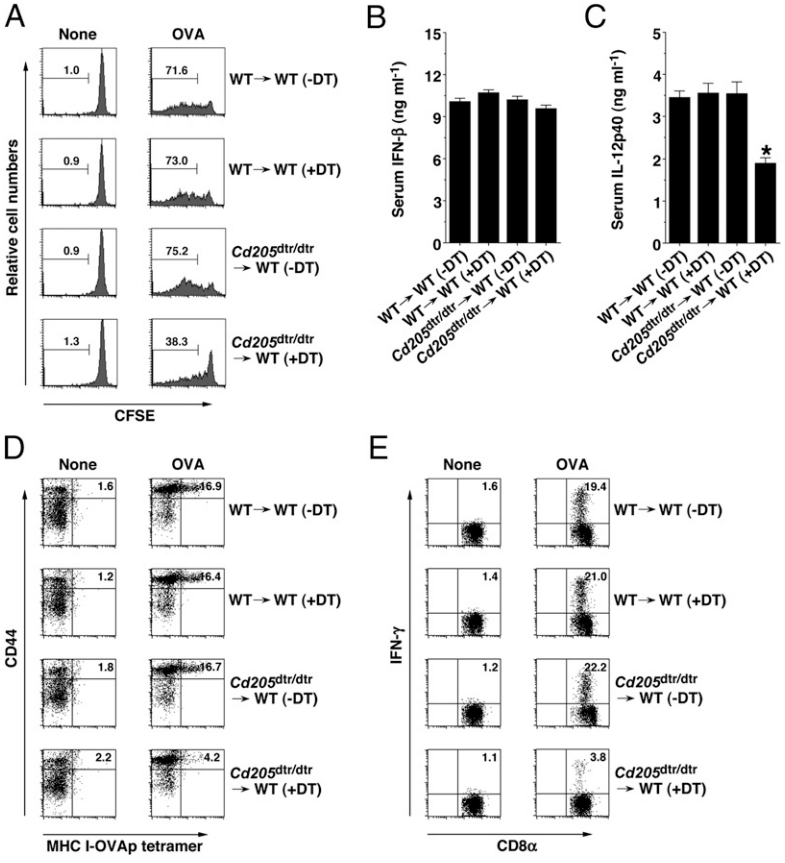

Fig. 3. Ablation of CD205^{+} cDCs reduces antigen-specific CD8^{+} T-cell responses in vivo. (*A*) CD45.1^{+}OT-I CD8^{+} T cells were transferred into WT→WT chimeras (n = 6) and *Cd205*$^{dtr/dtr}$→WT chimeras (n = 6) that had been treated with DT (0.5 μg/mouse), and then the mice were immunized with or without OVA protein. Antigen-specific division of CD45.1^{+}OT-I CD8^{+} T cells was analyzed at 3 d after the immunization by flow cytometry. Data are represented by a histogram, and numbers represent the proportion of CFSE dilution among gated CD45.1^{+}OT-I CD8^{+} T cells in each quadrant. (*B–E*) WT→WT chimeras (n = 6) and *Cd205*$^{dtr/dtr}$→WT chimeras (n = 6) that had been treated with DT (0.5 μg/mouse) were immunized with or without OVA protein in combination with poly (I:C) plus anti-CD40 mAb. (*B* and *C*) Serum levels of IFN-β (*B*) and IL-12p40 (*C*) were measured at 3 h after injection by ELISA. *$P < 0.01$ compared with WT→WT chimeras. Data are the mean ± SD (*D* and *E*) At 6 d after the immunization, splenocytes were analyzed for the generation of MHC I-OVA tetramer^{+}CD44^{high}CD8^{+} T cells (*D*) and for intracellular IFN-γ–producing CD8^{+} T cells (*E*) by flow cytometry. Data are represented by a dot plot, and numbers represent the proportion of MHC I-OVA tetramer^{+}CD44^{high} cells (*D*) and IFN-γ^{+} cells (*E*) among gated CD8^{+} T cells in each quadrant. The results are representative of three independent experiments.

adoptively transferred with CFSE-labeled OVA-specific OT-I $CD8^+$ T cells (15) were immunized with soluble OVA protein, and their antigen-specific division was measured in Spl 3 d after immunization (Fig. 3*A*). DT-treated *Cd205*$^{dtr/dtr}$→WT chimeras showed a marked reduction of antigen-specific division of OT-I $CD8^+$ T cells under steady-state conditions compared with WT→WT chimeras.

To directly prove the influence of the lack of $CD205^+$ cDCs on the generation of CTLs through the cross-presentation of soluble antigen in vivo, chimeric mice were immunized with OVA protein combined with poly (I:C) (a TLR3 ligand) and agonistic anti-CD40 mAb, and we quantified the generation of antigen-specific $CD8^+$ T cells in Spl based on binding with the MHC I-OVA tetramer and OVA-specific intracellular expression of IFN-γ at 6 d later. Immunization of WT→WT chimeras resulted in a marked elevation in serum concentrations of IFN-β and IL-12p40 3 h after the immunization (Fig. 3 *B* and *C* and Fig. S8*C*), and the prominent generation of MHC I-OVA tetramer$^+$ $CD44^{high}CD8^+$ T cells and $CD8^+IFN\text{-}\gamma^+$ T cells (Fig. 3 *D* and *E*). However, DT-treated *Cd205*$^{dtr/dtr}$→WT chimeras showed decreased production of serum IL-12p40 (Fig. 3*C*) and the impaired generation of OVA-specific CTLs (Fig. 3 *D* and *E*).

Ablation of $CD205^+$ cDCs Controls T-Cell Homeostasis in Vivo. To determine the impact of the ablation of $CD205^+$ cDCs on T-cell homeostasis, we measured the frequency and number of $CD4^+$ $Foxp3^+$ nTregs and $CD4^+$ Teff cells in chimeric mice (Fig. S9). DT-treated *Cd205*$^{dtr/dtr}$→WT chimeras exhibited a lower frequency and absolute number of thymic $CD4^+Foxp3^+$ Tregs than WT→WT chimeras, and the population of $CD4^+Foxp3^+$ Tregs in Spl, as well as the lamina propria of the small intestine, was higher in DT-treated *Cd205*$^{dtr/dtr}$→WT chimeras than in WT→WT chimeras. On the other hand, DT-treated *Cd205*$^{dtr/dtr}$→WT chimeras had an increased proportion and absolute number of Th1 cells and IL-17-producing $CD4^+$ T cells (Th17 cells) compared with WT→WT chimeras.

Ablation of $CD205^+$ cDCs Alters Host Defense Against Bacterial Infection in Vivo. $CD11c^+$ DCs have been suggested to control host defenses against intracellular bacterial infections (14, 16, 17). We therefore examined host immune responses to an infection with *Listeria monocytogenes* expressing OVA (LM-OVA) in the absence of $CD205^+$ cDCs. Similar to microbial septic shock (18), a high dose of LM-OVA (1×10^7 CFU/mouse) was lethal to WT→WT chimeras (Fig. 4*A*), and was associated with the marked

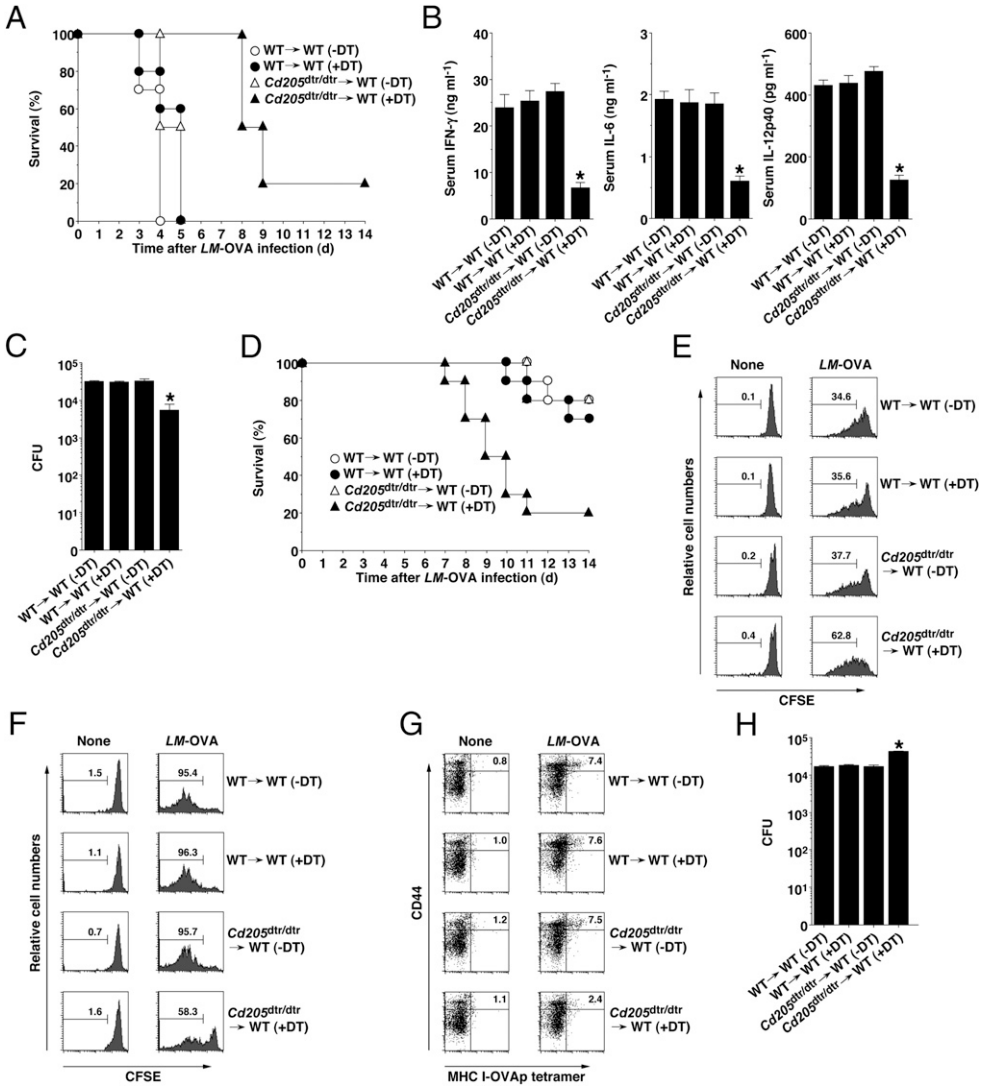

Fig. 4. Ablation of $CD205^+$ cDCs controls host defenses against bacterial infections in vivo. (*A–C*) WT→WT chimeras ($n = 10$) and *Cd205*$^{dtr/dtr}$→WT chimeras ($n = 10$) that had been treated with DT (0.5 μg/mouse) were infected with LM-OVA (1×10^7 CFU/mouse). (*A*) Survival was monitored for 14 d. (*B*) Serum levels of IFN-γ, IL-6, and IL-12p40 were measured at 24 h after infection by ELISA. (*C*) Bacterial burden in the Spl was determined as CFU 2 d after infection. $^*P < 0.01$ compared with WT→WT chimeras. Data are the mean ± SD. (*D*) WT→WT chimeras ($n = 10$) and *Cd205*$^{dtr/dtr}$→WT chimeras ($n = 10$) that had been treated with DT (0.5 μg/mouse) were infected with LM-OVA (5×10^5 CFU/mouse), and survival was monitored for 14 d. (*E* and *F*) $CD45.1^+$OT-II $CD4^+$ T cells (*E*) or $CD45.1^+$OT-I $CD8^+$ T cells (*F*) were transferred into WT→WT chimeras ($n = 6$) and *Cd205*$^{dtr/dtr}$→WT chimeras ($n = 6$) that had been treated with DT (0.5 μg/mouse), and then the mice were uninfected or infected with LM-OVA (5×10^5 CFU/mouse). Antigen-specific division of $CD45.1^+$OT-II $CD4^+$ T cells or $CD45.1^+$OT-I $CD8^+$ T cells was analyzed at 3 d after infection by flow cytometry. Data are represented by a histogram, and numbers represent the proportion of CFSE dilution among gated $CD45.1^+$OT-II $CD4^+$ T cells or $CD45.1^+$OT-I $CD8^+$ T cells in each quadrant. (*G* and *H*) WT→WT chimeras ($n = 10$) and *Cd205*$^{dtr/dtr}$→WT chimeras ($n = 10$) that had been treated with DT (0.5 μg/mouse) were uninfected or infected with LM-OVA (5×10^5 CFU/mouse). (*G*) At 6 d after infection, splenocytes were analyzed for the generation of MHC I-OVA tetramer$^+$ $CD44^{high}CD8^+$ T cells by flow cytometry. Data are represented by a dot plot, and numbers represent the proportion of MHC I-OVA tetramer$^+CD44^{high}$ cells among gated $CD8^+$ T cells in each quadrant. (*H*) Bacterial burden in the Spl was determined as CFU 6 d after infection. $^*P < 0.01$ compared with WT→WT chimeras. Data are the mean ± SD, and the results are representative of three independent experiments.

production of serum proinflammatory cytokines 24 h after infection (Fig. 4*B* and Fig. S8*C*). In contrast, DT-treated *Cd205*$^{dtr/dtr}$→WT chimeras showed resistance for 5 d after infection ($P < 0.01$) (Fig. 4*A*), and significantly less production of proinflammatory cytokines and splenic bacterial burden than WT→WT chimeras (Fig. 4 *B* and *C*). However, their survival rates gradually declined 8 d after infection (Fig. 4*A*). On the other hand, DT-treated *Cd205*$^{dtr/dtr}$→WT chimeras were more susceptible to bacterial infection than WT→WT chimeras 7 d after infection with a low dose of LM-OVA (5×10^5 CFU/mouse) ($P < 0.01$) (Fig. 4*D*).

To clarify the role of CD205$^+$ cDCs in the antibacterial priming of CD4$^+$ T cells or CD8$^+$ T cells in vivo, antigen-specific division of CFSE-labeled OT-II CD4$^+$ T cells or OT-I CD8$^+$ T cells transferred into chimeric mice was measured in Spl 3 d after infection with a low dose of LM-OVA (5×10^5 CFU/mouse). DT-treated *Cd205*$^{dtr/dtr}$→WT chimeras exhibited the enhanced antigen-specific division of OT-II CD4$^+$ T cells following infection with LM-OVA (Fig. 4*E*), but they showed a decreased antigen-specific response of OT-I CD8$^+$ T cells compared with WT→WT chimeras (Fig. 4*F*).

We also examined the influence of the ablation of CD205$^+$ cDCs on the generation of antibacterial CTLs. DT-treated *Cd205*$^{dtr/dtr}$→WT chimeras had a reduced capacity to generate MHC I-OVA tetramer$^+$CD44^{high}CD8$^+$ T cells in Spl compared with WT→WT chimeras 6 d after infection with a low dose of LM-OVA (5×10^5 CFU/mouse) (Fig. 4*G*). In addition, DT-treated *Cd205*$^{dtr/dtr}$→WT chimeras showed a higher bacterial burden in Spl 6 d after infection a low dose of LM-OVA (5×10^5 CFU/mouse) than WT→WT chimeras (Fig. 4*H*).

Ablation of CD205$^+$ cDCs Impairs Host Defense Against Viral Infection in Vivo. The importance of CD11c$^+$CD8α$^+$ cDCs in antiviral immunity has been mainly demonstrated by ex vivo analysis examining the initiation of CD8$^+$ T-cell responses through the cross-presentation of viral antigens (9). To determine the contribution of CD205$^+$ cDCs to the cross-presentation of viral antigens for the activation of CD8$^+$ T cells in vivo, we evaluated the host protective immunity in chimeric mice infected with HSV-1. The infection of WT→WT chimeras with HSV-1 elicited the production of IFN-β and IL-12p40 6 h later (Figs. S8*C* and S10 *A* and *B*), and the efficient generation of MHC I-HSV-1 gB tetramer$^+$CD44^{high}CD8$^+$ T cells and HSV-1 gB-specific CD8$^+$IFN-γ$^+$ T cells in Spl 5 d after infection (Fig. S10 *C* and *D*). In contrast, DT-treated *Cd205*$^{dtr/dtr}$→WT chimeras showed a significant reduction in the production of IL-12p40, but not IFN-β (Fig. S10 *A* and *B*), but they had a marked reduction of anti–HSV-1 CTLs following infection (Fig. S10 *C* and *D*). In addition, a viral burden in Spl was detected in DT-treated *Cd205*$^{dtr/dtr}$→WT chimeras, but not in WT→WT chimeras, 5 d after infection (Fig. S10*E*).

Discussion

In this article, we are unique in reporting the use of a DTR-based knock-in system to generate mice that conditionally lack CD8α$^+$CD205$^+$ cDCs and CD8α$^-$CD205$^+$ cDCs to address the role for these cells in the control of innate and adaptive immunity. It had been shown that mice with mutations to several transcription factor genes, including *Id2* and *Irf8*, lack CD8α$^+$ cDCs but have additional immune defects (9, 19). Furthermore, mice deficient in the transcription factor Batf3 reportedly also constitutively lack CD8α$^+$ cDCs as well as CD103$^+$CD11b$^-$ cDCs, but not other DC subsets, in lymphoid and peripheral tissues (3, 20). Therefore, we demonstrated that the almost complete and specific conditional ablation of CD8α$^+$CD205$^+$ cDCs and CD8α$^-$CD205$^+$ cDCs provides for the study of their function in the regulation of immune responses in vivo. Previous studies have shown some controversies regarding the influence of the constitutive versus conditional elimination of CD11c$^+$ DCs or LCs on immune responses, implying that their chronic ablation allows for the emergence of compensatory mechanisms, which can confound any primary deficits (6, 7, 21, 22). Therefore, our system allowing inducible ablation of CD205$^+$ cDCs could take an advantageous means to analyze their spaciotemporal role for the control of immune responses.

The observations in Batf3-deficient mice showed that the constitutive lack of CD8α$^+$ cDCs had no effect on CD4$^+$ T-cell responses (3). We showed that the antigen-specific division of splenic CD4$^+$ T cells was impaired under the conditional ablation of CD8α$^+$CD205$^+$ cDCs following systemic antigenic stimulation alone. Therefore, CD8α$^+$CD205$^+$ cDCs could contribute to the antigen-specific priming of splenic CD4$^+$ T cells in vivo under steady-state conditions. Conversely, the conditional ablation of CD8α$^+$CD205$^+$ cDCs and CD8α$^-$CD205$^+$ cDCs in the lymphoid tissues and possibly in the skin (12) promoted antigen-specific activation of splenic CD4$^+$ T cells for proliferation and Th1-differentiation after subcutaneous antigenic inflammatory stimulation as well as systemic microbial infection. Furthermore, the conditional ablation of CD205$^+$ cDCs reduced the antigen-specific peripheral generation of CD4$^+$Foxp3$^+$ iTregs. In vitro and ex vivo observations have shown that CD11c$^+$CD8α$^+$ cDCs had the potential to produce CD4$^+$Foxp3$^+$ iTregs compared with CD11c$^+$CD8α$^-$ cDCs because of the endogenous production of TGF-β, and the in vivo delivery of antigen to CD11c$^+$CD8α$^+$ CD205$^+$ cDCs caused a more marked generation of CD4$^+$ Foxp3$^+$ iTregs than that to CD11c$^+$CD8α$^-$ cDCs (4, 8). Therefore, CD205$^+$ cDCs could be critical APCs for converting CD4$^+$ Foxp3$^-$ T cells into CD4$^+$Foxp3$^+$ iTregs to control the immune tolerance. On the other hand, CD11c$^+$CD8α$^-$ cDCs are reportedly more effective than CD11c$^+$CD8α$^+$ cDCs in the activation of the antigen-specific CD4$^+$ T cells, possibly because of their superior capacity in producing MHC class II-peptide complexes (2). Taking these data together, the enhanced antigen-specific CD4$^+$ T-cell responses by the conditional ablation of CD205$^+$ cDCs after inflammatory antigenic stimulation could be because of the impaired generation of antigen-specific CD4$^+$ Foxp3$^+$ iTregs as well as the increased proportions of the activated CD11c$^+$CD8α$^-$ cDCs.

Similar to the results in Batf3-deficient mice (3), the conditional ablation of CD8α$^+$CD205$^+$ cDCs led to the impaired generation of splenic CTLs following application with antigen plus maturation stimuli. It had been reported that type I IFN, but not IL-12, is required for the generation of CTLs (23). Taking these data together, we find that CD8α$^+$CD205$^+$ cDCs could play a prerequisite role in the cross-presentation of exogenous antigen for the generation of CTLs regardless of sufficient type I IFN production.

CD11c$^+$ DCs have been implicated in the control of the generation and homeostasis of CD4$^+$Foxp3$^+$ nTregs and CD4$^+$ Teff cells (5, 6, 24). Conditional ablation of CD8α$^+$CD205$^+$ cDCs reduced the production of thymic CD4$^+$Foxp3$^+$ nTregs. Therefore, the self-reactive interaction of CD4$^+$Foxp3$^-$ thymocytes with thymic CD8α$^+$CD205$^+$ cDCs and their costimulation through CD28 and CD80/CD86 (25) could be required for the development of thymic CD4$^+$Foxp3$^+$ nTregs. On the other hand, the conditional ablation of CD8α$^+$CD205$^+$ cDCs and CD8α$^-$CD205$^+$ cDCs increased population of both CD4$^+$Foxp3$^+$ nTregs and CD4$^+$ Teff cells in peripheral and mucosal lymphoid tissues, possibly because of the indirect effect through the enhanced proportions of CD11c$^+$CD8α$^-$ cDCs. Collectively, these findings suggest that CD205$^+$ cDCs are critical for the regulation of the equilibrium of CD4$^+$Foxp3$^+$ nTregs and CD4$^+$ Teff cells to maintain peripheral and mucosal immune homeostasis.

Although CD11c$^+$CD8α$^+$ cDCs might be required for efficient bacterial entry into the Spl (17), the relevance of this process to the subsequent immune responses remains to be understood. The conditional ablation of CD8α$^+$CD205$^+$ cDCs diminished the splenic colonization of bacteria, and that led to the limitation of lethal inflammation after infection with a high dose of bacteria. Therefore, splenic CD8α$^+$CD205$^+$ cDCs could provide a specific nitch for the bacterial spreading and replication in the Spl that initiate undesirable lethal inflammatory responses during the early phase of an extensive bacterial infection. Conversely, the

conditional ablation of CD8α$^+$CD205$^+$ cDCs caused a decrease in the survival of the infected mice that accompanied the abortive generation of antibacterial CTLs and the enhanced splenic bacterial burden after infection with a low dose of bacteria. Taken together, our findings suggest that splenic CD8α$^+$CD205$^+$ cDCs are involved in the initiation of microbial septic shock after the massive bacterial infection, although they are required for the protection against the bacterial organ failure mediated by the cross-priming of CD8$^+$ T cells for generating antibacterial CTLs to efficiently eliminate pathogenic microbes under the lack of lethal bacterial inflammation.

Published reports suggest that dermal CD11c$^+$CD103$^+$ cDCs are the dominant migratory APCs cross-presenting viral antigens to CD8$^+$ T cells after an infection in the skin or lungs (3, 20, 26, 27). Our results show that the conditional ablation of splenic CD8α$^+$CD205$^+$ cDCs abrogated the generation of antiviral CTLs after systemic viral infection independent of type I IFN production. These findings led us to hypothesize that CD8α$^+$CD205$^+$ cDCs located in T-cell areas of Spl predominantly phagocytose virus-infected cells to cross-present viral antigens for the optimal generation of antiviral CTL-responses to eliminate the virus during a systemic infection.

Collectively, our findings demonstrate that CD205$^+$ cDCs play a critical role in controlling T-cell immunity and homeostasis as well as inflammation in vivo. A series of studies have suggested that human blood BDCA3$^+$ DCs are homologous to mouse CD8α$^+$CD205$^+$ cDCs in terms of their specific gene signature and functionally equivalent for cross-presentation (28). Therefore, further elucidation of CD205$^+$ cDCs might provide insights into immune regulation and pathology, and aid therapeutic interventions for infectious diseases as well as autoimmune and inflammatory disorders.

Materials and Methods

Mice. The following mice were used in this study: C57BL/6 mice (Charles River Laboratories); B6.OT-I TCR transgenic mice harboring OVA-specific CD8$^+$ T cells (15); and B6.OT-II TCR transgenic mice harboring OVA-specific CD4$^+$ T cells (15). B6.CD45.1$^+$ OT-I mice and B6.CD45.1$^+$ OT-II mice were bred in-house by crossing B6.OT-I mice and B6.OT-II mice with CD45.1$^+$ B6 mice (15). *Foxp3*^{EGFP}CD45.1$^+$ OT-II mice were also generated by crossing B6.CD45.1$^+$ OT-II mice with B6.*Foxp3*^{EGFP} mice (15, 29). All mice were bred and maintained in specific pathogen-free conditions in the animal facility at RIKEN Research Center for Allergy and Immunology in accordance with institutional guidelines.

Immunization. Details of the immunization are described in *SI Materials and Methods*.

Bacteria, Virus, and Infection. Details of the bacteria, virus, and infection are described in *SI Materials and Methods*.

Statistical Analysis. Data are expressed as the mean ± SD. The statistical significance of the values obtained was evaluated by ANOVA and the Kaplan–Meier log-rank test. A P value of <0.01 was considered significant.

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