

Letter to the Editor

Compensatory role of Langerhans cells and langerin-positive dermal dendritic cells in the sensitization phase of murine contact hypersensitivity

To the Editor:

In the initiation of skin immune responses, cutaneous dendritic cells (DCs) play a central role by presenting antigen to naive T cells and supporting T-cell expansion and polarization. Formerly, it was generally believed that 2 types of DCs existed in the skin, epidermal langerin-positive (CD207⁺) Langerhans cells (LCs) and dermal DCs (dDCs). Recently, it has been revealed that dDCs are divided into 2 populations according to their expression of langerin: langerin-positive dDCs and langerin-negative dDCs.¹⁻³ LCs have long been regarded as essential cells for the establishment of sensitization in patients with contact hypersensitivity (CHS), but this concept is now being challenged by recent analyses using LC ablation murine models.³⁻⁵ In this study we have revealed that LCs and langerin-positive dDCs play a compensatory role in the sensitization phase of murine CHS.

At present, there are 2 types of LC ablation murine models. The first uses mice that express enhanced green fluorescent protein (eGFP) fused with a diphtheria toxin (DT) receptor (DTR) under the control of the langerin promoter, which are known as langerin-eGFP-DTR mice,^{3,4} and langerin-DTR mice.⁶⁻⁸ In these knock-in mice, both LCs and langerin-positive dDCs are detected based on their eGFP expression, and both are completely depleted by administration of DT. Once depleted, LCs do not repopulate for at least 4 weeks, whereas the number of langerin-positive dDCs in the skin recovers to the basal level about 14 days after depletion by DT.³⁻⁵ Using these mice, some studies have reported that depletion of LCs alone in the sensitization phase leads to an insufficient CHS response, suggesting the importance of LCs in sensitization, especially to low doses of hapten.^{6,7} Other studies have reported that depletion of LCs alone does not impair CHS; rather, only depletion of both LCs and langerin-positive dDCs attenuates CHS, suggesting that LCs are not necessarily essential

for sensitization and that langerin-positive dDCs are rather important mediators for sensitization in CHS response.^{3,4} The second LC ablation model uses transgenic langerin-DTA mice, which constitutively lack LCs.⁵ These LC-deficient mice exhibit enhanced CHS responses, suggesting that LCs play a regulatory role rather than a stimulating role. Therefore the role of LCs in sensitization is still controversial and has not yet been fully elucidated. Furthermore, the necessity of langerin-positive dDCs in the sensitization phase of CHS remains unknown.

In this study we sought to characterize the respective roles of LCs and langerin-positive dDCs in the sensitization phase of CHS. To this end, we have established an experimental system in which we sensitized mice under 3 different depletion conditions: in the absence of both LCs and langerin-positive dDCs, in the absence of LCs alone, and in the absence of langerin-positive dDCs alone. First, we generated mice lacking only langerin-positive dDCs using a bone marrow transplantation (BMT) system. LCs are radioresistant, and LCs of host origin persist in the epidermis of the recipient for at least 1 year after BMT.⁹ In addition, although a population of radioresistant dDCs has been reported,¹⁰ langerin-positive dDCs are radiosensitive and are reconstituted with donor-derived cells after BMT.² Accordingly, we irradiated 6-week-old congenic CD45.1⁺ CD45.2⁺ C57BL/6 mice with 9 Gy and transferred 2×10^6 bone marrow cells from CD45.1⁻ CD45.2⁺ langerin-eGFP-DTR mice⁴ into the irradiated mice. After 2 months, more than 95% of the blood cells in the recipient mice had been reconstituted with CD45.2 single-positive cells.

To confirm reconstitution, the epidermis and dermis of bone marrow chimeric (BMC) mice and langerin-eGFP-DTR mice were separated through treatment with trypsin/EDTA (Sigma-Aldrich, St Louis, Mo), and single-cell suspensions of epidermis and dermis were prepared through treatment with collagenase type 2 (Worthington, Lakewood, NJ) for 1 hour. These single-cell suspensions were stained with fluorescent-labeled antibodies (all from eBioscience, San Diego, Calif) and analyzed by using the FACSCanto II flow cytometric system (BD Bioscience, San

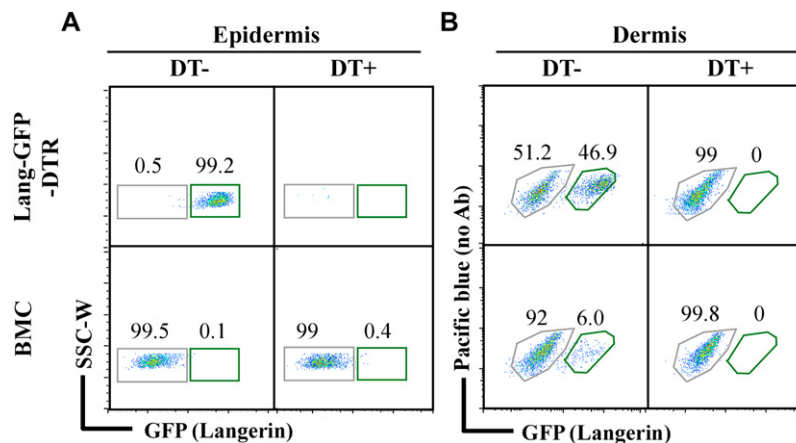


FIG 1. Effect of DT on GFP⁺ cells. The CD11c⁺ MHC class II⁺ cells of the epidermis and dermis from either langerin-eGFP-DTR (*Lang-GFP-DTR*) mice (*upper panels*) or BMC mice (*lower panels*) were analyzed by means of flow cytometry for the expression of GFP. The frequency of GFP⁺ and GFP⁻ cells in the epidermis (**A**) and dermis (**B**) before (DT-; *left panels*) and after (DT+; *right panels*) are shown.

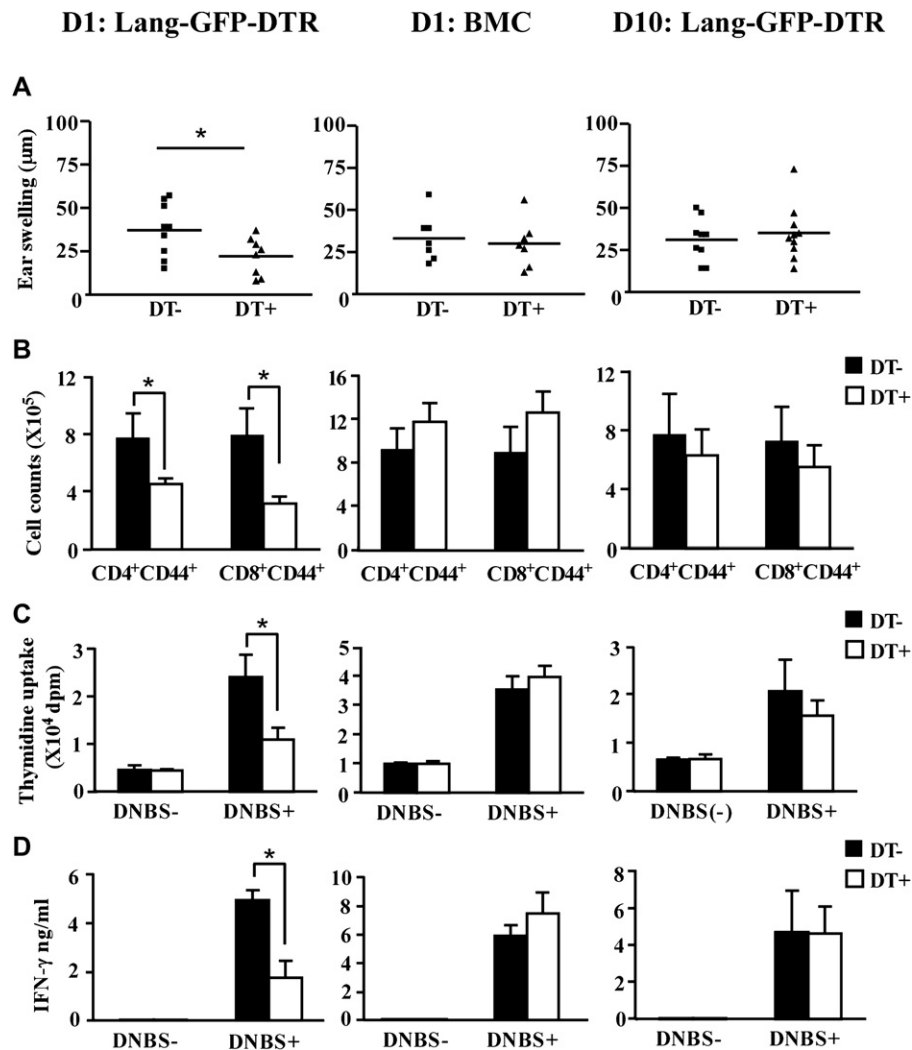


FIG 2. Role of LCs and langerin-positive dDCs in the sensitization phase of CHS. **A**, CHS response. **B**, The number of CD44⁺ T cells in draining lymph nodes 6 days after sensitization. **C**, DNBS-induced cell proliferation. LN cells (1×10^6) were cultured with or without DNBS stimulation in a 96-well plate for 72 hours; tritiated thymidine was added for the last 12 hours. **D**, IFN- γ level. The levels of IFN- γ in the culture medium 72 hours after incubation were measured with an ELISA kit. Data are presented as means $\pm$ SDs and are representative of 3 experiments with similar results. * $P < .05$.

Diego, Calif) and FlowJo software (Tree Star, Ashland, Ore; Fig 1). As reported previously, in langerin-eGFP-DTR mice almost all MHC class II⁺ CD11c⁺ DCs in the epidermis were langerin positive (LCs; Fig 1, A, upper left), and about half of MHC class II⁺ CD11c⁺ DCs in the dermis were langerin positive (migrating LCs and langerin-positive dDCs; Fig 1, B, upper left). Both langerin-positive subsets in the epidermis and dermis were depleted by intraperitoneal administration of 1 μg of DT (Sigma-Aldrich) into each mouse (Fig 1, A and B, upper right panels). All MHC class II⁺ CD11c⁺ LCs in the epidermis of BMC mice were eGFP negative (Fig 1, A, lower left), suggesting that LCs were not reconstituted by the donor cells. Furthermore, this LC subset was not depleted by treatment with DT (Fig 1, A, lower panels). On the other hand, an eGFP-positive subset was detected in MHC class II⁺ CD11c⁺ DCs in the dermis (langerin-positive dDCs; Fig 1, B, lower left); this subset completely disappeared 1 day after administration of DT (Fig 1, B, lower right). To further confirm the reconstitution of langerin-positive dDCs, we

evaluated the expression of eGFP in dermal MHC class II⁺ CD11c⁺ EpCAM⁺ CD103⁺ langerin-positive DCs and confirmed that more than 95% of them were eGFP positive (see Fig E1 in this article's Online Repository at www.jacionline.org), which suggests that langerin-positive dDCs were reconstituted quite well with langerin-eGFP-DTR bone marrow cells. In addition, this subset was almost completely depleted by administration of DT (see Fig E2 in this article's Online Repository at www.jacionline.org). These results indicate that only langerin-positive dDCs are reconstituted as a result of irradiation and depleted by DT administration.

Using these BMC mice, we investigated the role of langerin-positive dDCs in the sensitization phase of CHS. We used langerin-eGFP-DTR mice to evaluate the effect of depletion of both LCs and langerin-positive dDCs. Because it has been reported that CHS response was impaired in DT-treated langerin-eGFP-DTR mice sensitized with low-dose dinitrofluorobenzene (DNFB),³ we followed the same protocol: we sensitized

mice with 25 μ L of 0.3% (wt/vol) DNFB (Nacalai Tesque, Kyoto, Japan) in acetone/olive oil (4:1) on shaved abdominal skin and challenged the mice with 20 μ L of 0.15% DNFB in acetone/olive oil (4:1) on ear skin 5 days later. The ear-thickness change was measured at 1 mm medial from the periphery of the ear 24 hours after the challenge with a thickness gauge (Teclock PG-20, Nagan, Japan). As in previous reports,³ the CHS response was reduced in langerin-eGFP-DTR mice that had been treated with DT 1 day before sensitization (Fig 2, A). In addition to measuring ear-swelling response, which is affected not only by the intensity of the immune response but also by nonimmunologic factors, such as vascular leakage and keratinocyte irritability, we also assessed the induction of CD44⁺ memory T cells in draining lymph nodes, as well as antigen-specific T-cell clones and their differentiation into T_H1 cells. The numbers of CD4⁺ CD44⁺ and CD8⁺ CD44⁺ memory T cells in draining lymph nodes 6 days after sensitization were significantly reduced in DT-treated langerin-eGFP-DTR mice (Fig 2, B, left). *In vitro* antigen-specific cell proliferation and the amount of IFN- γ in the supernatant were also significantly reduced in the DT-treated group (Fig 2, C and D, left panels). As a control, wild-type C57BL/6 mice were also treated with DT, but DT treatment did not affect these parameters (data not shown). These results indicate that simultaneous depletion of LCs and langerin-positive dDCs impairs sensitization with low-dose DNFB.

We next applied this CHS protocol to BMC mice, either untreated or treated with DT 1 day before sensitization, and examined the same parameters. All parameters were comparable between vehicle-treated mice and DT-treated mice (Fig 2, middle panels), indicating that langerin-positive dDCs are not essential in the sensitization phase of CHS.

We also used this CHS protocol to examine the involvement of LCs. We treated langerin-eGFP-DTR mice with DT 10 days before sensitization and examined the same parameters in the same way. All the parameters were equivalent between the vehicle-treated group and the DT-treated group (Fig 2, right panels), indicating that LCs alone are not essential in the sensitization phase of CHS.

It has been reported that treatment with DT results in impairment of the CHS response in langerin-eGFP-DTR mice when the mice are sensitized with low doses of DNFB³ but not when they are sensitized with standard doses of DNFB.⁴ Therefore we followed the standard CHS protocol, namely sensitization with 25 μ L of 0.5% DNFB on the abdominal skin and elicitation with 20 μ L of 0.3% DNFB on the ear skin. We administered vehicle or DT to langerin-eGFP-DTR mice 1 day before sensitization and examined the parameters discussed above. All parameters were comparable between vehicle-treated mice and DT-treated mice (CHS, 122.2 $\pm$ 16.2 μ m [vehicle] vs 114.7 $\pm$ 14.6 μ m [DT], *n* = 6; cell proliferation, 31,564 $\pm$ 2,955 cpm [vehicle] vs 33,661 $\pm$ 3,364 cpm [DT], *n* = 3; and IFN- γ production, 14,210 $\pm$ 3,370 pg/mL [vehicle] vs 18,720 $\pm$ 3,560 pg/mL [DT], *n* = 3), suggesting that neither langerin-positive DCs nor LCs are essential in sensitization when standard DNFB concentrations are used.

Although langerin-positive dDCs are currently receiving attention as mediators of sensitization, our results suggest that antigen presentation by langerin-positive dDCs is not necessarily the main pathway involved in sensitization.

In conclusion, our results suggest that neither langerin-positive dDCs alone nor LCs alone are essential in sensitization; rather, both types play compensatory roles in the sensitization phase of CHS in a low-dose DNFB sensitization protocol, although not in a standard-dose DNFB sensitization protocol. Differences in antigens (eg, oxazolone in Bennett et al⁷ and DNFB in Bursch et al³), antigen concentrations, and depletion timing might explain the phenotypic differences among the LC ablation models.³⁻⁷ Further detailed analysis under different conditions is needed to reveal the functions and significance of LCs and langerin-positive dDCs in CHS.

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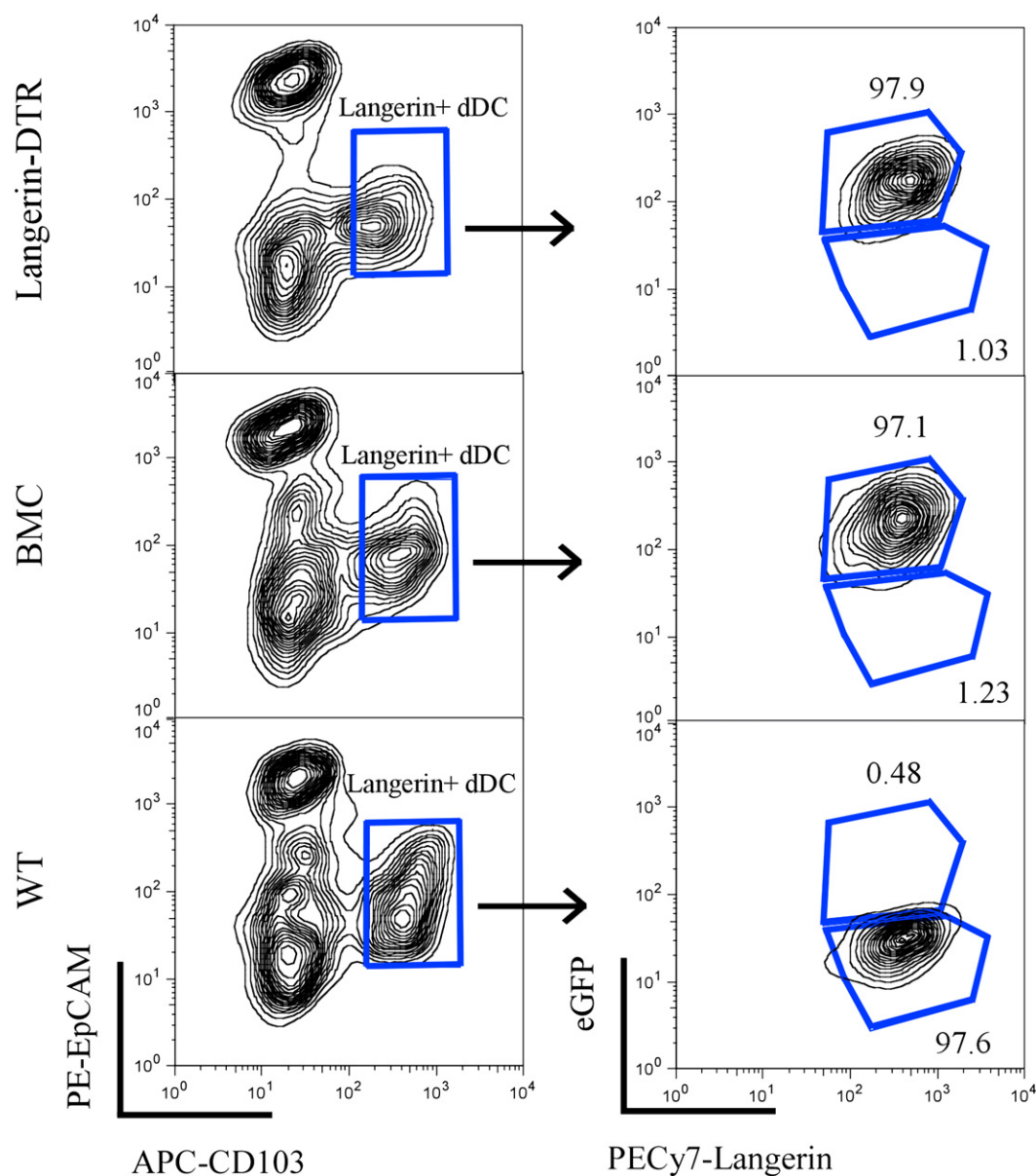


FIG E1. Chimerism of langerin-positive dDCs in BMC mice. The $CD11c^{+}$ MHC class II $^{+}$ EpCAM $^{-}$ CD103 $^{+}$ langerin-positive DCs of the dermis from langerin-eGFP-DTR, BMC, or B6 mice (*left panels*) were analyzed by means of flow cytometry for the expression of GFP. The frequencies (percentages) of GFP $^{+}$ and GFP $^{-}$ cells among langerin-positive dDCs are shown (*right panels*). APC, Allophycocyanin; PE, phycoerythrin; PECy7, phycoerythrin-Cy7; WT, Wild Type.

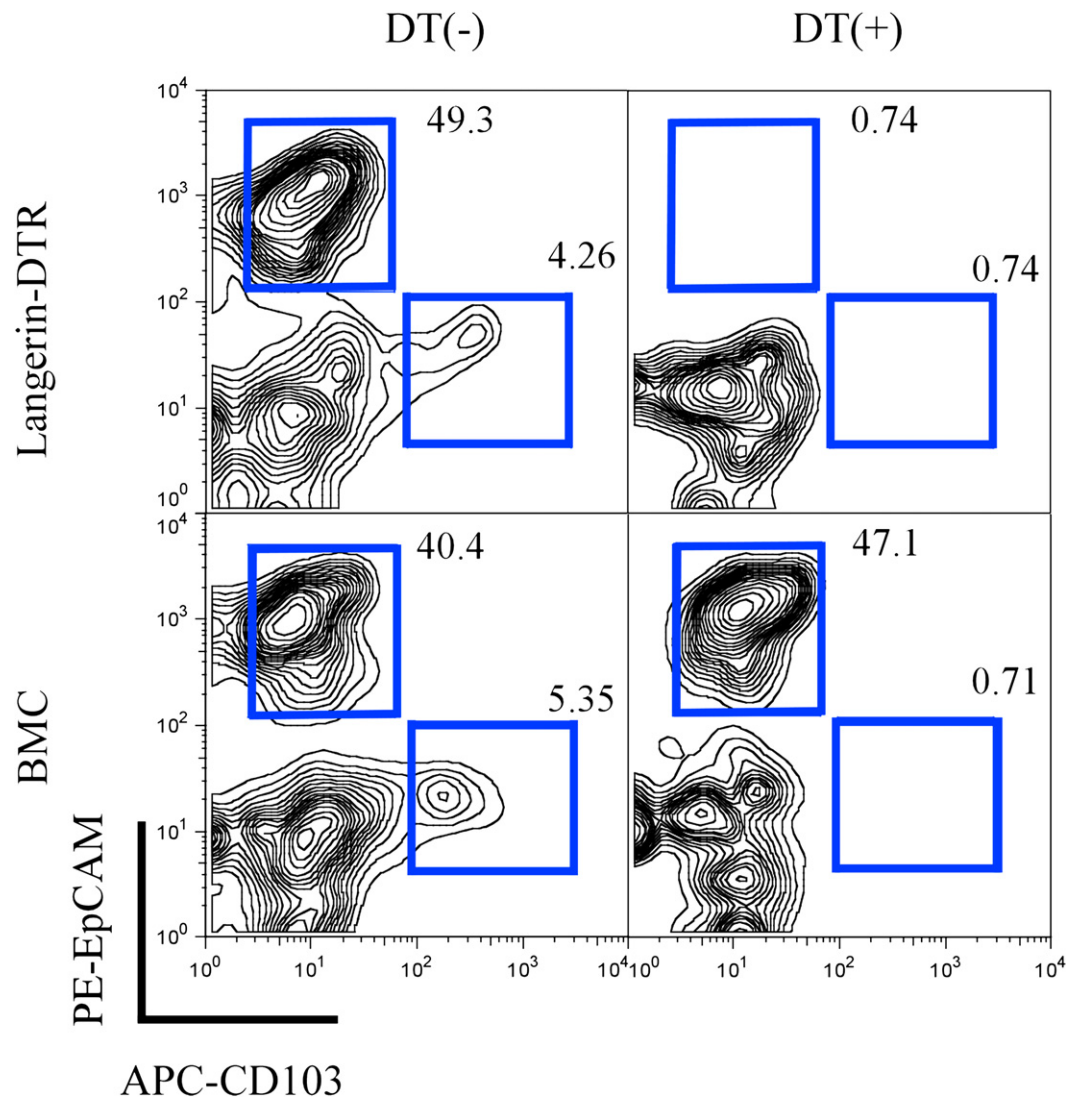


FIG E2. Effect of DT on reconstituted langerin-positive dDCs in BMC mice. Langerin-eGFP-DTR and BMC mice were treated with or without DT, and the CD11c⁺ MHC class II⁺ EpCAM⁻ CD103⁺ and CD11c⁺ MHC class II⁺ EpCAM⁺ CD103⁻ dDC subsets were analyzed by means of flow cytometry. The frequencies (percentages) of EpCAM⁺ CD103⁻ and EpCAM⁻ CD103⁺ cells among CD11c⁺ MHC class II⁺ DCs in the dermis are shown. APC, Allophycocyanin; PE, phycoerythrin.