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J Immunol published online 20 September 2010
http://www.jimmunol.org/content/early/2010/09/20/jimmunol.1001802

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Acute Ablation of Langerhans Cells Enhances Skin Immune Responses

Aleh Bobr, Irlanda Olvera-Gomez, Botond Z. Igyarto, Krystal M. Haley, Kristin A. Hogquist, and Daniel H. Kaplan*

Understanding the function of Langerhans cells (LCs) in vivo has been complicated by conflicting results from LC-deficient mice. Human Langerin-DTA mice constitutively lack LCs and develop exaggerated contact hypersensitivity (CHS) responses. Murine Langerin-diptheria toxin receptor (DTR) mice allow for the inducible elimination of LCs and Langerin+ dermal dendritic cells (dDCs) after administration of diptheria toxin, which results in reduced CHS. When Langerin+ dDCs have partially repopulated the skin but LCs are still absent, CHS returns to normal. Thus, LCs appear to be suppressive in human Langerin-DTA mice and redundant in murine Langerin-DTR mice. To determine whether inducible versus constitutive LC ablation explains these results, we engineered human Langerin-DTR mice in which diptheria toxin ablates LCs without affecting Langerin+ dDCs. The inducible ablation of LCs in human Langerin-DTR mice resulted in increased CHS. Thus, LC-mediated suppression does not require their absence during ontogeny or during the steady-state and is consistent with a model in which LCs actively suppress Ag-specific CHS responses. The Journal of Immunology, 2010, 185: 000–000.

Langerhans cells (LCs) reside in the epidermis of the skin and are considered to be the archetypal tissue-resident dendritic cells (DCs). In the steady-state, they acquire local Ag. Inflammation induces their migration from the skin to T cell areas in the regional lymph node (LN) where they present processed Ag to T cells (1, 2). In the classic model of LC function, LCs were thought to initiate adaptive immune responses. However, this view has been recently challenged based on in vivo studies using LC-deficient mice (3).

Murine Langerin-diptheria toxin receptor mice were generated by two independent groups using “knock-in” technology to introduce the primate receptor for diptheria toxin (DT) into the endogenous murine langerin gene (4, 5). Langerin is expressed by LCs, Langerin+ dermal DC (dDCs), as well as by DC subsets in other tissues (4, 6–10). Injection of DT leads to rapid and efficient ablation of these cells (4–6). At early time points after DT administration, Langerin-expressing DCs are absent and contact hypersensitivity (CHS) reactions are diminished (5, 6). However, Langerin+ dDCs and Langerin+ DTR+ DCs in other organs repopulate much more quickly than do LCs after DT administration. At 7 or 13 d after DT administration, LCs are still absent, but Langerin+ dDCs have partially recovered (6). CHS responses in this setting are normal, suggesting that Langerin+ dDCs are required for CHS responses, but LCs appear to be largely redundant (6, 11).

Human Langerin-DTA mice express the active subunit of diptheria toxin (DTA) under control of the human langerin genomic locus, which leads to a constitutive ablation of epidermal LCs (12). Other Langerin-expressing cells, including Langerin+ dDCs, are unaffected presumably due to variations between the human and mouse langerin promoters (6, 12). Unlike murine Langerin-diptheria toxin receptor (muDTR) mice, human Langerin-DTA (huDTA) mice develop exaggerated CHS responses, which suggests that LCs actually function to suppress the CHS response (12). Similar evidence of LC-mediated suppression was also observed with minor-mismatched skin grafts and T cell responses to tick infestation (13, 14). Examination of the mechanism has revealed that LC-mediated suppression of CHS occurs during the sensitization phase and requires direct cognate interaction between LCs and CD4 cells as well as LC-derived IL-10 (15).

Although muDTR mice 7–13 d after DT injection and huDTA mice both selectively lack LCs, CHS responses are divergent. This is a major inconsistency between the two LC-deficient models. One difference is the constitutive absence of LCs from birth in huDTA but not muDTR mice. LCs migrate to skin-draining LNs in the steady-state and have been proposed to participate in the maintenance of peripheral tolerance (16, 17). The absence of this process in huDTA mice may account for the observed exaggerated CHS responses. Alternatively, Langerin+ dDCs in muDTR mice 7–13 d after DT injection are still reduced in number, which may lessen CHS responses that otherwise would be exaggerated in the absence of LCs.

To distinguish between these possibilities, we have generated human Langerin-DTR (huDTR) mice. These mice allow us to selectively ablate only epidermal LCs just prior to immunization and test whether the acute ablation of LCs affects cutaneous immune responses.

Materials and Methods

Generation of huDTA mice

The primate DTR cDNA was generated by PCR as described (18). Recombination into the 3′-untranslated region (UTR) of Langerin in bacterial
artificial chromosome (BAC) clone RP11-50401 was performed as described (12). Primers used for generation of the recombinase cassette were: 5′ A box, 5′-TTAATTCAAGTCGTTATGCCTTAAGCCTGCTGGAAC-3′; 3′ A box, 5′-AGACGCTTTGGTGTGCGCATATTCATCGTG-3′; 5′ B box, 5′-AATCTCCGACTGATTTATCGTGATTTTCTTTTGTGC-3′; 3′ B box, 5′-TTATAGCGCTCATGTTCTGAGGTGAGA-3′; 5′ I box, 5′-ATGGCCCAACCCATCAAGCCTGCTGCGTCGTCGTCGTCG-3′; 3′ I box, 5′-CGTGCAAAGTCATTGAGGGAATTA-3′. Transgenic injections were performed into pronuclei of C57BL/6 and FVB mice by the University of Minnesota Mouse Genetics Laboratory.

Mice

huDTR mice generated in FVB and C57BL/6 backgrounds were maintained, respectively, on FVB or C57BL/6 wild-type mice obtained from The Jackson Laboratory (Bar Harbor, ME). All experiments were performed on age- (7–12 wk) and sex-matched mice. Mice were housed in microisolator cages and fed irradiated food. The Institutional Animal Care and Use Committee approved all mouse protocols.

Abs

The following Abs were used: murine (mu)Langerin-bio and anti-human (hu)Langerin-AP647 (Dendritics, Lyon, France); CD11c-PE, CD11c-Pacific Blue, CD45-Pacific Blue, MHC class II (MHC-II)-AF700 (BioLegend, San Diego, CA); and CD103-PE (eBioscience, San Diego, CA). Cells were stained for extracellular and intracellular markers as described (12). All flow cytometry was performed using an LSR II (BD Biosciences, San Jose, CA).

Flow cytometry

Single-cell preparations from LN, spleen, thymus, dermis, and epidermis were prepared as previously described (12). In brief, LN, spleen, and thymus were digested with collagenase D (Roche Diagnostics, Indianapolis, IN). Epidermis was digested with 0.3% trypsin for 120 min and removed from the dermis. The dermis was further digested with collagenase XI (4830 U/ml) and hyaluronidase (260 U/ml) (both from Sigma-Aldrich, St. Louis, MO) for 120 min. Data were analyzed with FlowJo software (Tree Star, Ashland, OR).

Immunofluorescence

For immunofluorescence, epidermal sheets were prepared by treating mouse ears with Nair (Chursh and Dwight, Princeton, NJ) for 3 min followed by affixing them to slides (epidermis side down) with double-sided adhesive (3M, St. Paul, MN). Slides were incubated in 10 mM EDTA in PBS for 1 hr at 37°C followed by physical removal of the dermis. Tissue was fixed and stained as previously described (19).

Contact hypersensitivity

Allergic contact dermatitis was induced in mice as previously described (12). Mice were sensitized with 0.5% 2,4-dinitro-1-fluorobenzene (DNFB; Sigma-Aldrich) for 120 min. Data were analyzed with FlowJo software (Tree Star, Ashland, OR).

Epicutaneous immunization

CD44low (naive phenotype) CD8+Thy1.1+ T cells were purified from OT-I mice by the University of Minnesota Mouse Genetics Laboratory.

Results

Generation and validation of huDTR mice

Using an approach similar to the one used to develop Langerin-DTA mice (12), human BAC clone RP11-50401 was modified by homologous recombination in Escherichia coli. A cassette encoding an IRES sequence followed by cDNA for the primate DTR was introduced into the 3′-UTR of the langerin gene (Fig. 1A). Successful recombination was confirmed by PCR and restriction digest (A. Bobr, unpublished observations). A 73-kb NotI linear fragment containing 26 kb upstream of the langerin gene was used to generate four transgenic founders. The founders were phenotypically similar, and data from a single founder are presented below.

To assess transgene expression in the epidermis, a single-cell suspension of epidermal cells was prepared and stained for MHC-II, CD45, and huLangerin. Because the insertion of DTR did not disrupt the langerin gene, the expression of huLangerin as detected with a species-specific Ab was used to identify cells expressing the transgene. All LCs (CD45+, MHC-II+) from huDTR mice demonstrated clear expression of transgenic huLangerin compared with transgene-negative littermate controls (Fig. 1B, 1C). Expression of huLangerin by CD45+, MHC-II+ dendritic epidermal T cells (DETCs) was not observed (unpublished observation). Administration of 1 μg of DT to huDTR mice efficiently eliminated virtually all LCs but did not alter numbers of DETCs (Fig. 1D, 1E). Immunofluorescent imaging of whole-mounted epidermal sheets stained for MHC-II (red) revealed a complete absence of LCs in DT-treated huDTR mice (Fig. 1F). As expected, the density and distribution of LCs in untreated mice and the density and distribution of DETCs expressing CD3 (green) in both treated and untreated mice were unaltered. Thus, transgene expression is specific for LCs that are efficiently ablated by administration of DT.

Langerin+ DCs unaffected in huDTR

In the dermis, in addition to LCs migrating from the epidermis to the cutaneous LN (CLN), Langerin is also expressed by a subset of dDCs. We have previously shown in huDTRA mice that transgene expression is limited to epidermal LCs and does not target Langerin+ dDCs (6). To determine whether Langerin+ dDCs are ablated in huDTR mice, we generated single-cell suspensions from the dermis of wild-type (WT), huDTR, and muDTR mice that had been treated with DT. As expected, cells gated based on expression of muLangerin were virtually absent in muDTR mice compared with WT mice (Fig. 2A). In huDTR mice, Langerin+ dDCs, identified as CD103bright and CD11bdim, are unaffected while epidermal LCs (CD103dim, CD11bbrigh) are absent.

DCs expressing Langerin are found in tissues other than the skin. In the spleen and thymus, Langerin is expressed primarily by CD8+ DCs (4, 10, 21–23). Expression of huLangerin is not evident in either tissue (Fig. 2B). In the CLN, Langerin is expressed by LCs, Langerin+ dDCs, and CD8+ DCs. CD11c+ DCs expressing huLangerin are present in the CLN. These cells represent skin migratory LCs based on the absence of CD8 and CD103 expression (Fig. 2C). As was observed in the epidermis and dermis, DT administration efficiently ablates LCs in the CLN. Other DC and lymphoid subsets (i.e., B cells, T cells, macrophages) in the secondary lymphoid tissues are unaffected in DT-treated huDTR mice (A. Bobr, unpublished observations). Thus, DT administration efficiently and selectively ablates LCs in huDTR mice.

Time course of LC ablation

To determine the optimal time after DT administration at which to test the effects of LC ablation, the kinetics of LC repopulation after DT administration was next examined. On day +2, there was
a dramatic decrease in the number of LCs found in the epidermis and CLN, which continued through day +7 (Fig. 2D). By day +14, a small number of LCs can be observed in the epidermis, which increases to ∼50% by day +28. Repopulation in the LN is delayed compared with the epidermis. This is consistent with nascent LCs repopulating the epidermis prior to migrating to the CLN. The rate of LC repopulation is similar to that observed in muDTR mice (4, 5). Importantly, there is a window from day +2 through at least day +7 when LCs are absent, during which functional assays can be performed.

**Langerin**^+^ dDCs functionally intact in huDTR mice

Hogquist and colleagues (24) recently demonstrated that optimal epicutaneous immunization of whole protein on the flanks of mice requires the presence of Langerin**^+^** dDCs. To determine whether Langerin**^+^** dDCs are functionally altered in huDTR mice, congenic CD90.1**^+^** OT-I T cells were adoptively transferred into huDTR, muDTR, and littermate control mice. Mice were treated with DT and immunized with OVA on day +2. Five days later, CLNs were harvested and the number of OT-I cells was determined by flow cytometry based on a CD90.1 and CD8 gate. As expected, muDTR mice showed an ∼10-fold decrease in the number of LCs found in the epidermis and CLN, which continued through day +7 (Fig. 2D). By day +14, a small number of LCs can be observed in the epidermis, which increases to ∼50% by day +28. Repopulation in the LN is delayed compared with the epidermis. This is consistent with nascent LCs repopulating the epidermis prior to migrating to the CLN. The rate of LC repopulation is similar to that observed in muDTR mice (4, 5). Importantly, there is a window from day +2 through at least day +7 when LCs are absent, during which functional assays can be performed.

**Discussion**

In this study, we described the generation of huDTR mice that allow for the highly selective and efficient inducible ablation of LCs. Langerin**^+^** dDCs are not depleted and remain functionally intact. The acute ablation of LCs promotes increased CHS responses and recapitulates our previous findings with constitutive depletion of LCs in huDTA mice. These results clearly demonstrate that the absence of LCs during ontogeny and/or the steady-state is not required for exaggerated CHS responses. Instead, LC-mediated suppression occurs at the time of immunization.

We have recently demonstrated that LCs suppress CHS responses and the development of hapten-specific effectors via a mechanism that requires cognate interaction with CD4 T cells and elaboration of IL-10 (15). We favor a model in which LCs and Langerin**^+^** dDCs both transport haptenated Ag to the LN. Dermal DCs arrive in the LN within 24 h and initiate anti-hapten responses. LCs arrive ∼72–96 h after sensitization and actively limit the development of Ag-specific effectors.

Although the duration of LC ablation was an attractive hypothesis to explain the different CHS responses in huDTA and muDTR mice, our present results exclude this possibility. Thus, the question of why enhanced CHS responses have not been observed in muDTR mice remains. As has been discussed, functional analysis of LCs in muDTR mice is complicated by the concomitant ablation of Langerin**^+^** dDCs. This has been overcome by relying on differences in the rate of repopulation of LC versus Langerin**^+^** dDCs.
dDCs after DT administration (6). CHS on day +2 after DT administration when LCs and Langerin+ DCs are absent is greatly reduced. CHS is normal on days +7 and +13 when LCs are absent but when other Langerin+ DCs have partially recovered. A key caveat, however, is that at day +7 and +13 Langerin+ dDCs are still only 30 and 50% of their predepletion levels, respectively. The still-decreased numbers of Langerin+ dDCs would be expected to reduce the total proinflammatory capacity of Langerin+ dDCs. We suggest that the resulting decreased response may compensate for the increased response due to the absence of LCs and results in CHS that appears unchanged.

Importantly, the mechanism of LC suppression has only been carefully studied in CHS to DNFB and in response to tick bites, which are predominantly a Th1/Th17-type response (14, 15). In both settings, the extent of Th1/17 effectors was exaggerated by the absence of LCs. A compensatory increase or decrease in Th2 cytokines (e.g., IL-4 and IL-13) was not observed, suggesting that LCs only affect Th1/Th17 responses. However, the possibility that

**FIGURE 2.** DT ablates LCs, leaving other Langerin+ DCs unaffected. A, Flow cytometry of dermal single-cell suspensions showing expression of CD11b and CD103 from WT, huDTR, and muDTR mice 2 d after DT administration. Cells were gated on muLangerin+, CD11c+ cells. Numbers represent the percentage of cells in the indicated gate. B, CLN, spleen, and thymus from huDTR mice treated with DT, huDTR mice treated with vehicle and WT mice treated with DT stained for huLangerin and CD11c cells. C, CLN from untreated huDTR mice gated on muLangerin showing expression of huLangerin, CD103, and CD8, cells. D, The depletion of huLangerin+ cells from epidermis and CLN at the indicated times after DT administration normalized to littermate controls. All data are representative of at least three independent experiments.

**FIGURE 3.** Langerin+ dDCs are functionally intact in huDTR mice. CD90.1 OT-I CD8 T cells (2.5 × 10⁵) were adoptively transferred into huDTR, muDTR, and control mice. One day after administration of DT, mice were epicutaneously immunized with 500 μg of OVA or vehicle. Five days later, the number of OT-I cells from CLN was determined based on expression of CD90.1. *p < 0.05. cntl, control; ~ova, without OVA.

**FIGURE 4.** Acute depletion of epidermal LCs leads to enhanced CHS. A, WT and huDTR mice were sensitized with 0.5% DNFB on day +2 after DT or PBS administration. Five days later, mice were challenged with 0.2% DNFB on the ear. Ear swelling 1 d after challenge (A) and over time (B) was measured. All data are representative of at least three independent experiments. *p < 0.05.
LCs may be specialized to promote Th2 responses in other settings cannot be excluded. Indeed, selectively targeting Ag to LCs with a gene gun led to the production of Th2-related Ab isotypes (25). LCs stimulated in vitro also promoted the development of Th2 cells (26, 27), and topical application of a vitamin D analog appears to selectively activate LCs to promote Th2-type responses (28). Additionally, CHS responses to low doses of oxazalone are reduced in the absence of LCs, although the effect on Th differentiation has not been reported (29). Examination of Ag-specific responses from LC depletion in models other than CHS may help address this issue.

Recently, experiments using bone marrow chimeric mice to selectively deplete Langerin+ dDCs suggest that LCs may be capable of priming CHS responses in certain circumstances (11). Thus, a trend that is becoming increasingly clear is that LC behavior can depend on the methods used to assay their function. Seemingly small differences in technique can give diametrically opposite results. This is abundantly evident from work using different LC-deficient mouse strains. The generation of hu-DR+ along with hu-DTA mice provided tools to examine the effects of LC depletion without having to rely on manipulations that affect other DC populations. We expect that these mouse lines, in combination with novel methods to immunize mice through intact skin, will provide tools to examine the effects of LC depletion in models other than CHS.

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