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*J Immunol* 2011; 187:5069-5076; Prepublished online 12 October 2011; doi: 10.4049/jimmunol.1101880

http://www.jimmunol.org/content/187/10/5069

Supplementary Material  http://www.jimmunol.org/content/suppl/2011/10/12/jimmunol.1101880.DC1

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Conditional Deletion of TGF-βR1 Using Langerin-Cre Mice Results in Langerhans Cell Deficiency and Reduced Contact Hypersensitivity

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The critical role of Langerhans cells (LC) in contact hypersensitivity (CHS) was recently questioned in studies using different LC-depletion mouse models. On one hand, inducible ablation of LC led to diminished ear swelling, suggesting functional redundancy between LC and (Langerin+) dermal dendritic cells (DC). On the other hand, constitutive or acute depletion of LC resulted in an enhanced reaction, supporting a regulatory role of LC in CHS. To address this controversy by conditional gene targeting, we generated Langerin-Cre knockin mice. Breeding these mice to a Cre-reporter strain demonstrated robust and specific DNA recombination in LC, as well as other Langerin+ tissue DC. In agreement with the vital requirement of TGF-β signaling for LC development, crossing Langerin-Cre to mice homozygous for a loxP-flanked TGF-βR1 allele resulted in permanent LC deficiency, whereas the homeostasis of dermal Langerin+ DC was unaffected. In the absence of LC, induction of CHS in these Langerin+ DC-specific TGF-βR1-deficient mice elicited decreased ear swelling compared with controls. This novel approach provided further evidence against a regulatory function of LC in CHS. Moreover, these Langerin-Cre mice represent a unique and powerful tool to dissect the role and molecular control of Langerin+ DC populations beyond LC. The Journal of Immunology, 2011, 187: 5069–5076.

Langerhans cells (LC) belong to the heterogeneous family of Ag-presenting dendritic cells (DC) that balance the induction of tolerance and immunity (1). They reside as sentinels in the epidermis and constantly probe their environment for self- and foreign Ags (2). LC are characterized by the expression of the C-type lectin Langerin/CD207 (3, 4), which they share with a small subset of dermal DC (5–7). LC can be distinguished by expression of epithelial cell adhesion molecule (EpCam) from the Langerin+ dermal DC population (8), which can be further subdivided into a CD103+ and CD103− subset (9). Moreover, LC require TGF-β signaling for their development (10), whereas Langerin+ dermal DC differentiation is TGF-β independent (8, 11). Both in the steady state and during inflammation, epidermal LC and dermal DC migrate to skin-draining lymph nodes (sLN) (12), where they induce naïve T cells to become regulatory or effector cells. Functional specialization or redundancy of epidermal LC and, in particular, Langerin+ dermal DC, has been a matter of intense investigation and debate in recent years (13).

Langerin, which was originally considered a specific marker of LC, is expressed by a subset of dermal DC, as well as by a population of DC in the lung (14). These may be part of a CD103+ DC subset residing in nonlymphoid tissues defined by expression of the transcription factor Batf3 (15), although their level of Langerin expression varies between organs (16). Accumulating data suggest distinct roles for the different Langerin+ DC subsets in specific immunological settings. For example, epidermal DC act as negative regulators of the anti-Leishmania response and are required for UV radiation (UVR)-induced immunosuppression (17–19). A report claiming that LC are dispensable for UVR-induced suppression of contact hypersensitivity (CHS) remains questionable, because the UVR treatment failed to mobilize the cells from the epidermis (20). Dermal Langerin+ CD103+ DC are specialized in cross-presentation of cell-associated self- and viral Ag (21), a feature they share with lymphoid organ-resident CD8α+ DC (22). In the lung, Langerin+ CD103+ DC are essential for effective clearance of influenza virus infection (23) and responsible for reactivation of Th2 responses in postasthmatic mice (24). However, the molecular cues that confer LC and, in particular, the Langerin+ CD103+ DC in the dermis and lung with their unique capacity to initiate regulatory and/or effector T cell responses remain elusive.

CHS to topically applied low molecular weight chemicals (hapten) is a relevant mouse model for allergic contact dermatitis (25). Painting of a contact sensitizer onto the back skin leads to mobilization of skin DC and priming of hapten-specific T cells in draining lymph nodes (LN) during the asymptomatic sensitization phase. Re-exposure to the same hapten on the ear induces...
a transient ear swelling reaction mediated by IFN-γ-producing CD8+ T cells and contained by CD4+ T cells secreting IL-4 and IL-10 (26). For a long time, LC were considered essential for the initiation of CHS (27). Therefore, it came as a surprise when ear swelling was similar or diminished, but not abolished, upon inductive ablation of LC, suggesting that dermal DC contribute to CHS (12, 28). Subsequent work established the functional redundancy of LC and (Langerin+) dermal DC in mediating the response (29, 30). Conversely, mice with constitutive LC deficiency mounted enhanced ear swelling reactions (31). The reason for this augmented reactivity is unclear, but it may be due to failing peripheral tolerance mechanisms in the absence of LC throughout life (1, 11, 32). More recently, aggravated CHS was failing peripheral tolerance mechanisms in the absence of LC for this augmented reactivity is unclear, but it may be due to the previous work in these Langerin+ DC-specific TGF-β–reduced mice (33). How to reconcile the incongruent results obtained with the different inducible LC-depletion models remains elusive. Moreover, it emphasizes the need for novel approaches to dissect the diverse functions of different (skin) DC populations.

In this article, we report the generation of a Langerin-Cre knockin mouse strain that efficiently targets LC and other Langerin+ DC, in particular, in the dermis and the lung. Crosses to mice with a loxP-flanked TGF-βRII allele reproduce the profound LC deficiency obtained in the different LC-depletion mouse models, whereas TGF-β–independent Langerin+ dermal DC are present at normal frequencies. In the absence of LC, CHS reactions in these Langerin+ DC-specific TGF-βRII–deficient mice are diminished, providing further evidence against a regulatory role for LC in CHS.

Materials and Methods

Mice

Mice were housed in the animal facilities of the Academic Medical Center, Erasmus Medical Center, and Institute for Immunology, Ludwig-Maximilians-University (Munich, Germany). The RosA26–tandem-dimer red fluorescent protein (tdRFP) Cre reporter strain (34) was a gift of H.J. Febling (University Clinics Ulm, Ulm, Germany) and kindly provided by T. Brocker (Ludwig-Maximilians-University). Langerin-Cre mice were crossed to floxed TGF-βRI (Alk5) mice (35), made available by S. Karlsson (Lund University Hospital, Lund, Sweden), to generate LDC-TGFR1+ mice. LC-depleted Langerin-DTR mice (28) were used as controls, where indicated. All animal experimentation was in compliance with European Union and national laws and approved by the local ethics committees.

Generation of Langerin-Cre mice

The targeting construct was cloned, as previously described (28), except that in the last cloning step the crebpA CDNA containing a nuclear localization signal (36) was inserted into exon 2 of the langerin gene. Nonlinearized targeting vector (25 μg DNA/103 embryonic stem [ES] cells) was used for electroporation of V6.5 ES cells (415; C57BL/6 × 129Sv/J) (37). Targeted ES cell clones were identified by Southern blot analysis of KpnI-digested genomic DNA using a 3′ external probe (28). Three correctly targeted ES cell clones were selected for the production of chimeras. Germline transmission was achieved from one clone and verified by Southern blot and PCR analysis. The FRT-flanked Neo+ cassette was deleted by breeding founder mice to the ACTFlpe deleter purchased from The Jackson Laboratory (38). F1 offspring were analyzed by genomic PCR, and the presence or absence of the Neo+ cassette was indicated by a 1.7-kb or a 480-bp band, respectively. Langerin-Cre mice are routinely genotyped by PCR with a 270-bp band identifying the cre knockin allele. The Langerin-Cre mutation has been backcrossed to C57BL/6 for ≥10 generations.

Epidermal sheets and fluorescence microscopy

Split mouse ears were floated on 20 mM EDTA/PBS at 37°C for 90 min. Epidermis was separated from the dermis, fixed with 4% paraformaldehyde or 100% EtOH, rehydrated in PBS, and blocked with PBS/0.5% BSA. The sheets were stained with Abs MHC class II (MHC II)-Alexa Fluor 647 (M5/114; BioLegend) and Langerin–Alexa Fluor 488 (929.F3; Dendritics), according to standard procedures, and mounted on slides with ProLong Gold mounting medium (Invitrogen). Images in Fig. 2 were taken using a BX41TF-5 microscope equipped with an F-View II Digital camera and CELL-BND-F software (Olympus). Images in Fig. 4 were generated with a DMRA fluorescent microscope (objectives 10× or 25×; Leica) and a Kx14 camera (Apogee Instruments) and analyzed with Image-Pro Plus software.

Cell isolation and flow cytometry

Single-cell suspensions from epidermis and dermis were prepared and stained, as described (11). The lungs and lymphoid organs were processed with collagenase D (type IV; Worthington Biochemical) for 45–90 min and supplemented with 10 mM EDTA for 5 min before being passed through a 70-μm cell strainer (BD Falcon). For flow cytometry, the cells were blocked with anti-CD16/CD32 (2.4G2; BD Pharmingen) and stained with MHC II–allophycocyanin (M5/114; BD Pharmingen); MHC II-Pacific blue (M5/114; BioLegend); CD45–allophycocyanin–Cy7 or CD45–PE–Cy7 (30-F11; BD Pharmingen); CD11c–allophycocyanin, CD11c–PE–Cy7, and CD11c–PE (N418; BD Pharmingen); CD11b–allophycocyanin (M1/70; BioLegend); EpCam-Alexa Fluor 647, EpCam-Alexa Fluor 488, or EpCam–PerCP–Cy5.5 (G8.8; BioLegend); CD103–A647; signal-regulatory protein α (SIRPα)–allophycocyanin (P84; BD Pharmingen); CD3e–allophycocyanin (145-2C11; BD Pharmingen); NK1.1–FITC (DX5; BD Pharmingen); CD19–allophycocyanin (1D3; BD Pharmingen); or Gr-1–Ly6G-Pacific blue (RB6-8C5; BioLegend). For intracellular staining with Langerin–Alexa Fluor 647 or Langerin–Alexa Fluor 488 (929.F3; Dendritics), cells were fixed with 4% paraformaldehyde and stained in the presence of 0.5% saponin. FACS data represent genuine Langerin-Cre–driven tdRFP expression. Experiments were performed on a FACS Canto (Becton Dickinson) and analyzed using FlowJo software (Tree Star).

FITC painting, skin inflammation, and CHS

The dorsal sides of both ears were painted with 25 μl 1% FITC (Sigma-Aldrich) dissolved in a 1:1 mixture of DMSO and acetone/dibutylphthalate (Sigma-Aldrich). Draining LN were harvested 48 h later for flow cytometric analysis. To study inflammation-induced LC repopulation, 25 μl 2% oxazolone in acetone/olive oil (v/v 4:1) was topically applied on the right ear of LDC-TGFR1+ mice and control mice. For the induction of CHS, wild-type (WT), Langerin-Cre, LC-depleted Langerin-DTR, and LDC-TGFR1+ mice (n = 7–8) were sensitized on the shaved abdomen with 50 μl 2% oxazolone and challenged 5 d later with 25 μl 0.5% oxazolone painted onto the right ear. Swelling was measured in a blinded fashion at 24 and 48 h after hapten challenge by comparing treated (right) and untreated (left) ears using a caliper (Digimatic Quick Micrometer, Mitutoyo, Japan). Because the degree of ear swelling induced by unspecific irritation from oxazolone painting was similar in WT and all transgenic mice (data not shown), negative (“challenged-only”) controls were omitted in the CHS experiments to reduce the number of animals used. To achieve selective depletion of LC, Langerin-DTR mice were injected i.p. with 400 ng diphtheria toxin in PBS 2 wk before sensitization (29).

Statistics

Statistical analysis was performed with GraphPad Prism, using ANOVA or a two-tailed Student t test.

Online supplemental material

Supplemental Fig. 1 illustrates reduced tdRFP expression after fixation and permeabilization of epidermal LC and the absence of tdRFP+ cells among immune cell populations of sLN.

Supplemental Fig. 2 depicts absent LC repopulation of the epidermis upon skin inflammation in LDC-TGFR1+ mice.

Results

Generation of Langerin-Cre knockin mice

To apply conditional gene targeting (39) to LC and other Langerin-expressing DC populations, we generated Langerin-Cre knockin mice (Fig. 1). Following the same strategy that we previously applied to produce the Langerin-DTR strain (28), the targeting vector was constructed such that the cre CDNA was inserted into the second exon of the langerin gene via homologous recombination in ES cells (Fig. 1A). All ATG start codons upstream of cre
were mutated to ensure efficient translation of the Cre protein. To facilitate translocation into the nucleus, cre was genetically engineered to encode a nuclear localization signal (36). The linearized targeting construct was electroporated into V6.5 ES cells (37), and correctly targeted ES cell clones were identified by genomic Southern blot using a 5\textsuperscript{9} external probe outside the short arm of homology (Fig. 1B). Three independent targeted ES cell clones were injected into blastocysts for the generation of chimeric mice, one of which resulted in germline transmission of the mutation. The FRT-flanked neomycin resistance (Neo\textsuperscript{R}) cassette was removed in vivo by breeding to the ACTFlpe deleter strain (38). Successful deletion of the neo\textsuperscript{R} gene was confirmed by PCR (Fig. 1C). Heterozygous Langerin-Cre mice were bred onto a pure C57BL/6 background and are routinely genotyped by PCR (Fig. 1D).

Langerin-Cre mice exhibit specific Cre activity in LC and other Langerin\textsuperscript{+} DC

To determine specificity and efficiency of langerin-driven Cre expression, Langerin-Cre mice were crossed to a tdRFP reporter strain (34). In these reporter mice, Cre-mediated removal of a loxP-flanked STOP cassette enables ROSA26-driven expression of an extrabright tdRFP protein. Langerin-Cre/tdRFP double-mutant mice were analyzed for tdRFP expression in the epidermis, dermis, sLN, and lung in comparison with tdRFP\textsuperscript{-} littermates.

MHC II staining of epidermal sheets of Langerin-Cre/tdRFP mice revealed an intact LC network, with almost complete overlap of tdRFP and MHC II expression (Fig. 2A). Flow cytometric analysis of the epidermis confirmed that nearly all MHC II\textsuperscript{+} LC were tdRFP\textsuperscript{+} (93.7 ± 1.6%; Fig. 2B, Table I). Notably, the fixation and permeabilization procedure required for intracellular Langerin staining to identify epidermal LC significantly quenched the tdRFP signal (Supplemental Fig. 1A). Similarly, intracellular staining for Langerin impaired the identification of tdRFP\textsuperscript{+} DC in dermis and lung tissue (data not shown). Therefore, we performed side-by-side analysis of tdRFP expression in unfixed cells and Langerin expression in fixed material of individual mice to demonstrate Cre activity in Langerin\textsuperscript{+} DC in the dermis, sLN, and lung.

In the dermis, we detected 10.7 ± 1.5% Langerin\textsuperscript{+} DC and 7.3 ± 1.3% tdRFP\textsuperscript{+} cells, which corresponds to 71% of dermal Langerin\textsuperscript{+} DC with Cre-mediated tdRFP expression (Table I). Further analysis of the different DC subsets revealed that, in addition to transmigrating epidermal LC (Langerin\textsuperscript{+} EpCam\textsuperscript{+}), both Langerin\textsuperscript{+} EpCam\textsuperscript{-} CD103\textsuperscript{+} and Langerin\textsuperscript{+} EpCam\textsuperscript{-} CD103\textsuperscript{-} dermal DC expressed tdRFP (Fig. 2C). Consistent with published data, 21.1 ± 5.8% of the skin-immigrant DC (MHC II\textsuperscript{hi} CD11c\textsuperscript{int}) in the sLN were Langerin\textsuperscript{+} (Table I) (9). Because a comparable fraction (21.5 ± 6.2%) of MHC II\textsuperscript{hi} CD11c\textsuperscript{int} DC was positive for tdRFP, our data indicated that all skin-immigrant Langerin\textsuperscript{+} DC express active Cre recombinase (Table I). Based on CD103 and SIRP\alpha expression, we could identify Cre-driven tdRFP expression in epidermal LC and both dermal Langerin\textsuperscript{+} DC subsets in the sLN (Fig. 2D).

Another prominent Langerin\textsuperscript{+} DC population has been identified in the lung (14). Examination of pulmonary CD103\textsuperscript{+} DC (CD45\textsuperscript{+} MHC II\textsuperscript{hi} CD11c\textsuperscript{int}) in Langerin-Cre/tdRFP mice revealed 23 ± 2.4% tdRFP\textsuperscript{+} cells, whereas 28.5 ± 2.6% of the DC expressed Langerin (Fig. 2E, Table I). From these data, we calculated 81% deletion efficiency in Langerin\textsuperscript{+} pulmonary DC. In contrast, we did not detect any tdRFP expression in other immune cells of Langerin-Cre/tdRFP mice (Supplemental Fig. 1B).
data demonstrated robust and specific Cre activity in LC and Langerin+ DC in the dermis and lung of Langerin-Cre mice. Langerin-Cre knockin does not affect the function of Langerin+ skin DC

Introduction of the cre cDNA into one langerin allele resulted in decreased Langerin protein expression in LC (Fig. 3A). To ensure that the mutation of the langerin locus does not interfere with the migration of Langerin+ skin DC to sLN, heterozygous Langerin-Cre and Cre–/– littermates were painted with FITC onto the dorsal side of the ears. The sLN were isolated 48 h later to determine the frequency of FITC+ Langerin+ DC by flow cytometry. In Langerin-Cre mice, the percentage of total CD11c+ MHC IIhigh Langerin+ FITC+ DC that migrated to the sLN was comparable to WT mice (17 ± 0.1% versus 20 ± 1.3%, respectively) (Fig. 3B). Subsequently, we tested the Langerin-Cre mice in a CHS reaction to exclude an altered function of the Langerin+ DC due to the insertion of cre. A side effect in Fig. 3C, 24 and 48 h after hapten challenge, the ear-swelling response of Langerin-Cre mice was indistinguishable from nontransgenic (non-TG) littermates. From these results, we concluded that the Langerin-Cre knockin does not affect the migratory potential of Langerin+ skin DC or their ability to induce an adaptive-immune response, which is in agreement with observations in Langerin knockout (KO) mice (40).

**Permanent absence of LC and reduced CHS in LDC-TβRI−/− mice**

Langerin-Cre and TGF-βR1−/− mice were crossed to generate animals lacking the TGF-βR1 on all Langerin+ DC (LDC-TβRI−/− mice). The crucial role of TGF-β signaling in LC development (10) was reflected by the gene dosage-dependent absence of LC in the epidermis of LDC-TβRI−/− mice, as quantified by flow cytometry (Fig. 4A). Heterozygous TGF-βR1–deficient mice exhibit a 50% reduction in the number of LC, whereas homozygous TGF-βR1 deficiency results in near-complete depletion of the

| Table I. Frequency (%) of tdRFP-expressing LC and Langerin+ DC in Langerin-Cre/tdRFP mice |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Epidermis | Langerin+ | tdRFP+ | Langerin+ | tdRFP+ | Langerin+ | tdRFP+ | Langerin+ | tdRFP+ |
| MHC II+ tauRFP+ | 93.7 ± 1.6 | 10.7 ± 1.5 | 7.3 ± 1.3 | 21.1 ± 5.8 | 21.5 ± 6.2 | 28.5 ± 2.6 | 23 ± 2.4 |

The frequency of MHC II+ tauRFP+ LC was determined in epidermal single-cell suspensions (mean ± SD; n = 3 mice). In the dermis, sLN, and lung, expression of Langerin and tauRFP was determined in parallel (mean ± SD, n = 3 mice) and used to calculate the efficiency of target gene deletion (percentage of Langerin+ tauRFP+ cells, bottom row). One of at least two independent experiments is shown for each organ.
Discussion

LC were long considered to be prototypic DC uniquely required for the induction of skin immune responses (41, 42). With the generation of LC-depletion mouse models, the significant contribution of dermal DC to cutaneous immune reactions became clear (12, 28). Moreover, Langerin+ DC distinct from classical LC were discovered in the dermis and sLN (5–7), as well as in skin-unrelated organs, such as the lung, thymus, and spleen (4, 43). Elegant bone marrow chimera experiments established that the skin and sLN harbor at least five distinct DC subsets based on the expression of Langerin, CD103, and CD11b among MHC IIhigh (epidermis and dermis) and MHC IIhigh CD11cint (sLN) cells (9, 44). In addition to Langerin+ EpCam− Sirpα− CD103− LC, two Langerin+ EpCam− Sirpα+ DC subsets can be distinguished in the dermis, which are either CD103+ or CD103−, both before and after their migration to the nodes. By the time the Langerin+ dermal DC subsets have reached the sLN, they have gained expression of EpCam and Sirpα, whereby these markers lose their discriminatory power to distinguish Langerin+ dermal DC from migratory Langerin+ EpCam+ Sirpα+ LC in sLN of nonchimeric mice.

The question of functional specialization of these Langerinexpressing DC subsets has been actively investigated in recent years (1). The role of lymphoid organ-resident Langerin+ DC remains elusive, largely owing to their paucity in C57BL/6 mice (45), the most common background of transgenic and KO mice. Similarly, information on the prominent Langerin+ DC population residing in the lung is sparse (23, 24). The emerging and sometimes conflicting evidence of a functional specialization of epidermal LC and Langerin+ dermal DC largely stems from in vivo cell-ablation experiments (1, 46). Alternatively, conditional gene targeting using the Cre/loxP system represents an effective means to dissect the specific contribution and, in particular, the molecular control of different DC subsets in immune regulation (13). Although CD11c+ DC- and LC-specific Cre transgenic mice are already available (47, 48), Langerin+ DC have escaped this powerful in vivo approach because of the lack of appropriate Cre mice. To this aim, we generated Langerin-Cre knockin mice that mediate specific and robust target gene deletion in LC (Fig. 2A, 2B) and other Langerin+ DC subsets, in particular, in nonlymphoid tissues (i.e., the dermis and lung) (Fig. 2C–E).

Efficient Cre-mediated DNA recombination was demonstrated by tdRFP reporter gene expression in 94% of epidermal LC and all MHC II^high Langerin+ DC that migrated to the sLN (Fig. 2A, 2B, Table I). In dermal cell suspensions, we calculated that 71% of the Langerin+ DC express tdRFP (Table I). Based on CD103 EpCam (Fig. 2C) and CD103 Sirpα (Fig. 2D) staining, we demonstrated Langerin-Cre-driven tdRFP expression in all three Langerin+ DC subsets in the dermis and sLN, respectively. Similarly, among Langerin-expressing DC in the lung, the calculated frequency of tdRFP+ cells is 81% (Fig. 2E, Table I). Luche et al. (34) reported an inverse relationship between red fluorescence intensity and proliferation status of a cell. Notably, DC in the dermis and lung have a higher turnover compared with the long-lived LC in the epidermis. Therefore, the efficiency of target gene recombination in dermal and pulmonary Langerin+ DC is quite likely greater than
the 71 and 81% respectively that we calculated from the tdRFP
data. Because of the absence/low frequency of lymphoid organ-
resident Langerin+ DC populations in C57BL/6 mice (45), these
were not addressed in our analysis.

Insertion of the cre cDNA into the langerin locus did not interfere
with the migration and function of Langerin+ skin DC (Fig. 3).
Despite the reduced expression of Langerin on DC with one dis-
rupted langerin allele, mutant skin DC migrate to the sLN at a similar

FIGURE 4. Permanent LC deficiency and reduced CHS in LDC-TβR1del mice. A, Frequency of LC in the epidermis. Epidermal cell suspensions of control, LDC-TβR1+/-, and LDC-TβR1del mice were stained for MHC II to quantify LC by flow cytometry. B, Epidermal sheets of LDC-TβR1del and non-
TG littermates were stained for MHC II (red) and Langerin (green) and analyzed by fluorescent microscopy. Scale bar, 20 μm. One representative of six mice per group is depicted. C, Single-cell suspensions of epidermis from aging LDC-TβR1del and non-TG mice were stained with MHC II to determine the frequency of LC by flow cytometry. Data points represent individual mice (n = 1–3/time point). D, DC subsets in the dermis of non-TG and LDC-TβR1del mice. CD45+ cells were gated on CD11c and Langerin to identify Langerin+ DC (non-TG: 21.0 ± 4.0%; KO: 1.8 ± 0.8%). The frequencies of EpCam+ LC (non-TG: 93.2 ± 2.0%; KO: 62.8 ± 8.4%) and both CD103+ (non-TG: 2.1 ± 0.5%; KO: 13.1 ± 5.1%) and CD103+ (non-TG: 3.9 ± 1.7%; KO: 23.8 ± 11.5%) Langerin+ dermal DC were determined (mean ± SD; n = 4–5). E, Skin immigrant DC in the draining nodes. Skin-derived DC in sLN were
identified as CD11c+ MHC II high cells. The frequencies of Langerin+ DC (non-TG: 40.2 ± 4.6%; KO: 27.2 ± 3.2%), Langerin+ CD103+ dermal DC (non-
TG: 47.5 ± 4.4%; KO: 71.2 ± 3.7%), and SIRPa+ cells (non-TG: 50.3 ± 4.1%; KO: 26.1 ± 2.8%) comprising both LC and Langerin+ CD103+ dermal
DC, were measured (mean ± SD; n = 4–5). F, CHS responses in LDC-TβR1del, non-TG, and LC-depleted Langerin-DTR mice. Ear swelling was
determined 24 h after hapten challenge. One representative of two experiments is depicted in D–F. *p < 0.05, **p < 0.01, ***p < 0.001.
rate as WT cells. Furthermore, Langerin-Cre mice mount a similar CHS response as non-TG littermates (Fig. 3C). These findings are in line with physiologic LC function in Langerin KO mice (40).

As expected, based on the crucial role of TGF-β for LC development and homeostasis in the epidermis (10, 11), LDC-TβRI<sup>del</sup> mice lack epidermal LC (Fig. 4A, 4B). In another model using human Langerin-Cre bacterial artificial chromosome transgenic mice to ablate TGF-βRII, 20–60% of LC that escaped Cre-mediated deletion of the receptor repopulated the epidermis as early as 8 wk after birth (48). In contrast, we observed a permanent absence of epidermal LC in LDC-TβRI<sup>del</sup> mice >7 mo of age (Fig. 4C). In contrast, the development of dermal Langerin<sup>+</sup> DC is independent of TGF-β (8, 11). Despite efficient Cre activity in Langerin<sup>+</sup> dermal DC, LDC-TβRI<sup>del</sup> animals exhibit a similar composition of the dermal DC compartment as non-TG controls (Fig. 4D, 4E). These data confirmed that our Langerin-Cre knockin mice are a powerful and reliable tool to target genes in LC and (dermal) Langerin<sup>+</sup> DC.

Experiments using different mouse models that lacked epidermal LC and/or Langerin<sup>+</sup> dermal DC due to diphtheria toxin-mediated cell ablation yielded incongruent results in CHS reactions to topical hapten (1, 13, 46). Although the inducible depletion of all Langerin<sup>+</sup> skin DC resulted either in diminished or similar ear swelling responses compared with WT (12, 28, 49), the constitutive absence of only LC from birth onward produced aggravated CHS (31). Initially, these data were interpreted to reflect a regulatory role of LC and a stimulatory function of Langerin<sup>+</sup> dermal DC in CHS (7). More recent results revealed that the magnitude of a CHS response depends on the hapten dose and the number of skin DC available to prime naive T cells in the draining LN, rather than on the specific function of a particular skin DC subpopulation (29, 30). Consequently, LC and (Langerin<sup>+</sup>) dermal DC serve redundant roles during CHS. The reason for enhanced reactivity observed thus far only in human Langerin<sup>+</sup> epidermal chromogranin transgenic mice with constitutive or acute ablation of LC remains enigmatic (31, 33), in particular, because CHS responses were unaffected in Batf3-deficient mice specifically lacking Langerin<sup>+</sup> CD103<sup>+</sup> dermal DC (15). In these mice, CHS should have been attenuated if LC had a regulatory function, as has been suggested (31, 33). In a mouse model independent of diphtheria toxin-mediated cell ablation, we demonstrated that CHS responses are reduced (and not enhanced) in the selective absence of LC (Fig. 4F), providing further evidence against a regulatory role for LC in CHS. Moreover, the diminished ear swelling reaction in LDC-TβRI<sup>del</sup> mice indicated that TGF-β signaling does not govern Langerin<sup>+</sup> dermal DC function in CHS.

In conclusion, mice specifically lacking epidermal LC as a result of deficient TGF-β signaling in Langerin<sup>+</sup> DC mount attenuated ear swelling reactions, providing further evidence against a regulatory role for LC in CHS. The Langerin-Cre knockin mice that we generated represent an efficient and unique tool to study the molecular control of epidermal LC and Langerin<sup>+</sup> DC in nonlymphoid tissues by conditional gene targeting and cell lineage tracing.

Acknowledgments
We thank the members of the Clausen laboratory for many helpful discussions and support; Sabina Onderwater, Erwin van Rijn, and Ellen De Feyter for expert technical assistance; and Julia Ober-Blöbaum and Jon Laman for critical reading of the manuscript. We are particularly grateful to Stefanie Meier and Thomas Brocker for dedicated help with the analysis of Langerin-Cre/dTRFP mice.

Disclosures
The authors have no financial conflicts of interest.

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