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J Immunol 2011;186;1377-1383; Prepublished online 27 December 2010;
doi:10.4049/jimmunol.1002557
http://www.jimmunol.org/content/186/3/1377

Langerin$^+$ Dermal Dendritic Cells Are Critical for CD8$^+$ T Cell Activation and IgH γ-1 Class Switching in Response to Gene Gun Vaccines

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The C-type lectin langerin/CD207 was originally discovered as a specific marker for epidermal Langerhans cells (LC). Recently, additional and distinct subsets of langerin$^+$ dendritic cells (DC) have been identified in lymph nodes and peripheral tissues of mice. Although the role of LC for immune activation or modulation is now being discussed controversially, other langerin$^+$ DC appear crucial for protective immunity in a growing set of infection and vaccination models. In knock-in mice that express the human diphtheria toxin receptor under control of the langerin promoter, injection of diphtheria toxin ablates LC for several weeks whereas other langerin$^+$ DC subsets are replenished within just a few days. Thus, by careful timing of diphtheria toxin injections selective states of deficiency in either LC only or all langerin$^+$ cells can be established. Taking advantage of this system, we found that, unlike selective LC deficiency, ablation of all langerin$^+$ DC abrogated the activation of IFN-γ–producing and cytolytic CD8$^+$ T cells after gene gun vaccination. Moreover, we identified migratory langerin$^+$ dermal DC as the subset that directly activated CD8$^+$ T cells in lymph nodes. Langerin$^+$ DC were also critical for IgG1 but not IgG2a Ab induction, suggesting differential polarization of CD4$^+$ T helper cells by langerin$^+$ or langerin-negative DC, respectively. In contrast, protein vaccines administered with various adjuvants induced IgG1 independently of langerin$^+$ DC. Taken together, these findings reflect a highly specialized division of labor between different DC subsets both with respect to Ag encounter as well as downstream processes of immune activation. ***The Journal of Immunology*****, 2011, 186: 1377–1383.**

Langerhans cells (LC) are APC found predominantly in the skin and mucosal epithelia. Owing to their ideal anatomic location, LC have long been regarded as a prototypic example of professional APC and have become one of the best characterized APC subsets since the unique ability of dendritic cells (DC) to activate naive T cells was recognized (1–5). The C-type lectin langerin/CD207 has been identified as a unifying marker for LC in humans and mice (6, 7). Molecular cloning of langerin and the development of mAbs has greatly facilitated the research on LC and DC subset organization (8). Recently, knock-in mice were generated that express either enhanced GFP (langEGFP mice) or the human diphtheria toxin receptor-EGFP fusion molecule under transcriptional control of the langerin gene locus (langDTR mice) (9, 10). Because wild-type (WT) mice lack a high-affinity receptor for diphtheria toxin (DT), injection of DT into langDTR mice results in selective ablation of langerin-expressing cells. These mouse models have rapidly become popular and yielded a series of novel and surprising insights in the field of DC research. First, epidermal LC turned out to be much less important for the induction of immune reactions against skin-borne Ags than previously assumed (9, 10). Second, after ablation, the dermis was repopulated with langerin$^+$ cells much faster than the epidermis. Thus, langerin$^+$ dermal DC (LdDC), which had previously been regarded as migrating epidermal LC on their way to cutaneous lymph nodes (cLN), were now identified as an additional and distinct DC subset (11–13). Third, a more recent work identified three different langerin$^+$ DC in the skin that can be divided into epidermal LC and langerin$^+$ CD103$^+$ or langerin$^+$ CD103$^-$ dermal DC, as well as two langerin$^-$ dermal DC subsets, characterized by differential expression of CD11b (14). After ablation, epidermal LC do not reappear until at least 10 d, whereas LdDC start to repopulate the dermis already after ~3 d. Thus, appropriate timing of ablation regimens facilitated the differential analysis of skin DC subsets. Using this approach, LdDC were identified as exquisite activators of CD8$^+$ T cells in mouse models of contact hypersensitivity (11), leishmaniasis (15), or herpes infection (16). Cross-presentation of skin-borne Ags by CD103$^+$ LdDC was also demonstrated in transgenic mouse models of keratinocyte-restricted expression of membrane-bound OVA (14). In contrast to CD8$^+$ T cells, CD4$^+$ T cell activation seems to be independent of a langerin$^+$ DC subset (15, 16).

DNA vaccines, particularly when administered by gene gun technology, have proven highly efficient inducers of CD8$^+$ T cell-

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Received for publication July 29, 2010. Accepted for publication November 25, 2010.

This work was supported by Biomay (Vienna, Austria), the Christian Doppler Research Association (Vienna, Austria), and the Austrian Science Fund (Fonds zur Förderung der Wissenschaftlichen Forschung) (Grant W1213-B05).

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The online version of this article contains supplemental material.

Abbreviations used in this article: B6, C57BL/6 mouse strain; cLN, cutaneous lymph node; DC, dendritic cell; DT, diphtheria toxin; DTH, delayed-type hyperreactivity; βgal, β-galactosidase; EGFP, enhanced GFP; i.d., intradermal; langDTR, human diphtheria toxin receptor knock-in into 3′-untranslated region of the langerin gene locus; LC, Langerhans cell; LdDC, langerin$^+$ dermal dendritic cell; LN, lymph node; pCI, a eukaryotic expression vector; WT, wild-type.

mediated immunity, at least in experimental mouse models (17–20). Unlike immunization with protein Ags, DNA vaccines require transfection of host cells that, in turn, express the encoded Ag. The precise mechanisms of how DC gain access to the Ag are still not clear. There is experimental evidence for both mechanisms, that is, direct transfection of APC as well as uptake of liberated Ag with subsequent presentation and/or cross-presentation by DC (21–26). Moreover, the fact that Ab production is commonly observed with DNA vaccines suggests that intact Ag might somehow be released from transfected cells to facilitate B cell activation (27).

Although epicutaneous gene gun vaccination targets primarily cells in the epidermis, we previously found that LC were not required for the induction of humoral or cell-mediated immunity (28). In the current study, we examined the role of LdDC for gene gun-induced immune reactions. We show that the activation and functional differentiation of $CD8^+$ T cells were exclusively mediated by LdDC, whereas $CD4^+$ T cell activation appeared, at first view, independent of this DC subset. However, the Ab isotype profile changed fundamentally in the absence of LdDC, suggesting that Th cells were differentially polarized by different DC subsets. In sharp contrast to gene gun immunization, LdDC did not play a role for the humoral response against protein vaccines.

Materials and Methods

Mice

C57BL/6 and BALB/c mice were obtained from Charles River Laboratories (Sulzfeld, Germany). OT-I, OT-II, and langerin-DTR-EGFP (langDTR) (10) mice were backcrossed onto C57BL/6 (B6) background for six generations and maintained at the local animal facility. Mice were used for experiments between 8 and 16 wk of age. Animal care and experimentation were conducted in accordance with European Union guidelines 86/609/EWG and were approved by national authorities (permission no. GZ 66.012/0004-II/10b/2009).

Ablation of langerin$^+$ DC

For ablation of langerin$^+$ DC, 1 μg DT (Sigma-Aldrich) in 100 μl pyrogen-free PBS was injected i.p. 1 d before immunization. For continuous depletion of langerin$^+$ DC, subsequent DT injections were given at intervals of 3 d for the entire duration of experiments.

Analysis of DC from skin and cLN

For whole skin DC analysis, hair was plucked and fatty tissue was removed from back skin. Precut skin was digested with 0.15 mg/ml Liberase (Roche) and 0.12 mg/ml DNAse I (Roche) in HBSS for 1 h at 37°C with agitation. For lymph node (LN) DC, mechanically disrupted LN tissue was digested with 1 mg/ml collagenase D (Roche) and 0.12 mg/ml DNAse I (Roche) in HBSS for 40 min at 37°C with agitation. Cells were filtered through 70-μm nylon mesh filters before flow cytometric analysis.

Flow cytometry

Skin and LN cells were stained with Alexa Fluor 648 anti-MHC class II (clone KH74; BioLegend), PE/Cy7 anti-CD11c (clone N418; BioLegend), allophycocyanin anti-CD8α (clone 53-6.7; American Type Culture Collection), and biotinylated anti-CD103 (clone M290; BD Pharmingen) followed by streptavidin-PE (BD Pharmingen). For langerin staining with Alexa 488 anti-langerin (clone 929F3.01; Dendritics), cells were fixed with 4% formaldehyde and permeabilized with 0.1% saponin. Cells were recorded on a BD FACSCanto II and analyzed with the BD FACSDiva software (all from BD Biosciences).

Immunization

Gene gun immunization was performed as previously described (29). One dose comprised two nonoverlapping shots onto the shaved abdominal skin. With each shot, 1 μg pCI-OVA (28) or pCI-β-galactosidase (βgal) (30) plasmid DNA, immobilized onto 0.5 mg gold particles, was delivered with pressurized helium gas at 400 psi using a Helios gene gun (Bio-Rad, Richmond, CA). For protein immunization, mice were injected intradermally (i.d.) with 2 μg recombinant *Escherichia coli* βgal protein in 100 μl sterile PBS, distributed to four spots on the abdomen twice with a 1-wk interval. As an adjuvant, we used either 50 μl alum (Alu-Gel-S; Serva, Heidelberg, Germany), 2 μg LPS (*E. coli* O55:B5; Sigma-Aldrich, Vienna, Austria), or 10 μg CpG oligodeoxynucleotide 1826 (Biomers, Ulm, Germany) (31).

Serum Abs

Serum was prepared from tail vein blood 1 wk after the last immunization. Ag-specific serum IgG was detected by ELISA with isotype-specific peroxidase-conjugated detection Abs, followed by chromogenic development. Ab titers were determined by endpoint titration and are expressed as the dilution factor yielding a response equal to the quantification limit (i.e., mean + 3 × SD of 16 blank values).

ELISPOT for IFN-γ– or IL-4–secreting splenocytes

Spleen cells, 2×10^5 per well, were cultured for 24 h with or without 10 μM immunodominant CTL epitope peptide ($\beta gal_{96–103}$, DAPIYTNV) (32) or 20 μg recombinant βGal protein on a 96-well filter-bottom MultiScreen plate (Millipore) coated with anti–IFN-γ mAb (clone AN18; BD Pharmingen) or anti–IL-4 mAb (clone 11B11; BioLegend). Culture medium was MEM supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 1% heat-inactivated FCS (PAA Laboratories, Austria), 50 μM 2-ME, and antibiotics. Cytokine spots were detected by use of biotinylated anti–IFN-γ mAB (clone R46A2; BD Pharmingen) or anti–IL-4 mAb (clone BVD6-24G2; BioLegend), followed by streptavidin-HRP and 3-amino-9-ethylcarbazol as a chromogenic substrate. Spots were counted manually from flatbed scans of duplicate wells.

In vivo proliferation assay

Erythrocyte-depleted spleen cells from naive OT-I or OT-II donor mice that are transgenic for a TCR with specificity for H2-K^b–restricted $OVA_{257–264}$ (SIINFEKL) or I-$A^{b/d}$–restricted $OVA_{323–339}$, respectively, were stained with 3 μM CFSE (Molecular Probes) for 10 min at 37°C in PBS. After washing, 2×10^6 cells in 100 μl PBS were injected i.v. into langDTR or WT mice, respectively, that had been left untreated or treated with 1 μg DT. Next day, mice were gene gun immunized with pCI-OVA, and cells were isolated from draining LN and spleen 3 d later. Cells were stained with PE-conjugated anti-Vβ5.1,5.2 TCR mAb (clone MR9-4; BD Pharmingen) together with either allophycocyanin-conjugated anti-CD8 (clone 53.67) or anti-CD4 (clone GK1.5) mAb for OT-I or OT-II transfers, respectively, and analyzed by flow cytometry.

In vivo CTL assay

CTL activity was determined in vivo as previously described (33), with minor modifications. In brief, two samples of erythrocyte-depleted spleen cells from B6 donor mice were stained with CFSE at either 5 or 0.3 μM, respectively. The latter fraction was pulsed in culture medium with 10 μM immunodominant MHC class I-restricted βgal CTL peptide DAPIYTNV for 1 h and combined with the first fraction at a 1:1 ratio. Of this mixture, a total of 5×10^6 cells in PBS was injected i.v. into recipient mice. After 16 h, splenocytes were analyzed by flow cytometry and percentage specific lysis was calculated from the reduction of peptide-pulsed $CFSE^{dim}$ cells relative to nonpulsed $CFSE^{hi}$ reference cells.

Delayed type hyperreactivity reaction

LangDTR or C57BL/6 WT mice, either naive or gene gun-immunized with pCI-βgal, were challenged with pCI-βgal or pCI-OVA by a gene gun shot onto both the dorsal and ventral side of the left ear, whereas the right ear was left untreated. Ear thickness was determined with a micrometer caliper after 1 and 2 d. Cell infiltrates were analyzed after collagenase digestion of ear tissue by flow cytometry using FITC-conjugated anti-CD4 (clone GK1.5), allophycocyanin-conjugated anti-CD8 (clone 53.67), PE-conjugated Gr-1 (clone RB6-8C5), and PE-Cy7–conjugated anti-CD45 (clone 30-F11) mAbs. For some experiments, $CD8^+$ and $CD4^+$ T cells were depleted by i.v. injection of 100 μg anti-CD4 mAb (clone GK1.5) and anti-CD8 mAb (clone 53-6.7), respectively, in 100 μl PBS 24 h before ear challenge.

Statistical analysis

Results of in vivo proliferation, CTL activity, Ab titers, ELISPOT, and delayed-type hyperreactivity (DTH) reactions of different groups of mice (n = 4–5) are presented as arithmetic means ± SD. Data were tested for statistical significance by unpaired t test with Welch's correction. Values of $p < 0.05$ were considered statistically significant.

Results

Langerin$^+$ DC are completely ablated for 3 d after a single DT injection

In langDTR mice, langerin-expressing cells disappear within 24 h after DT injection. However, unlike LC, LdDC and LN-resident langerin$^+$ DC start to repopulate tissues within a few days (11). Because it was not clear how many reappearing LdDC would suffice for adequate Ag presentation, we established a protocol to maintain LdDC deficiency long enough to conduct vaccination experiments. After a single dose of DT, we found that 93% of langerin$^+$ cells were ablated in cLN within 1 d and 99% on day 3 (Fig. 1*A*, 1*C*). In the skin of untreated mice, 38% of MHC class II$^+$ cells were langerin$^+$CD103$^-$ and 5% were langerin$^+$CD103$^+$. DT treatment eliminated both subsets to 0.1 or 0.4%, respectively, within 24 h, with a slight but statistically not significant increase during the next two days (Fig. 1*B*, 1*D*). Thus, repetitive DT injection at 3-d intervals seemed appropriate to keep mice continuously devoid of langerin$^+$ DC. From previous work we knew that langDTR mice tolerate at least five DT injections without obvious side effects. Moreover, although DT-specific Abs were detected in serum (see Supplemental Fig. 1), even the last of five weekly doses was still sufficiently active to keep LC ablated (28). Thus, five doses given at 3-d intervals provide a 2-wk window of langerin$^+$ DC deficiency for experiments.

LdDC are critical for gene gun-induced CD8$^+$ but not CD4$^+$ T cells

To investigate the dependence on LdDC of T cell activation by skin-borne Ag, we performed CFSE dilution experiments with adoptively transferred OT-I or OT-II splenocytes into WT or langDTR recipients. Parallel groups of recipient mice were either, left untreated or injected with one dose of DT at the day of cell transfer and a second dose 2 d later. To induce skin-restricted expression of Ag, recipients were gene gun immunized with pCI-OVA the day after cell transfer. Three days later, cLN cells were analyzed for proliferating donor T cells. As expected, CD4$^+$ (OT-II) as well as CD8$^+$ (OT-I) T cells proliferated in langDTR mice without DT treatment and, irrespective of DT treatment, in B6 WT controls. In contrast, proliferation of CD8$^+$ but not CD4$^+$ T cells was almost completely abrogated in DT-treated langDTR mice (Fig. 2). From this, we conclude that LdDC were required for the activation of CD8$^+$ T cells, whereas CD4$^+$ T cells could also be activated by langerin$^-$ DC.

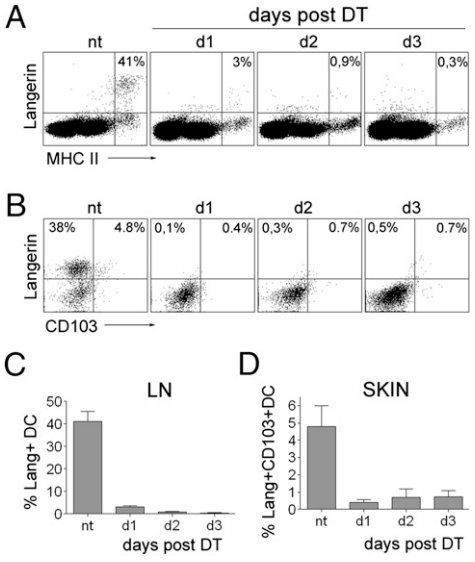

FIGURE 1. Frequency of langerin$^+$ DCs in cLN and skin after DT treatment. LangDTR mice were either left untreated (nt) or injected once with DT. At time points indicated, cLN and skin were analyzed by flow cytometry. *A*, Representative dot blots and (*C*) summary for cLN cells. Percentages given represent percentage of langerin$^+$ cells among MHC class II^{hi} cells as defined by quadrant settings. *B*, Representative dot blots and (*D*) summary for skin cells, gated on MHC class II$^+$ cells and analyzed for langerin and CD103 surface expression. Percentages are percentage of langerin$^+$CD103$^+$ cells among MHC class II$^+$ skin cells. Data in *C* and *D* are means ± SD of $n = 3$ mice/group. Three independent experiments were performed for cLN, and two for skin.

CD8$^+$ T cells are primed by direct interaction with migratory LdDC

For herpes infection where Ag expression is confined to skin cells, Allan et al. (34) have demonstrated that only LN-resident CD8$^+$ DC, but not immigrated skin DC, were able to activate T cells ex vivo. However, migratory DC were still important, because pharmacological blockade of DC migration abrogated T cell activation. Based on these observations, the authors proposed a mechanism of Ag handover from migratory DC to LN-resident CD8$^+$ DC. We were interested in whether this might also apply to our system. To this end, we depleted langerin$^+$ cells not before but, rather, 3 d after gene gun immunization. With this strategy, Ag-bearing DC had a chance to arrive at the LN and, eventually, hand over antigenic material to LN-resident DC. One day after DT treatment, recipients were injected with CFSE-labeled OT-I cells, and cLN were analyzed 3 d later. Again, depletion of langerin$^+$ cells totally abrogated the proliferation of adoptively transferred cells in langDTR mice, suggesting that migratory LdDC activated CD8$^+$ T cells by direct interaction (Fig. 3*A*, 3*B*). LangDTR mice had been developed from 129Sv embryonic stem cells, that is, a mouse strain in which a fraction of LN-resident CD8$^+$ DC express langerin (10, 35, 36). However, after back-crossing onto B6 background for six further generations, the mice used in our study had lost this population (Fig. 3*C*).

Functional activation of endogenous CD8$^+$ T cells by gene gun vaccines relies on LdDC

Because LdDC were important for the activation of adoptively transferred OT-I cells, we next examined whether they were also required to induce endogenous effector T cells. WT or langDTR mice, either left untreated or DT treated during the entire duration of the experiment, received two gene gun vaccinations with pCI-βgal at a 1-wk interval. One week later, IFN-γ–producing CD8$^+$ T cells were quantified by ex vivo restimulation with an immunodominant MHC class I-restricted CTL peptide. The frequency of IFN-γ–producing cells was virtually identical in untreated langDTR and WT mice, and DT did not affect IFN-γ production in WT mice. However, when langerin$^+$ DC had been continuously depleted during immunization, mice failed to generate IFN-γ–producing CD8$^+$ T cells in response to CTL peptide (Fig. 4*A*). In contrast, restimulation with βgal protein instead of CTL peptide revealed no difference in the frequency of IFN-γ–secreting bona fide CD4$^+$ T cells (data not shown). Moreover, gene gun vaccination induced cytolytic activity against CTL peptide-loaded target cells in both WT and untreated langDTR. This cytotoxic activity was almost completely abrogated when mice had been immunized under continuous depletion of langerin$^+$ DC (Fig. 4*B*).

To investigate immune effector functions in the periphery, we examined DTH responses in gene gun-sensitized mice. The assay employed was based on the assumption that in previously immunized mice, Ag-specific CTL would home to skin areas in which transfected cells express Ag in response to a gene gun challenge.

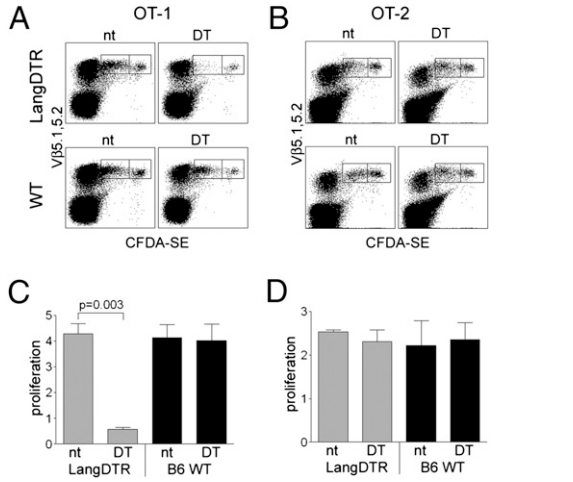

FIGURE 2. Gene gun-induced proliferation of adoptively transferred CD8+ but not CD4+ T cells depends on langerin+ dermal DC. LangDTR or WT mice, either untreated (nt) or treated with DT twice (DT, day 0 and +2), received CFSE-labeled OT-I or OT-II splenocytes, respectively, on day 0. On day +1, mice were gene gun immunized with pCI-OVA, and 3 d later proliferation of viable OT-I and OT-II cells in cLN was analyzed by flow cytometry. *A* and *B*, Representative dot plots of cLN. *C* and *D*, Proliferation of OT-I and OT-II cells calculated as the ratio of dividing to nondividing cells. Data are means ± SD of groups of four mice. Three (OT-II) and five (OT-I) independent experiments were performed, each with groups of four recipients per mouse strain.

Upon interaction with such target cells CTL would release cytokines that initiate a localized inflammatory reaction, very much in the same way that CTL do in response to intracellular pathogens or Th1 cells in a classical DTH reaction against extracellular microbial Ags (37).

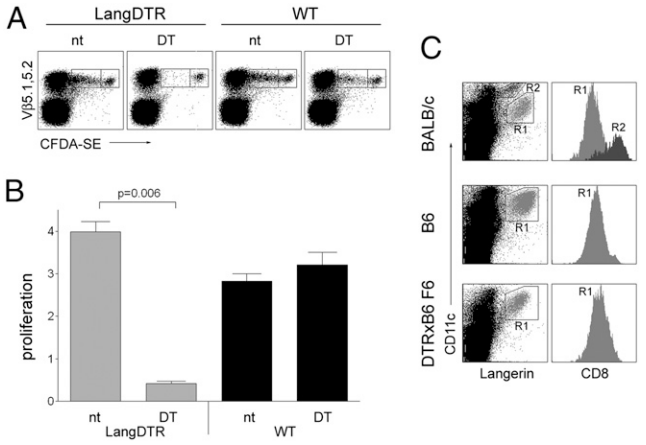

FIGURE 3. CD8+ T cells are directly activated by langerin+ migratory DC and not LN-resident CD8α+ DC. LangDTR or WT mice were gene gun immunized with pCI-OVA (day 0) prior to DT treatment, so that all DC subsets can contribute to Ag transport into LN. Then, mice were either left untreated (nt) or DT-treated twice (DT, day +3 and +5) to ablate langerin+ cells. On day 4, mice received CFSE-labeled OT-I splenocytes, and 3 d later, proliferation of OT-I donor T cells was analyzed by flow cytometry. *A*, Dot plots representative of four mice. *B*, Proliferation of OT-1 cells is calculated as the ratio of dividing to nondividing cells. Data are means ± SD of groups of four mice. *C*, LangDTR mice, after six generations of back-cross onto B6 background (LangDTR × B6:F6), lack an LN-resident langerin+CD8α+ DC subset. Histograms show CD8α+ DC (R2, filled histogram) and CD8α− DC (R1, shaded histogram) among langerin+ LN cells of BALB/c, B6, and LangDTR × B6:F6 mice. Data shown are representative of two independent experiments performed.

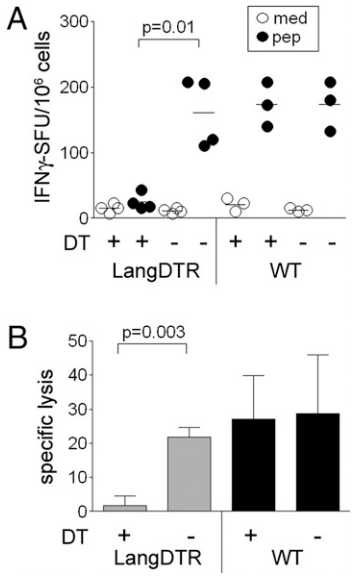

FIGURE 4. Gene gun-induced activation of endogenous CTL depends on langerin+ dermal DC. LangDTR or WT mice were left untreated (−) or treated five times with DT (+) at 3-d intervals, starting 1 d before gene gun immunization. Mice received two gene gun immunizations with pCI-βgal at a 1-wk interval. *A*, One week after the boost, splenocytes were restimulated in vitro with DAPIYTNV or medium and analyzed for IFN-γ secretion by ELISPOT technique. *B*, Specific lysis of DAPIYTNV-pulsed syngeneic target cells injected into langDTR mice 1 wk after the boost. WT mice served as a control. Data are means ± SD of three to four mice per group. Data shown are representative of three independent experiments.

When gene gun-immunized mice were challenged by a further gene gun shot with the same vaccine on both sides of the left ear, inflammation and ear swelling were observed within 48 h. Only moderate reactions were seen in naive mice or mice challenged with an unrelated Ag. Gene gun-induced DTH appeared to rely on CD8+ T cells, because depletion of CD8+ but not CD4+ T cells on the day before challenge reduced ear swelling to the levels observed in naive animals (Fig. 5*A*, 5*B*). Flow cytometric analysis of collagenase-digested ear tissue revealed that the inflammatory infiltrate consisted mainly of CD45+Gr-1+ granulocytes and minor fractions of CD4+ and CD8+ T cells (data not shown). Gene gun-induced DTH reactions were also observed in nondepleted langDTR mice. However, ablation of langerin+ DC during sensitization abrogated ear swelling and markedly reduced the fraction of CD8+ cells in the inflammatory infiltrate (Fig. 5*C–E*).

Production of IgG1 Abs relies on LdDC for gene gun but not protein vaccines

For a variety of different Ags, gene gun vaccines have been reported to induce primarily Th2-dominated humoral immune reactions (38, 39). One exception to this rule is *E. coli* βgal that additionally induces Ab isotypes such as IgG2a or IgG2b (28, 40). To investigate the role of LdDC in Ab formation, we immunized mice with a βgal-encoding gene gun vaccine. DT-treated or untreated WT mice as well as nondepleted langDTR mice produced similar amounts of IgG1, IgG2a, or IgG2b Abs against βgal. Continuous ablation of langerin+ cells during the entire period of immunization barely affected IgG2a or IgG2b Ab titers. In contrast, the induction of IgG1 was almost completely abolished under these conditions (Fig. 6*A*). To search for potential reasons for this effect, we analyzed splenocytes for cytokine production. For preferential targeting of CD4+ T cells we used recombinant protein Ag for

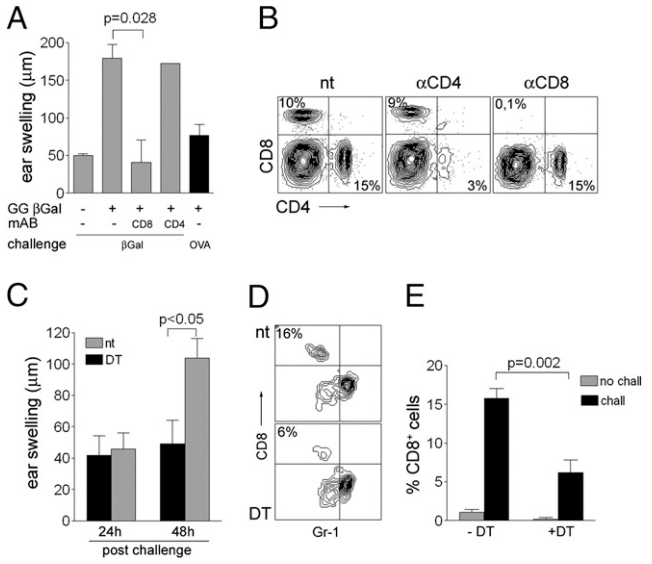

FIGURE 5. Langerin$^+$ dermal DC are the relevant APC for gene gun-induced DTH responses. *A*, DTH effector reactions depend on CD8$^+$ T lymphocytes. Three groups of B6 WT mice were gene gun-immunized with pCI-βgal three times at 1-wk intervals. For CD4$^+$ or CD8$^+$ T cell depletion, mice received 100 μg respective mAb i.v. 1 wk after the last boost, and a third group of mice was not T cell depleted. One day later, mice were challenged with gene gun shots onto both sides of the left ear, and ear swelling was measured after 48 h. Naive mice that were only challenged, and sensitized mice challenged with an irrelevant Ag (OVA, black bar) served as negative controls. *B*, FACS analysis of peripheral blood from untreated (nt) or CD4$^+$ or CD8$^+$ cell-depleted mice. *C*, DTH reaction after DT treatment. LangDTR mice, either under continuous DT treatment (DT) or left untreated (nt), were gene gun-immunized with pCI-βgal twice at a 1-wk interval. One week after the boost mice were gene gun-challenged on both sides of the left ear and ear swelling was measured after 24 and 48 h. *D*, Diminished infiltration of CD8$^+$ T cells in mice gene gun-immunized in the absence of langerin$^+$ DC. Ear cells from *C*, gated on CD45$^+$ cells and analyzed for Gr-1 and CD8 expression. *E*, Percentage of CD8$^+$ T cells detected in gene gun-challenged (chall) or nonchallenged (no chall) ears of langDTR mice either DT treated (+DT) or untreated (−DT). Data represent means ± SD of groups of five mice.

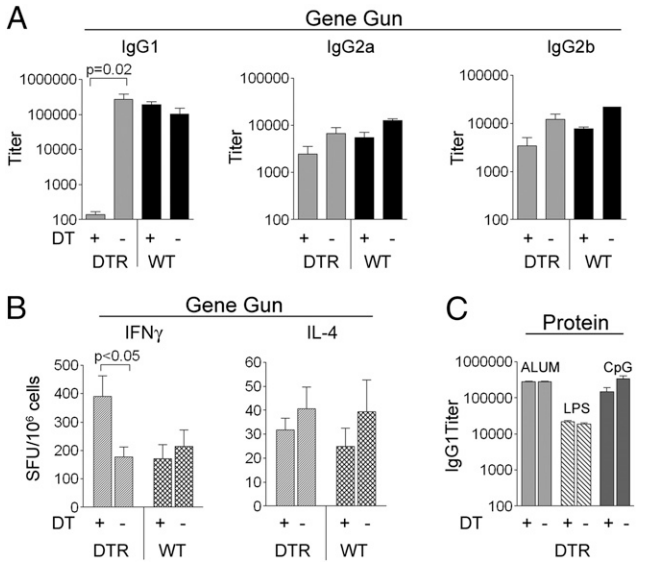

FIGURE 6. Gene gun-induced but not protein vaccine-induced IgG1 production relies on langerin$^+$ DC. LangDTR or WT mice, either under continuous DT treatment (+) or left untreated (−), were gene gun-immunized with pCI-βgal twice at a 1-wk interval. *A*, Serum IgG isotypes were analyzed by ELISA 1 wk after the boost. *B*, IFN-γ and IL-4 ELISPOT assays of spleen cells of the same mice, restimulated with recombinant βGal. *C*, DT-treated or untreated (nt) langDTR mice were immunized with βgal protein i.d. together with the indicated adjuvant (ALUM, LPS, and CpGs) twice at a 1-wk interval. Serum IgG1 was analyzed 1 wk after the boost. Data represent means ± SD of groups of four to five mice. Gene gun-immunized mice were analyzed in two independent experiments, and protein immunization was investigated in three independent experiments, each with a different adjuvant.

restimulation. There were no significant changes in the frequency of IL-4–producing cells. However, IFN-γ–secreting cells were at least 2-fold increased in DT-treated langDTR mice (Fig. 6*B*). Because of the unexpected loss of IgG1, we extended our experiments to examine the role of LdDC for IgG1 production in response to protein vaccines. To this end, langDTR mice were injected i.d. with two doses of recombinant βgal protein with alum, LPS, or CpG oligonucleotides, respectively, as adjuvants, given at a 1-wk interval. One week after the boost, βgal-specific IgG1 was found with each of the adjuvants. In contrast to gene gun vaccines, there was no difference in IgG1 production between nondepleted mice and mice that had been kept devoid of langerin$^+$ cells during the entire experiment (Fig. 6*C*). From this we conclude that LdDC are required for the production of IgG1 in response to gene gun, but not protein, vaccines.

Discussion

The recent discovery of novel DC subsets has shed light onto the complexity of skin immunity. Until a few years ago, epidermal LC have been regarded as the major APC subset of the skin, particularly for epicutaneous Ags such as contact-sensitizing agents or gene gun vaccines. In a previous study on gene gun vaccination, we found that LC deficiency did not affect the development of cellular, CD8$^+$ T cell-dependent, or humoral, CD4$^+$ Th cell-dependent, immunity (28). Our results were in good agreement with studies employing contact hypersensitivity models that, apart from some quantitative variation, did not fully depend on LC either (9, 10, 41). However, LC seemed to become less important with higher doses of contact-sensitizing agents, probably because of increased haptenization of dermal cells (42). Analogously, with our standard conditions for gene gun immunization, a minor fraction of gold particles had penetrated the basement membrane. Thus, we cannot exclude that some transfected cells in deeper skin layers might have provided Ag to dermal DC. Employing langDTR mice, three groups reported the discovery of a novel subset of langerin$^+$ dermal DC (11–13). Unlike LC, this novel subset was readily demonstrated to play important roles not only in contact hypersensitivity but also other models, for example, leishmaniasis, herpes infection, or immune reactions against a keratinocyte-restricted membrane-bound variant of OVA (14–16). In these studies, LdDC were shown to be highly efficient in cross-presentation and activation of CD8$^+$ T cells. Analogously, langerin$^+$ DC from tissues other than skin, too, seem to be important for the activation of CD8$^+$ T cells. For example, influenza virus clearance from the lung of langDTR mice was delayed when langerin$^+$ DC were depleted by intratracheal administration of DT, and this was correlated with aggravated clinical symptoms and reduced numbers of Ag-specific CTL (43).

In the present article, we provide evidence that LdDC are the critical DC subset for the activation and functional differentiation of CD8$^+$ IFN-γ–secreting cytolytic T cells, as well as IgG1 Ab production in response to gene gun vaccines. To this end, we established a regimen for the ablation of all langerin$^+$ cells in langDTR mice over a period sufficient to monitor endogenous lymphocyte activation. The challenge in achieving this goal is in

the rapid repopulation of LdDC, which requires repetitive DT administration at short intervals. A 3-d interval turned out to be appropriate to keep langerin$^+$ DC <1–10% of that found in untreated controls. Notably, this included the minor subset of CD103$^+$ LdDC that has a unique capability for the cross-presentation of skin-borne Ag (14, 16). However, multiple injections of DT induced anti-DT Abs, raising concerns about potentially neutralizing activities (A. Stoecklinger, unpublished data). Moreover, with more than five doses at 3-d intervals we observed side effects such as weight loss in some, though not all, animals (A. Stoecklinger, unpublished observations). Therefore, we restricted our protocol to a period of 2 wk, sufficient to apply 2 weekly vaccine doses and examine the activation of endogenous T cells and at least the early phase of Ab formation.

Adoptive transfer of TCR-transgenic T cells to gene gun-immunized hosts revealed that CD8$^+$ T cell priming strictly required langerin$^+$ DC, whereas CD4$^+$ T cells were activated predominantly by langerin$^-$ DC. This was in line with published observations in other models, which suggested a general functional diversity among cutaneous DC subsets for the management of skin-borne Ag (14–16, 44, 45). However, adoptive transfer experiments may not accurately reflect the fate of endogenous lymphocytes. TCR-transgenic T cells are exceptionally sensitive to cognate peptides, and the number of transferred cells exceeds that of an endogenous naive T cell pool with a given peptide specificity usually by magnitudes (46, 47). Clearly more relevant was that LdDC were the only DC subset capable of activating endogenous CD8$^+$ T cells. Gene gun vaccines are excellent inducers of CD8$^+$ T cell-dependent effector functions, such as Ag-specific cytolytic activity, IFN-γ secretion, and inflammation in peripheral tissues containing Ag-bearing cells. Such functions were all dramatically reduced, if not abrogated, when langerin$^+$ DC were absent.

Generally, DC sample Ag in the periphery and then migrate to LN to interact with T cells. However, an alternative mechanism has been proposed by Allan et al. (48) for herpes infection. According to their study, migratory dermal DC would transport viral Ag to the LN but not prime T cells. Instead, they hand Ag over to LN-resident CD8α$^+$ DC that, in turn, activate CD8$^+$ T cells. We were interested whether gene gun-induced immune reactions would also make use of this mechanism. Our langDTR mice were on B6 background, a strain in that CD8$^+$ DC do not express langerin and, hence, are resistant to DT (49, 50). Taking advantage of this feature, we depleted langerin$^+$ cells at a time when Ag-bearing dermal DC had arrived in the draining LN. In this setting, Ag transfer to CD8$^+$ DC would allow for OT-I proliferation in the absence of langerin$^+$ DC. However, OT-I proliferation was prevented in this setting, suggesting that a langerin$^+$ DC subset directly interacted with CD8$^+$ T cells. Theoretically, migratory skin DC could have transferred Ag to DT-sensitive LC progeny in the LN. However, this seems unlikely since, in the herpes model, this subset was clearly not a target of Ag exchange (16, 34, 48). Also, it does not seem very likely that soluble Ag draining from gene gun-transfected tissue into LN would be cross-presented exclusively by DT-sensitive LC progeny. If so, we should have observed a reduction in CD8$^+$ T cell responses in an earlier study where the regimen of DT treatment kept LC ablated not only in skin but also in LN (28). Therefore, we favor the view that Ag-bearing LdDC migrate to draining LN and stimulate CD8$^+$ T cells by direct interaction.

Because CD4$^+$ OT-II cells responded to gene gun vaccines independently of langerin$^+$ DC, we assumed that Ab production would remain unchanged. Unexpectedly, depletion of langerin$^+$ DC caused a complete loss of Ag-specific IgG1. Likewise, IgE was not detectable in DT-treated animals, but it was also relatively low in nondepleted mice, probably due to the short time span of the experiment (data not shown). In depleted mice we found a 2-fold increase in IFN-γ–secreting spleen cells. However, it remains unclear whether this is sufficient to explain the almost complete loss of IgG1 Abs, particularly in view of the unaltered number of IL-4–secreting splenocytes. In langerin$^+$ DC-depleted mice, adoptively transferred OT-II cells proliferated as efficiently as in untreated individuals. However, this does not preclude that the acquisition of effector functions by endogenous Th cells could be impaired. A general impairment of T cell help could account for decreased B cell activation, but it does not explain why selectively IgG1 (and perhaps IgE) was affected whereas other isotypes were not. In a recent article, the selective ablation of LC by a single DT injection given 13 d before gene gun immunization was sufficient to reduce IgG1 production over a period of 4 wk (51). These data are in apparent conflict with our previous study where even continuous LC deficiency did not significantly affect Ab induction toward two different Ags (28). Admittedly, we do not have a plausible explanation for these discrepancies, except that these studies were employing langDTR strains of different origin. Nevertheless, taken together, these data suggest that gene gun-induced Ab formation is mediated and/or modulated by multiple and distinct DC subsets. Thus, langerin$^-$ DC are sufficient for the production of Th1-related Ig isotypes, whereas LdDC are critically required for IgG1 and, probably, IgE formation.

However, this apparent division of labor among distinct skin DC subsets cannot be generalized. In fact, it was specifically observed with gene gun vaccines but not protein vaccines. When recombinant βgal was injected i.d., IgG1 production was completely independent of LdDC, irrespective of whether the adjuvant was alum, LPS, or CpG. This indicates that langerin$^-$ DC are generally capable of inducing Th cells that, in turn, mediate B cell class switching to type 2-associated Ig isotypes. Theoretically, it cannot strictly be excluded that i.d. injected soluble Ags might drain to the LN without the help of dermal DC, for example, after mechanical trauma or due to hydrostatic pressure at injection sites. Therefore, these observations could also involve direct Ag presentation by LN-resident DC.

Collectively, our results demonstrate that for gene gun vaccines LdDC are the critical DC subset in the periphery that activates both CD8$^+$ T cell-mediated cytolytic and inflammatory effector mechanisms, as well as selectively the formation of Th2 but not Th1 Ig isotypes. The observation that protein vaccines did not depend on LdDC demonstrates the intricacy of Ag presentation by distinct DC subsets present in the skin, depending on the nature of the Ag.

Acknowledgments

We thank G. Posselt for critically reading the manuscript, A. Mehrl, A. Post, and A. Resch for expert animal care, and T. Stepanoska for genotyping.

Disclosures

The authors have no financial conflicts of interest.

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