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Epidermal Langerhans Cells Are Not Required for UV-Induced Immunosuppression

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UV light can be highly beneficial in the treatment of skin disorders such as psoriasis. It is thought to cause immunosuppression by depleting or altering the function of epidermal Langerhans cells (LC). Our previous studies identified a novel langerin+ dendritic cell in the dermis, distinct from LC in phenotype, circulation, and function. In this study, we determined the role of LC and dermal langerin+ cells in UV suppression. UV light suppressed the CD8 T cell response to both contact hypersensitivity and epicutaneous protein immunization, and resulted in a dramatically altered phenotype of LC. UV light did not alter early CD8 T cell activation in the lymph nodes, but rather reduced CD8 T cell expansion at later time points. We found that dermal langerin+ cells, but not LC, were essential for the CD8 T cell response. Furthermore, in the selective absence of LC, UV light still caused suppression of both CD8 T cell expansion and contact hypersensitivity. The Journal of Immunology, 2009, 183: 5548–5553.

The use of UV light therapy is common to treat skin disorders such as psoriasis. The effect is thought to be due to UV-induced immunosuppression (1, 2). UV suppression is most commonly studied in mice using the contact hypersensitivity (CHS)3 model to hapten. Epidermal Langerhans cells (LC) are the predominant dendritic cell (DC) subset in the epidermis and thus are thought to be an important APC affected by UV light in this model (3). UV irradiation was first reported to decrease the abundance of epidermal LC in the skin in 1980 (4). This was corroborated and extended in other studies, which showed that UV irradiation enhances the migration of epidermal LC from skin to s.c. lymph nodes (5). Furthermore, LC that were UV-treated in isolation and transferred back into animals were able to suppress CHS (6). Since these findings, it has been generally accepted that LC are critical for UV-induced immunosuppression.

In our previous research, we identified a novel subset of langerin+ CD103+ DC in the skin, which are distinct from epidermal LC in terms of phenotype, microanatomic localization, and circulation (7). This research was confirmed by two other independent studies (8, 9). Unlike the conventional concept that epidermal LC were the only langerin+ DC in skin and that they could be found in the dermis when migrating to the lymph nodes, this newly characterized bone marrow-derived dermal langerin+ CD103+ DC constitutively resides in the dermis. Furthermore, two-thirds of langerin+ DC in the s.c. lymph nodes are derived from dermal langerin+ DC, and one-third are derived from epidermal LC (7). Importantly, we showed that dermal langerin+ DC have distinct biological functions from epidermal LC and were able to promote a CD8 T cell response to OVA protein, through epicutaneous immunization and the CHS response to hapten, even in the absence of epidermal LC (7, 10). In contrast, evidence is emerging that epidermal LC may also regulate or suppress immune responses (11, 12). Mice that lack epidermal LC, but still retain dermal langerin+ DC, show an enhanced CHS response to hapten (13), and skin grafts from these mice are more efficiently rejected (11).

Thus, to better understand the therapeutic and pathogenic effects of UV irradiation, we wanted to clarify the roles of both epidermal LC and dermal langerin+ DC in promoting skin immune responses and in UV-induced immunosuppression. To examine this question, we first investigated the UV-induced alteration of epidermal LC using a knock-in mouse with the enhanced GFP (EGFP) expressed in the langerin locus (Lang-EGFP mouse) (14). In addition, we conditionally depleted either epidermal LC or dermal langerin+ DC, and investigated the ability of UV irradiation to suppress CHS or the CD8 T cell response to OVA protein applied on the skin. We found that dermal langerin+ DC are required for both responses. In contrast, epidermal LC were not required for either response or for UV suppression of the response.

Materials and Methods

Animals

C57BL/6 (B6) mice were purchased from the National Cancer Institute and bm1 mice were purchased from The Jackson Laboratory. Lang-EGFP knock-in mice and Lang-DTR (a mouse in which the diphtheria toxin receptor (DTR) was introduced into the endogenous langerin locus) knock-in mice were previously described (14). OT-I TCR transgenic mice (15) were crossed to B6.PL-Thy1.1 congenic OT-1.PL. All mice were treated in accordance with federal guidelines, and protocols were approved by the University of Minnesota Institutional Animal Care and Use Committee.

UVB irradiation

UVB irradiation was provided by a bank of two TL 20W/12RS lamps (Philips). Mice were anesthetized with ketamine and xylazine, and a shaved area (the sides of the flanks in epicutaneous immunization and the backs in the CHS experiment) was exposed to 45 mJ/cm2 of UVB light daily for 3 consecutive days. The ears and eyes of the mice were protected by guest on April 20, 2017 http://www.jimmunol.org/ Downloaded from
with CFSE (Molecular Probes). A total of $2.5 \times 10^5$ or $2 \times 10^6$ purified cells were i.v. injected into recipient mice. On the day of immunization, mice were anesthetized and the flanks were hydrated for 15 min with water. The hydrated areas were painted with 10 μg of OVA protein or 469 μg of OVA in 25 μl of PBS (Sigma-Aldrich). These mice were then covered with an occlusive patch (DuoDERM Extra Thin; ConvaTec).

**Preparation of epidermal suspension**

Epidermal cell suspensions from flank skin were prepared by limited trypsinization and dissociation of epidermal sheets by pipetting in DNase, as previously described (7). Epidermal langerin$^+$ cells were identified after this digestion by expression of EGFP.

**Radiation bone marrow chimeras**

Single cell suspensions of bone marrow were prepared and depleted of mature T cells by complement-mediated cytotoxicity with 30H12 (anti-Thy1.2; American Type Culture Collection), as previously described (7). Single cell suspensions of bone marrow were depleted of mature T cells by complement-mediated cytotoxicity with 30H12 (anti-Thy1.2; American Type Culture Collection), as previously described (7).

**In vivo depletion of langerin$^+$ cells**

In all experiments, langerin$^+$ cells were ablated by injection of 1 μg of diphtheria toxin i.p. either on day −4 and −1 or on day −7, with day 0 defined as the day of immunization.

**CHS challenge**

All mice were shaved at least 1 day before immunization. A total of 25 μl of 0.3% 2,4-dinitrofluorobenzene (DNFB; Sigma-Aldrich) in a mixture of acetone and olive oil (4:1) was painted on the backs of the mice. On day 5, all mice were challenged with 5 μl of 0.15% DNFB on both sides of one ear. Ear thickness was measured before and 24 h after challenge, with a spring-loaded micrometer (Mitutoyo).

**Results**

**UV irradiation suppresses the CD8 T cell response to epicutaneous immunization with OVA protein**

UV suppression is commonly studied in animal models of CHS to hapten. However, because it is difficult to track and study Ag-specific T cells in this model, we wanted to determine whether UV could suppress the CD8 T cells response to epicutaneously applied protein. We used a hapten CHS model to determine the optimal dose of UVB irradiation to use for suppression of CD8 T cell-dependent immune responses. C57BL/6 mice were primed and challenged with hapten as shown (Fig. 1A). The immune response to DNFB measured by ear swelling was routinely decreased by >85% from UV exposure at this dose (Fig. 1A). We split the administration of UV light over 3 days to avoid the occasional skin lesions observed with a single dose. This UV irradiation dose and timing was then applied for all of the experiments (Fig. 1B). Epicutaneous OVA protein elicits a modest CD8 T cell proliferative response that can be tracked by adoptive transfer of CD8 T cells from OVA-specific TCR transgenic mice (OT-I) (11). Thus, mice were adoptively transferred with $2.5 \times 10^5$ OT-I CD8 T cells immediately after the third UV irradiation dose, and then immunized with OVA protein on the skin surface. The immune response was checked 6 days after immunization (Fig. 1B). UV irradiation inhibited the CD8 T cell response to this protein immunogen. In the UV-treated group, ~15–20% of transferred OT-I cells remained undivided, and the expansion was reduced ~5-fold in comparison to those in the untreated group (Fig. 1B).

UV irradiation did not deplete epidermal LC, or prevent T cell activation in the lymph nodes, but altered LC phenotype

The association of CHS suppression with the disappearance of epidermal LC induced by UV irradiation was first shown in 1980 (4). Since then, epidermal LC have been generally accepted to be one of the mediators in UV-induced immunosuppression. Further studies suggested that the epidermal LC disappearance might result from UV-induced DNA damage (16) and impaired migration from epidermis to s.c. lymph nodes as well (5). In addition, UV irradiation was observed to alter the phenotype of epidermal LC including the reduction of ATPase and class II MHC (17), inhibition of ICAM-1 (18), and reduced expression of the costimulatory molecules B7-1 and B7-2 (19). In this study, we used Lang-EGFP mice created by Kisslenpfennig et al. (14) to investigate the effect of UV on epidermal LC in vivo. In these mice, EGFP was expressed under the control of langerin promoter, and EGFP expression accurately reflected langerin expression. One side of the Lang-EGFP mouse was irradiated, whereas the other side was protected from UV irradiation as control. Epidermal suspensions from both sides were prepared and stained separately 24 h after the last UV exposure. Unlike previous reports in which UV irradiation

![FIGURE 1.](http://www.jimmunol.org/)

**A**. Groups of mice were irradiated consecutively for 3 days with 45 mJ/cm² UV at each time point, then immunized and challenged according to the experimental scheme shown. DNFB immunization was performed on the flank, whereas challenge was on the ear. Ear thickness was measured 24 h later. Error bars represent SD and data (for n = 3 mice) are representative of at least four independent experiments. B. Mice irradiated as in A, but mice were i.v. injected with $2.5 \times 10^3$ CFSE-labeled CD44$^{low}$ (naive) OT-I.PL CD8 T cells and 24 h later epicutaneously immunized with OVA protein. Mice were then harvested 6 days after immunization. Total OT-I CD8 T cell numbers from spleen and s.c. lymph nodes were determined using flow cytometric analysis. Data are expressed as mean ± SD for n = 18–24 mice/group from eight independent experiments. The CFSE histogram indicated the division of OT-I CD8 T cells.
depleted epidermal LC, UV irradiation under these conditions did not reduce the abundance of epidermal LC, comparing the percentage of EGFP-positive cells to those from the unirradiated side or an unirradiated control animal (Fig. 2, A and B). However, UV irradiation did up-regulate the expression of class II MHC and other costimulatory molecules (Fig. 2C). The absence of LC depletion is likely due to the UV dose used, as LC depletion is highly sensitive to dose in this range (17), and we sought to use the lowest possible dose that would still give suppression to avoid direct damage to the skin. It is possible that LC depletion could have occurred after 24 h, but we chose 24 h for the analysis because that was the time point at which animals were epicutaneously immunized.

The alteration of phenotype indicated that UV irradiation skewed epidermal LC toward an activated or mature state. We therefore tested whether these UV conditioned epidermal LC resulted in greater CD8 T cell activation in lymph node. In epicutaneously immunized mice, CD69 on Ag-specific CD8 T cells was up-regulated in the draining lymph nodes 48 h after immunization, whereas in the same mouse, CD69 from the nondraining lymph nodes remained at the background level (Fig. 2D). As did CD69 on the non-Ag-specific CD8 T cells (data not shown). However, there was no significant difference between UV-irradiated and unirradiated animals in terms of CD69 up-regulation in lymph nodes at this time point (Fig. 2D, left). This finding suggests that the tempo of DC migration and initiation of T cell activation are not grossly altered by UV irradiation. However, 72 h after immunization, only 14 ± 3.5% of CD8 T cell in the draining nodes had divided (CFSE low) in UV-irradiated animals in comparison to 45 ± 5% divided in unirradiated animals, indicating a significant reduction caused by UV exposure (Fig. 2E). This selective effect on cell division, in the face of relatively normal initial activation, is consistent with a potential role for regulatory T cells, as suggested by other studies (20, 21).

Bone marrow-derived (dermal) langerin+ cells are essential for epicutaneous immunization to OVA protein

Epidermal LC had been thought to be the principal APC in the skin to promote both innate and adaptive immune responses (22, 23). However, this premise has been challenged recently by analysis of CHS in mice in which epidermal LC were either conditionally depleted (14, 24) or permanently ablated (13). The latter mice lack epidermal LC from birth, and CHS was actually increased (13), suggesting that LC suppress and not promote CHS. In the conditional deficiency models, one group found CHS was decreased (24), whereas the other group did not observe any change (14). We and others subsequently identified a novel population of dermal
bm1 mice, a C57BL/6 mouse strain carrying a mutant Kb molecule

tuated bone marrow-ablated C57BL/6 mice with bone marrow from
whereas epidermal LC are radioresistant (7–9, 25). We reconsti-
for epicutaneous immunization.

CD44low OT-I CD8 T cells were adoptively transferred into bone marrow

OT-I CD8 T cell expansion.

conditional deficient strain (7–9). Furthermore, we found that
langerin

FIGURE 3. Bone marrow-derived (dermal) langerin
DC are essential for epicutaneous immunization. A, A total of 2.5 \times 10^5 CFSE-labeled
CD44low OT-I CD8 T cells were adoptively transferred into bone marrow chimeric mice with the ability to present OVA protein was limited either to radioresistant epidermal LC (bm1 into B6) or to bone marrow-derived DC (B6 into bm1). Representative plot from three experiments shows CFSE dilution 6 days after immunization. B, Adoptive transfer into bone marrow chimeric mice as in A except that bone marrow chimeras of Lang-DTR into B6 mice and B6 into Lang-DTR mice were i.p. treated with two dose of 1 \mu g of diphtheria toxin at 96 and 24 h before immunization. Results show OT-I CD8 T cell expansion. C, Histogram shows the OT-I CD8 T cell division 6 days after immunization.

langerin

FIGURE 4. UV exposure suppresses the CD8 response to epicutaneous Ag even in the absence of epidermal LC. A, The individual mice and chimeras were UV irradiated, transferred with 2.5 \times 10^5 CFSE-labeled CD44low OT-I CD8 T cells, and immunized with OVA protein. OT-I CD8 T cell expansion was calculated 6 days later. B, UV irradiation as in A, but all of the bone marrow chimeric mice were treated with diphtheria toxin at 96 and 24 h before immunization. One group of mice was UV irradiated and followed with OT-I CD8 T cells transfer and immunization. Results show OT-I CD8 T cell expansion 6 days after immunization. C, Representative histogram shows CFSE dilution of CD8 T cells from three experiments in n = 3 mice/group.

UV suppresses the CD8 T cell response to epicutaneous Ag even in the absence of epidermal LC

Given that epidermal LC were not required to prime a CD8 T cell response to epicutaneous OVA (Fig. 3B), we were able to evaluate whether these cells are required for UV suppression of the response. We tested this hypothesis using chimeric mice. Bone marrow chimeras were UV irradiated on 3 consecutive days as described in Fig. 1B, followed by cell transfer and OVA protein immunization. CD8 T cell expansion and division were measured 6 days after immunization. UV application suppressed the response to OVA to a similar extent in both B6→bm1 and B6→Lang-DTR chimeras as in control mice (Fig. 4). The inhibition was indicated both by the CD8 T cell expansion (Fig. 4, A and B) and cell division (Fig. 4C). This result suggests that neither LC Ag presentation nor epidermal LC themselves are required to mediate UV suppression.

UV irradiation suppressed CHS in the absence of epidermal LC

UV-induced immunosuppression has been generally tested using the CHS model, thus we wished to test the role of epidermal LC in that system. Our previous data demonstrated that dermal langerin
DC are required for promoting the CHS to hapten (7), similar to that shown for OVA in this study. Furthermore, previous data showed that after the depletion of all dermal langerin
cells with diphtheria toxin, dermal langerin
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LC ARE NOT REQUIRED FOR UV-INDUCED SUPPRESSION

FIGURE 5. UV irradiation suppresses CHS even in the absence of epidermal LC. Lang-DTR mice were untreated or treated with diphtheria toxin 7 days before treatment to selectively deplete epidermal LC, whereas allowing dermal DC to repopulate. Mice were then UV irradiated for 3 consecutive days, and 24 h after the last UV treatment, immunized with 25 μl of 0.3% DNFB on the shaved back. At 5 days after immunization, mice were challenged with 5 μl of 0.15% DNFB on the ear. Ear thickness was measured before and 24 h after challenge. The immune response equals ear swelling (after challenge − before challenge).

~50% (7). In contrast, epidermal LC remained >95% depleted at day 7. Replenishment was not observed until at least 2 wk (7, 14), and did not reach 50% even 28 days later (data not shown). Thus, we herein took advantage of the time window of repopulation in the skin to test the hypothesis that UV suppression of CHS requires epidermal LC. Lang-DTR mice were treated with 1 μg of diphtheria toxin i.p. 7 days before immunization to create conditions in which epidermal LC were absent, but the dermal langerin+ cell compartment was partially recovered. Mice were UV irradiated, immunized with DNFB, and then challenged with low-dose DNFB. The immune response measured by ear swelling was significantly reduced in comparison to the UV unirradiated group in both the diphtheria toxin-treated mice, as well as controls (Fig. 5).

Thus, epidermal LC are not required for UV-induced immunosuppression of CHS either.

Discussion

The finding that UV suppression still occurs in the absence of LC is somewhat surprising, as LC have been suggested to be an important target of UV suppression. The number (4, 26), migration (2, 27–29), phenotype (17–19), and Ag-presenting properties (18, 19) of LC have all been shown to be altered by UV treatment. These are correlative findings, of course. However, a gain of function approach, in which LC were UV irradiated and loaded with hapten in vitro, resulted in suppression of immune responses upon reintroduction into animals (6). Furthermore, other gain of function approaches suggested that LC can influence the migration of suppressive regulatory T cells (21). In contrast, our loss of function experiments clearly showed that LC are not required for UV suppression. One possibility is that there is more than one mechanism by which UV exposure causes immune suppression. Indeed, several soluble mediators have been shown to contribute to UV suppression, including IL-10 (30–32), TNF-α (30), calcitonin gene-related peptide (30), cis-urocanic acid (33), prostaglandins (34), and others (3). Although epidermal LC can produce some of these mediators, other cell types may as well, including keratinocytes. Furthermore, the induction of regulatory T cells has been suggested to play a role in UV suppression (35, 36) and the induction of a regulatory T cell response could occur independently of LC. Thus although LC may contribute to UV suppression, they are not required for it, at least in this model.

In this study we also report that dermal langerin+ cells are essential for epicutaneous immunization to OVA protein. This finding is consistent with previous results from our lab showing that dermal langerin+ CD103+ DC were sufficient to promote CHS (7) and the response to OVA protein when epicutanously applied (10). In this study, however, we further show that dermal langerin+ DC, but not epidermal LC or any other DC population, are essential for the CD8 response to epicutaneous immunization with OVA protein. A profound requirement for dermal langerin+ DC is somewhat surprising given that only about half of the DC in the dermis are langerin+. However, we and others have noted a slight difference in the localization of langerin+ DC in the dermis, compared with other DC (generally more perifollicular) (7, 9). Langerin+ DC were recently shown to be required for the CD8 T cell response to influenza in the lung (37), despite the presence of several other DC types, and dermal langerin+ DC were required for optimal production of β-galactosidase-specific IgG2a/c and IgG2b after gene gun immunization (38). Finally, Heath and colleagues (39) recently showed that langerin+ CD103+ dermal DC are the main migratory subtype able to cross-present viral and self-Ags. Thus, the langerin+ CD103+ interstitial DC population may be specialized for carrying Ags to local lymph nodes and initiating CD8 T cell responses.

Because of the unique functions ascribed to the langerin+ CD103+ dermal DC subset in the mouse, it will be important to determine whether human skin bears a population that is functionally equivalent. Three prominent DC populations have been described in human skin, epidermal LC, which are CD1a+ langerin+, and two dermal DC populations: one that is CD1a+ CD14−, the other that is CD14+ CD1a+ (40). Human dermal CD1a+ DC might be equivalent to dermal langerin+ DC in the mouse, as they have been shown to be distinct from migrating epidermal LC (41). However, the expression of langerin is not prominent on either dermal DC subset and the expression of CD103 has not been reported. Further analysis is clearly needed on this subject.

In summary, we show in this study that UV irradiation with the dose used in this study did not reduce the abundance of epidermal LC, but did alter their phenotype and presumed activation status. However, this alteration did not affect the initiation of a CD8 T cell response in the lymph node. This is likely due to the fact that dermal langerin+ DC, but not epidermal LC are essential for initiating the CD8 T cell response to protein and hapten applied to the skin. Finally, UV was fully effective in inhibiting the CD8 T cell response to protein and hapten, even in the absence of epidermal LC. These findings are important in the context of attempts to develop epicutaneous vaccine strategies, and they improve our understanding of the therapeutic and pathogenic aspects of UV-induced immunosuppression.

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Disclosures

The authors have no financial conflict of interest.

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