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*J Immunol* 2010; 185:4633-4640; Prepublished online 15 September 2010; doi: 10.4049/jimmunol.1000246

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Langerhans Cells Serve as Immunoregulatory Cells by Activating NKT Cells

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Ultraviolet exposure alters the morphology and function of epidermal Langerhans cells (LCs), which play a role in UV-induced immune suppression. It is generally believed that UV exposure triggers the migration of immature LCs from the skin to the draining lymph nodes (LNs), where they induce tolerance. However, because most of the previous studies employed in vitro UV-irradiated LCs, the data generated may not adequately reflect what is happening in vivo. In this study, we isolated migrating LCs from the LNs of UV-irradiated mice and studied their function. We found prolonged LC survival in the LNs of UV-irradiated mice. LCs were necessary for UV-induced immune suppression because no immune suppression was observed in LC-deficient mice. Transferring LCs from UV-irradiated mice into normal recipient animals transferred immune suppression and induced tolerance. We found that LCs colocalized with LN NKT cells. No immune suppression was observed when LCs were transferred from UV-irradiated mice into NKT cell-deficient mice. NKT cells isolated from the LNs of UV-irradiated mice secreted significantly more IL-4 than NKT cells isolated from nonirradiated controls. Injecting the wild-type mice with anti–IL-4 blocked the induction of immune suppression. Our findings indicate that UV exposure activates the migration of mature LC to the skin draining LNs, where they induce immune regulation in vivo by activating NKT cells. The Journal of Immunology, 2010, 185: 4633–4640.

Epidermal Langerhans cells (LCs) are immature dendritic cells (DCs) residing in the skin that are distinguished from other DCs by the presence of cytoplasmic organelles, known as Birbeck granules (1), and by strong expression of the transmembrane type II cell-surface lectin langerin/CD207 (2). LCs capture Ags in the skin, undergo maturation, and migrate to lymph nodes (LNs) (3). LCs are traditionally thought to play a crucial role in activating adaptive cutaneous immune responses, such as contact hypersensitivity (CHS). Recent studies using LC-deficient mice, however, have caused a re-evaluation of the exact role of LCs in CHS because three distinct results were obtained when these mice were sensitized with hapten: a diminished CHS response (4), an enhanced CHS response (5), or a CHS response that was no different from that found in the wild-type controls (6). The concept that LCs may not play a major role in cutaneous immunology is further supported by data demonstrated that dermal DCs (dDCs) and not LCs act as the principal APC in leishmaniasis (7) and CHS (8).

LCs also play a role in regulating the immune response. The enhanced CHS response in LC-deficient mice was an early hint that LCs can function as immune regulatory cells (5). LCs are required for graft acceptance across an HY minor histocompatibility barrier, indicating they regulate tolerance induction in vivo (9). Similarly, LCs regulate the induction of graft-versus-host disease in vivo (10). Furthermore, Waithman and colleagues (11) found that presentation of self-Ags (OVA constitutively expressed under the control of the K5 promoter in OVA-transgenic mice) by LCs resulted in the deletion of Ag-specific T cells, resulting in immune tolerance.

Exposing skin to UV radiation, prior to hapten sensitization, suppresses the induction of CHS in both humans (12) and mice (13). Because the morphology and function of epidermal LCs is profoundly altered by UV irradiation (14), their role in UV-induced immune suppression has been intensively studied (15). Hapten-bearing cells, isolated from the draining LNs of UV-irradiated mice, fail to induce CHS when transferred to normal recipient mice; rather they induce immunological tolerance (16). In vitro UV exposure of LCs renders them incapable of presenting Ag to Th1 cells (17). Similarly, in vitro UV exposure downregulates the expression of CD80 and CD86 on LCs (18), suggesting that one mechanism by which UV exposure induces immune suppression and tolerance is by promoting the migration of immature DCs to the LN. Studies by Kripe and colleagues (19, 20) indicate that UV-induced DNA damage, specifically pyrimidine dimer formation, depresses LC APC function.

Although much is published about the role of LCs in UV-induced immune suppression, two major caveats must be kept in mind when reviewing the data in this area. First, studies using in vitro-irradiated LCs may not adequately reflect what happens when LCs are exposed to UV radiation in vivo. Second, in experiments in which hapten-bearing DCs are isolated from the LNs of UV-irradiated mice, FITC is often used. Because FITC is a weak contact allergen, a relatively large volume is required to induce
CHS, raising the concern that free hapten gets to the LN and is taken up and presented to T cells by resident DCs.

Because the UV radiation in sunlight is the primary cause of skin cancer (21), and because UV-induced immune suppression is a major risk factor for skin cancer induction (22), a major focus of our research is to delineate the mechanisms involved. In view of the developing controversy regarding the role of LCs in cutaneous immunology, we decided to examine the mechanism(s) by which UV-irradiated LCs induce immune suppression and tolerance. We were particularly interested in studying the immune function of LCs that migrated from the skin to the LN. Rather than using fluorescent hapten to track migrating LCs, we employed cell sorting to isolate LCs from the LN of UV-irradiated mice. As expected, transferring UV-irradiated LCs to normal mice suppressed CHS and induced immunologic tolerance. Moreover, we noted that LCs colocalized with NKT cells in the LN, and NKT cells isolated from the LN secreted IL-4. Moreover, when LCs from UV-irradiated wild-type mice were transferred into CD1d<sup>-/-</sup> or Jα<sup>18/-/-</sup> NKT-deficient mice, a vigorous CHS reaction was generated. Our findings indicate that LCs play an immune regulatory role and induce immune regulation by activating NKT cells.

Materials and Methods

Animals

Specific pathogen-free female C57BL/6J (CD45.2<sup>+</sup>) and congenic female CD45.1<sup>+</sup> (B6.SJL-Pipp<sup>+</sup> Pep<sup>+</sup>/BoyJ) mice were purchased from The Jackson Laboratory (Bar Harbor, ME), Dr. Dipeng Zhou (University of Texas, MD Anderson Cancer Center, Houston, TX) supplied us with the Jα<sup>18</sup>-deficient mice; CD1d-deficient mice were obtained from Dr. Luc Van Kaer (Vanderbilt University Medical Center, Nashville, TN), and 7 d later, LNs were removed. The CD11c<sup>+</sup>, CD8<sup>+</sup>α<sup>+</sup>-deficient mice; CD1d-deficient mice were obtained from Dr. Luc Van Kaer (Vanderbilt University Medical Center, Nashville, TN). The back of mice was shaved and then irradiated with UV. During irradiation, the mice were anesthetized, and the ears were covered with black adhesive tape.

Abs and reagents

mAbs recognizing CD8<sup>α</sup>, CD11c<sup>+</sup>, CD3<sup>+</sup>, NK1.1<sup>+</sup>, CD1d<sup>+</sup>, IL-4<sup>+</sup>, and I-A/I-E<sup>+</sup>, corresponding isotype controls, and secondary reagents were purchased from BD Biosciences (San Jose, CA). mAbs specific for I-A/I-E, CD80<sup>+</sup>, and CD86<sup>+</sup>, and CD103<sup>+</sup> were purchased from eBioscience (San Diego, CA). mAbs specific for Langerin (CD207) were purchased from eBioscience (clone RMUL.2), Dendritics (clone 929F3) and the monoclonal recognizing the extracellular domain of Langerin (clone 205C1) (23) was purchased from AbCys (Paris, France). The monoclonal specific for epithelial

FIGURE 1. LC migration from the skin post UV radiation. A, Epidermal sheets were obtained from mice 1, 3, 7, 21, or 63 d posttreatment with 1 kJ/m<sup>2</sup> UVB radiation. They were stained with anti-CD207. Scale bar, 100 μm. Original magnification ×200. B, Mice were exposed to 400 J/m<sup>2</sup> ( ), 1 kJ/m<sup>2</sup> ( ), or 2 kJ/m<sup>2</sup> ( ) UVB radiation, and the density of LCs was determined. The data are expressed as percentage of control versus normal LC density. Results are expressed as means ± SD. Each experiment was repeated twice; representative data are shown.
cell adhesion molecule (EpCAM) was purchased from Biolegend (San Diego, CA). Alexa Fluor 594 goat anti-rat IgG (H+L) and streptavidin-conjugated Alexa Fluor 350 were purchased from Invitrogen (Carlsbad, CA). Dinitrofluorobenzene (DNFB) was purchased from Sigma-Aldrich (St. Louis, MO). The PE-conjugated CD1d tetramer loaded with PBS-57, an α-galactosylceramide analog, as well as the empty tetramer control was obtained from the National Institutes of Health Tetramer Core Facility (Atlanta, GA).

Preparation of murine epidermal sheets and immunofluorescence analysis

Murine epidermal sheets were prepared as described previously (24). Postfixation, the sheets were incubated at 23°C overnight with rat anti-mouse CD207 (clone RMUL.2). They were then washed with PBS and incubated at 23°C for 1 h with Alexa Fluor 594 goat anti-rat IgG (H+L). After washing with PBS, they were mounted using Vectashield HardSet Mounting Medium with DAPI (Vector Laboratories, Burlingame, CA). The samples were analyzed using a fluorescence microscope (Olympus, Melville, NY). The number of LCs found in the epidermis was determined by counting at least 10 fields/sample.

Generation of bone marrow chimeric mice

Bone marrow chimeras were prepared according to the method of Ginhoux et al. (25). Six- to 8-wk-old recipient C57BL/6 mice (CD45.2) were lethally irradiated with 1200 rad (660 rad/exposure; two exposures 3–4 h apart). The mice then received 10⁶ CD45.1 bone marrow cells via the tail vein. Six to 8 wk postreconstitution, chimerism was confirmed by measuring the number of CD45.1 cells in the peripheral blood, as described previously (8).

Suppression of CHS and tolerance induction by UV radiation

A modification of the procedure described by Miyazaki et al. (26) was used to induce immunosuppression, and a modification of the procedure described by Matsunaga et al. (27) was used to induce tolerance. The dorsal hair of the mice was removed, and the mice were exposed to 1 kJ/m² UVB.

Suppression of CHS and tolerance induction by UV radiation

One to 35 d later, the mice were sensitized by applying 50 μl 0.5% DNFB solution diluted in acetone/olive oil (4:1) to the UV-irradiated skin. Six days later, the thickness of the right ear was measured with a micrometer (Mitutoyo, Kawasaki, Japan), and 5 μl 0.2% DNFB solution was applied to the dorsal and ventral aspects of the ear. One day later, the thickness of right ear was remeasured. Three weeks later, the mice were resensitized by applying 50 μl 0.5% DNFB solution to the unirradiated shaved abdominal skin. Six days later, the left ear was measured, and 10 μl 0.2% DNFB solution was applied. One day later, ear swelling was measured. LCs were depleted by injecting Lang-EGFP-DTR mice with 1 μg DT (i.p. injection, Calbiochem, San Diego, CA).

In some experiments, immune suppression was transferred by LCs that had migrated to the LN. Inguinal LN from control or UV-irradiated mice were removed 7 d after UV and stained for CD11c, CD8α, and CD207. The CD11c⁺ CD8α⁻ CD207⁺ cells and CD11c⁺ CD8α⁺ CD207⁻ cells were sorted using a FACSARia (BD Biosciences). The sorted cells (1 × 10⁶ to 2 × 10⁷ DCs) were injected s.c. into the backs of wild-type, CD1d-deficient, or Jo-18-deficient mice. Three days later, the mice were sensitized with DNFB, and CHS was measured. In some experiments, 100 μg anti-IL-4 Abs (clone 11B11) and isotype-matched control Ab (eBioscience) were injected i.p. 1 d after transferring the LCs.

IL-4 production by NKT cells

CD4⁺ NK1.1⁺ LN cells from UV-irradiated or control mice were isolated by FACSARia (BD Biosciences). The cells (2 × 10⁶/culture) were stimulated for 16 h with plate-bound anti-CD3 (10 μg/ml) and soluble anti-CD28 (5 μg/ml). The supernatant was collected, and IL-4 production was measured by ELISA (R&D Systems, Minneapolis, MN).

Statistics

In the CHS experiments, the mean change in ear thickness (left ear + right ear divided by 2) was calculated for each animal in the group (n = 5). The change in thickness ± SEM was then calculated for the group. In experiments measuring changes in LN DC numbers, the number of cells per LN for each individual animal (n = 3) was calculated. The mean ± SD was then calculated for the group. Statistical differences between the control and experimental

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**FIGURE 3.** UV-induced LC migration and immune suppression. A. Total number of cells in the draining LN was measured at various times post UVB exposure (1 kJ/m²). *p < 0.05 Student t test versus time 0. B, Langerin (anti-CD207; clone 205C1) expression was measured on gated CD11c⁺, CD8α⁻ cells. White histograms represent CD207 expression and gray histograms represent isotype controls. C. The number of LCs in the LN is shown at different times post UVB exposure. *p < 0.05 versus time 0. D, Suppression of the primary CHS reaction in UV-irradiated mice. Mice were irradiated with 1 kJ/m² UVB radiation and, at the time indicated, were sensitized with hapten. All mice were challenged with hapten (on the right ear) 6 d post sensitization; mean ear swelling ± SEM (n = 5) is shown. *p = 0.011; **p = 0.003; †p = 0.0028 versus no UV. E, Tolerance induction. The mice used in D were rested and resensitized with DNFB 21 d after the first sensitization. They were challenged 6 d (left ear) post resensitization. Data represent mean ear swelling ± SEM; n = 5. Each experiment was repeated twice; representative data are shown. *p = 0.0082; **p = 0.0001; †p = 0.0001; ‡p = 0.0001 versus no UV.
groups were determined using an unpaired two-tailed Student t test (Prism Software version 4, GraphPad, San Diego, CA).

**Results**

**UV-induced migration and long-term survival of epidermal LCs in draining LNs**

Post UV irradiation of the skin, epidermal LC density decreases. Our initial focus was to determine the duration of UV-induced LC depletion (Fig. 1). Posttreatment with relatively low doses of UV radiation (1000 to 2000 J/m²), epidermal LC numbers started to decrease 1 d postirradiation, and 7 d later, almost no epidermal LCs were observed. LCs gradually repopulated the epidermis, and normal numbers were noted 63 d postirradiation (Fig. 1A, 1B). Lower UV doses (400 J/m²) had a similar effect, although the depression was not as severe, and repopulation occurred earlier (Fig. 1B).

Conventional wisdom suggests that UV-irradiated LCs migrate from the skin to the LNs, where they activate immune suppression. It is also clear that other DCs, particularly dDCs, migrate to the LN following trauma to the skin. Moreover, others have shown that dDCs express CD207 on their surface, adding to the complexity of differentiating LCs from dDCs (28). With this in mind, we took advantage of the fact that Merad and colleagues (25) demonstrated that LC precursors are radioresistant, whereas Hogquist and colleagues (28) have shown that dDC precursors are radiosensitive. We reconstituted lethally x-irradiated CD45.2+ mice with CD45.1+ bone marrow cells, which results in a mouse for which the LCs are CD45.2+ and dDCs are CD45.1+ (25). We then exposed the chimeric mice to 1 kJ/m² UVB radiation. Seven days postirradiation, the mice were killed, the LNs draining the dorsal skin were removed, and the phenotype of the CD45.1+ and CD45.2 cells was analyzed (Fig. 2). We gated on the CD45.1+ or CD45.2+ cells and then further gated on the CD8α− CD11c+ cells to discriminate the resident DCs from the infiltrating cells. CD45.2+ cells (LCs) were positive for CD207, CD24a, CD80, CD86, and MHC class II, and a small number of cells expressed CD103 (Fig. 2A). The CD207+, CD103+ cells may reflect a small population of radioresistant dDCs in the face of complete chimerism, recently described by Heni and colleagues (29). The CD45.1+ cells (dDCs) did not express CD207 or CD24a and stained positively for CD103, CD86, and MHC class II (Fig. 2B). Because CD24a and CD103 expression discriminate between LCs and dDCs (28, 30), and in light of the fact that in this chimera, the LCs are derived from radioresistant CD45.2+ precursors, we conclude that UV exposure induces the migration of both LCs and dDCs to the LN; the LCs are CD207+, CD24a+, and the dDCs are CD207− CD24a−.

![FIGURE 4. Migrating LCs induce immunosuppression by activating NKT cells. A. Lang-EGFP-DTR mice were injected with DT 7 d preirradiation with 1 kJ/m² UVB. The mice were sensitized with hapten 7 d post UV (DT+UVB+DNFB). Unirradiated Lang-EGFP-DTR mice (DT+DNFB) received DT 14 d presensitization. Wild-type mice (UVB+DNFB) were irradiated with 1 kJ/m² of UVB and sensitized 7 d postirradiation. The positive control mice (DNFB) were sensitized and challenged. Negative control mice (negative) were not sensitized but were challenged (DNFB versus UVB+DNFB; *p = 0.0012; n = 5). B. Epidermal cell suspensions were prepared from normal mice or mice exposed to 1 kJ/m² UVB. LCs were identified by gating on the CD11c+ I-Ahigh+ cells (blue box on dot blot). Blue lines indicate CD1d expression; red lines indicate isotype control. C. LNs were sectioned and stained with CD3 (blue), LNs in lymph nodes 7 d post UVB. LNs from normal mice (blue scale) were positive for CD207, CD24a, CD80, CD86, and MHC class II, and a small number of cells expressed CD103. D. LNs were positive for CD207, CD24a, CD80, CD86, and MHC class II. E. Transferring LCs isolated from the LNs of UVB-irradiated mice suppresses CHS. Seven days post UV, migrating LCs (UVB-LCs) and dDCs (UVB-dDCs) were collected. LNs isolated from NT-LCs were used as controls. Cells were injected into wild-type recipient mice, and 3 d later, the recipient mice were sensitized with hapten, and CHS was measured. One group of recipient mice received no cells but were exposed to UV (UVB 1kJ/m²). Positive indicates mice that were sensitized and challenged. Negative indicates mice that were not sensitized but were challenged. *p = 0.02 compared with NT-LC control; **p = 0.001 compared with NT-LC control. F. LCs from UV-irradiated wild-type mice (UVB-LCs) were transferred into CD1d−/− mice; 3 d later, the recipients were sensitized with hapten and CHS was measured. UVB 1kJ/m² shows the results in a group of control CD1d−/− mice exposed to UV radiation. G. UVB-LCs were transferred into Jα-18−/− mice; 3 d later, the recipients were sensitized with hapten and CHS was measured. UVB 1kJ/m² shows the results in a group of control Jα-18−/− mice exposed to UV radiation. Results are expressed as mean ear swelling ± SEM; n = 5. Each experiment was repeated twice; representative data are shown.**
Because the protocol employed (UV irradiation of chimeric mice) differs from most of the previous work described in the literature, we were concerned that the differences in the protocols may introduce unwanted artifacts. Therefore, we repeated the experiment using wild-type C57BL/6 mice. The animals were shaved and exposed to 1 kJ/m² UV radiation. Seven days later, the mice were killed, and their LNs were removed. As before, we gated on the CD11c⁺ CD8α⁻ population and stained these cells with CD207, CD24a, EpCAM, which is expressed on LCs but not dDCs (31), and CD103. Because the expression of the epitope recognized by anti-CD207 clone 929F3 does not change with maturation (28), this Ab was used in this experiment. We observed (Fig. 2C) that the CD207⁺ cells coexpressed CD24a and EpCAM, but not CD103. From these data, using both chimeric and normal mice, we conclude that UV exposure activates the migration of CD207⁺, CD24a⁺, EpCAM⁺, CD80⁺, CD86⁺, and CD103⁻ LCs into the draining LNs.

Next, we assessed the time course of LC migration into the LN. Mice were exposed to 1 kJ/m² UV radiation. One to 35 d later, LNs were collected. As expected, we noted a significant increase in the cellularity of the LN 3, 7, and 14 d postirradiation (Fig. 3A; *p < 0.05 versus time 0). This increase in cell number is undoubtedly due to the migration of a variety of myeloid cells into LNs, including dDCs and LCs (6, 8, 23), as well as other cells, such as mast cells (32). However, because we are primarily focused on the role of LCs in UV-induced immune suppression, we concentrated on LC migration (Fig. 3B). We noted a significant increase in CD207⁺ cells in the LN at days 7, 14, and 21 d postirradiation (Fig. 3C; *p < 0.05 versus 0 time control). To test the relation between LC migration and immunosuppression, DNFB was applied directly to the UV-irradiated skin (1 kJ/m²) at various times postirradiation. Immune suppression was most prominent when DNFB was applied 7 d post UVB irradiation (Fig. 3D). When DNFB was applied 21 or 35 d post UVB radiation, no suppression was generated, as the ear swelling response was not significantly different from the positive control (day 0; no UV). To measure UV-induced immune tolerance, the mice were rested and then resensitized with DNFB 21 d after the first sensitization. Tolerance induction was most striking in mice in which DNFB was initially applied 7 or 14 d post UV irradiation (Fig. 3E). These results indicate that UV-induced immunosuppression and tolerance is correlated with a decrease in epidermal LC numbers from the skin (Fig. 1) and correlates with the migration of DCs to the LN (Fig. 3).

**LCs are essential for UV-induced immune suppression**

To further confirm a role for LCs in UV-induced immune suppression, we used Lang-EGFP–DTR mice, in which LCs are depleted by administration of DT. Both epidermal LCs and CD207⁺ dDCs are depleted following DT injection. dDCs repopulate the skin 3–7 d post treatment, whereas LCs remain absent 14–28 d postinjection (28). LC-deficient mice were exposed to UV 7 d post DT treatment, and DNFB was applied to the skin 7 d post UV exposure. We saw no UV-induced immune suppression in these mice (Fig. 4A). In contrast, CHS was not affected by injecting nonirradiated mice with DT 14 d presensitization. These results indicate that epidermal LCs are essential for activating UV-induced immune suppression.

**Migrating epidermal LCs induce immune suppression by activating NKT cells**

During our examination of the cell surface markers expressed on migrating LCs, we noted that UV induced the upregulation of CD1d on the migrating LCs (Fig. 4B, 4C). Because of the prominent role that CD1d plays in activating NKT cells (33), and in light of previous findings showing that the transfer of NKT cells from UV-irradiated mice to nonirradiated controls transfers UV-induced immune suppression (34), we examined the ability of migrating LCs to activate NKT cells. Immunohistochemical analysis of LNs from UV-irradiated mice demonstrated that CD207⁺ LCs migrated to the T cell area of the node and were found in the vicinity of NK1.1⁺ CD3⁺ T cells (Fig. 4D, inset). To determine if LCs activate NKT cells, we performed the following experiments. LCs were isolated from the LNs of UV-irradiated mice. The LCs were then injected into the s.c. space underlying the back skin of normal syngeneic mice. Three days later, these mice were painted with hapten. Six days later, the mice were challenged, and CHS was measured (Fig. 4E). Injecting normal untreated LCs (NT-LCs) had no effect on the CHS reaction (*p = 0.16 versus the positive control). Injecting LCs isolated from the LN of UV-irradiated mice (UVB-LC) caused a dose-dependent suppression of CHS (Fig. 4E; **p = 0.02; ***p = 0.001 versus normal LC control). Injecting dDCs from UV-irradiated mice (UVB-dDCs) did not suppress CHS, indicating that UV does not activate immune regulatory dDCs. As expected, sensitizing mice through UV-irradiated skin (UVB 1 kJ/m²) induced immune suppression. To test the hypothesis that immune suppression results from CD1d⁺ LCs activating NKT cells, we transferred LCs from UV-irradiated wild-type mice into NKT-deficient animals. We noted no immune suppression when nonirradiated mice with DT 14 d presensitization. These results indicate that UV-induced immune suppression and tolerance is correlated with a decrease in epidermal LC numbers from the skin (Fig. 1) and correlates with the migration of DCs to the LN (Fig. 3)
LCs from UV-irradiated mice were injected into CD1d−/− recipients, lacking all NKT cells (Fig. 4F), or Jα-18−/− mice, which lack invariant NKT cells (Fig. 4G). Nor did direct irradiation of NKT cell-deficient mice induce immune suppression, as reported previously (34). These data indicate that transferring LCs isolated from the LNs of UV-irradiated mice activates immune suppression and does so in an NKT cell-dependent manner.

To measure immune tolerance, the mice were rested for 3 wk and then re sensitized on the unirradiated abdominal skin (Fig. 5). LCs isolated from the LNs of UV-irradiated mice activated immune tolerance in wild-type mice (Fig. 5A) but not in the CD1d-deficient or Jα-18−deficient mice (Fig. 5B). These data indicate UV-irradiated LCs induce immune suppression and tolerance and do so by activating NKT cells.

**NKT cells isolated from the LNs of UV-irradiated mice secrete IL-4**

NKT cells modulate immunity by secreting cytokines, such as IL-4 (35). Abs to IL-4 block UV-induced immune suppression, and IL-4−/− deficient mice are resistant to the immunosuppressive effects of UV radiation (36, 37). Therefore, we measured IL-4 secretion by NKT cells (CD4+ NK1.1+) sorted from the LNs of UV-irradiated mice (Fig. 6). The CD4+ NK1.1+ cells stained positively with a CD1d tetramer, confirming they are NKT cells (Fig. 6A). UV exposure significantly enhanced IL-4 secretion by NKT cells (Fig. 6B; p = 0.0006 UVB CD4+ NK1.1+ versus NT CD4+ NK1.1+). Transferring UVB-LCs into wild-type recipients suppressed CHS (Fig. 6C; p = 0.0005 UVB-LC + control Ab versus positive + control Ab). When anti–IL-4 was injected into mice that were injected with UVB-LCs, the suppression was reversed (Fig. 6C; **p = 0.002 UVB-LCs + control Ab versus UVB-LCs + anti–IL-4). Injecting anti–IL-4 had no effect on the induction of CHS in the positive control mice. These data indicate that migrating LCs induce NKT to secrete IL-4, a cytokine critical for UV-induced immunosuppression.

**Discussion**

Conventional wisdom suggests that LCs are the major APC of the skin, for which the function is to take up Ags, migrate to the LNs, and initiate an immune response. However, the observation that dDCs and not LCs are the primary APCs in leishmaniasis and CHS (7, 8) and the fact that a vigorous CHS reaction is found in LC knockout mice (4, 5) suggests that our understanding of LC function is incomplete. It has been recognized for years that UV irradiation of the skin modulates the morphology and function of LCs, transforming them from immune stimulatory cells into immune regulatory cells (38, 39). One suggested mechanism is the migration of immature LCs to the LNs, for which APC function is impaired, thereby resulting in tolerance (17, 18). In view of recent reports questioning the exact role of LCs in the generation of and/or the regulation of the immune response, we re-examined the role of LCs in UV-induced immune regulation. For some time, Langerin (CD207) expression has been used to identify LCs in the LN. However, it is now clear that dDCs also express Langerin (28). For this reason, we took advantage of the observation that LC precursors are radioresistant and dDC precursors are radiosensitive to generate bone marrow chimeric mice (CD45.1 bone marrow transplanted into CD45.2 x-irradiated recipient) to follow DC migration in response to UV exposure. The CD45.2+ cells were positive for CD207, CD24a, CD80, CD86, and MHC class II and expressed low levels of CD103. The CD45.1+ cells, in contrast, did not express CD207 or CD24a and stained intensely positive for CD103, CD86, and MHC class II. Because CD24a and CD103 expression discriminates between LCs and dDCs (28, 30), and in light of the fact that in this chimera, the LCs are derived from radioresistant CD45.2+ precursors, these data indicate that UV exposure induces the migration of both LCs and dDCs to the LN, but the CD207+ population found in the LNs of UV-irradiated mice are LCs. We confirmed the same migration pattern when wild-type mice were UV irradiated (Fig. 2C). The CD207+ cells simultaneously expressed CD24a and EpCAM and did not express...
CD103, indicating they are LCs. Moreover, the findings reported in this study are similar to those recently reported by Yoshiki et al. (31) and support their conclusion that UV exposure activates the migration of CD207+ LCs, but not CD207+ dDCs from the skin to the draining LN.

We found that LCs rapidly migrate to the LN following UV exposure and persist in the LNs for up to 63 d. We found that the suppression of CHS and tolerance induction strongly correlated with LC migration to the LN. The LCs isolated from the LN of UV-irradiated mice expressed I-A, CD80, CD86, and CD1d, indicating that they are mature cells, suggesting that the induction of immune suppression and tolerance cannot be attributed solely to the migration of immature LCs to the LN. Finally, we found that injecting LCs into normal recipient mice suppresses the induction of CHS in the recipient animals, and the induction of immune suppression is associated with the activation of IL-4–secreting NKT cells.

No immune suppression was noted in LC-deficient mice. At least three other groups have used Lang-EGFP-DTR mice to study the role of LCs in UV-induced immune suppression: one reports LCs are dispensable, whereas two others report they are essential (31, 40, 41). What is puzzling about the results reported by Wang and colleagues (40) is that although they report immune suppression, they see no epidermal LC depletion after exposing their mice to 1350 J/m² UVB radiation. This is in contrast to the results reported in this study, which used a similar dose of UV, and findings frequently reported in the literature (13, 14). It appears that the major difference between Wang’s study and the data demonstrating that LCs are essential for UV-induced immune suppression reflects differences in UV dosimetry.

Another hallmark of UV immunosuppression is the generation of regulatory cells in the LN (34, 42, 43). Because all of the energy contained within UV radiation is absorbed in the upper layers of the skin, it is still unclear how the immunosuppressive signal is transmitted from the epidermis to the LNs. Loser and colleagues (44) have shown that LC migration plays a role in the generation of classic T regulatory cells. In addition, Moodycliffe et al. (34) previously demonstrated that IL-4–secreting NKT cells can transfer UV-induced immune suppression, and El-Ghorr and Norval (37) failed to note immune suppression in UV-irradiated IL-4–deficient mice. Our present study expands on these previous observations by demonstrating that LC migration to the T cell area of the LN activates IL-4 secretion by NKT cells and activates immune suppression. Whether the NKT cells are the only source of IL-4 is open to question. Although we note a significant increase in IL-4 secretion by NKT cells isolated from UV-irradiated mice, and we can completely block immune suppression by neutralizing IL-4, the increase in IL-4 production by NKT cells is only 2-fold. Whether the NKT cells (or their secretions) are inducing other cells in the LN to release IL-4 remains to be seen.

NKT cell function and development is restricted by CD1d, which preferentially presents glycolipids to NKT cells (45). Although the identity of the natural ligand for NKT cells is still open to question, it is interesting to note that glucosylceramide is found in high concentrations in the skin, and UV exposure suppressed β-glucocerebrosidase activity, an enzyme that converts glucosylceramide to ceramide, thereby upregulating the concentration of glucosylceramides in the epidermis (46). We suggest that CD1d+ LCs carry the epidermal-derived glycolipids to the LN and activate NKT cells to secrete IL-4. This potential mechanism links epidermal damage with the induction of immune regulatory cells in the LN. This observation may have clinical relevance. Phototherapy with UVB has been used since the 1920s as an effective treatment for psoriasis, an inflammatory Th1–mediated skin disease. IL-4 therapy is also effective in treating psoriasis by inducing a Th2 response (47). Our findings provide a potential mechanism for the therapeutic effects of UV radiation in psoriasis.

Exposure to UV radiation in vivo depresses Th1 immunity, in part through the production of IL-4 and IL-10 (48). The data presented in this paper provide a mechanistic linking events in the skin to immune regulation. These findings may have implications beyond UV-induced immune suppression. In parasite infections, such as schistosomiasis, the induction of a Th2 response is protective. The most common route of human Schistosoma infection is through the skin. Recent findings have indicated the generation of an immune response to Schistosoma mansoni involves Ag presentation by CD1d+ DCs (49), which activate NKT cells to secrete IFN-γ, IL-4, and IL-5 (50). Perhaps the migration of CD1d-positive LCs, as described in this study, plays a role in activating the immune response to parasites that enter via the skin.

In summary, our findings demonstrate that LCs are essential for UV-induced immune suppression and immune tolerance, supporting the concept that these cells are important immunoregulatory elements. They demonstrate that CD1d+ LCs migrate to the LN and activate NKT cells to secrete IL-4. These findings provide a mechanistic link between events that occur in the skin and the activation of immune regulation in LNs.

Acknowledgments
We thank Drs. Dapeng Zhou, Luc Van Kaer, and Bernard Malissen for providing knockout or knockin mice.

Disclosures
The authors have no financial conflicts of interest.

References


