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Enhanced Epidermal Langerhans Cell Migration in IL-10 Knockout Mice¹

Binghe Wang, Lihua Zhuang, Hiroshi Fujisawa, Gayle A. Shinder, Claudio Feliciani, Gulnar M. Shivji, Hirotake Suzuki, Paolo Amerio, Paola Toto, and Daniel N. Sauder²

The migration of epidermal Langerhans cells (LC) to lymph nodes (LN) is critical in the initiation of contact hypersensitivity (CHS) responses. Studies suggest that contact allergen-induced epidermal proinflammatory cytokines, including IL-1 and TNF-α, play important roles in promoting LC migration. Contact allergens also induce epidermal anti-inflammatory cytokines such as IL-10. Since IL-10 down-regulates proinflammatory cytokine production and inhibits CHS, we hypothesized that IL-10 might inhibit LC migration. To test this hypothesis, IL-10 knockout (KO) mice were epicutaneously sensitized with the hapten, FITC, and 24 h later hapten-bearing cells in the draining LN were examined. The number of hapten-bearing cells in the LN was significantly greater in IL-10 KO mice than in wild-type mice. The mutant mice also had an exaggerated CHS to FITC. Pretreatment with anti-TNF-α Ab or IL-1R antagonist significantly reduced the number of hapten-bearing cells in the LN, suggesting that IL-10 modulation of LC migration involves IL-1 and TNF-α. Moreover, IL-10 KO mice demonstrated a greater increase in TNF-α, IL-1α, and IL-1β mRNAs in the allergen-exposed epidermis, and keratinocytes derived from the mutant mice were able to produce higher amounts of TNF-α and IL-1α protein. These data suggest that IL-10 plays an inhibitory role in LC migration and that this effect may occur via the down-regulation of TNF-α and IL-1 production. The Journal of Immunology, 1999, 162: 277–283.

Dendritic cells (DC)³ represent a family of so-called professional APCs. They localize in most peripheral tissues and act as the initiator and modulator of immune responses (1, 2). DC resident in the skin consist of epidermal Langerhans cells (LC) and dermal DC. LC, which represent the major APC within the skin, perform their critical functions in the initiation of cutaneous immune responses by capturing and processing epicutaneous Ag, and then migrating to the regional LN to present Ag to naive T cells (3).

The migration pathway of epidermal LC has been well documented (4, 5). Following epicutaneous application of haptns, hapten-bearing cells appear in the draining LN within 24 h. These hapten-bearing dendritic cells are Ia⁺, and some of them contain Birbeck granules. Furthermore, these hapten-bearing cells can sensitize naive syngeneic recipients for the development of CHS in an MHC-restricted manner. These studies suggest that the hapten-bearing cells (at least a part of these cells) are migratory LC. This migration pathway is further supported by DC transfer studies (6, 7). In a chimpanzee model, DC generated from peripheral blood were labeled with a fluorescent marker and then injected s.c. back into the chimpanzee. The injected DC rapidly migrated to draining LN and interdigitated with T cells in the parafollicular and paracortical zones in a manner very similar to that of endogenous LC (6). In a mouse study, OVA peptide-specific T cells and OVA-pulsed spleen DC were labeled with different color fluorescent markers and then injected into syngeneic naive mice. OVA-bearing DC as well as OVA peptide-specific T cells migrated to the paracortical region of LN to form clusters (7).

Although it is well known that LC migration is critical for the initiation of cutaneous immune responses, little is known about the molecular mechanisms involved. Adhesion molecules, including LFA-1/CD11a, ICAM-1/CD54, VLA6/CD49f (α6 integrin), E-cadherin, and CD44, as well as cytokines, such as proinflammatory cytokines and chemokines, are thought to be involved in such events (8–15).

Considerable evidence suggests that epidermal cell-derived proinflammatory cytokines, including IL-1 and TNF-α, play an important role in promoting LC migration during the induction phase of CHS (13, 16, 17). Under normal conditions, resting epidermal cells synthesize low levels of cytokines. However, stimuli including contact allergens induce epidermal cells to produce significant amounts of proinflammatory cytokines, including IL-1 and TNF-α. In the epidermis, keratinocytes (KC) are the main source of IL-1α and TNF-α, whereas IL-1β is mainly produced by LC (18). The mechanism of contact allergen-induced LC migration may involve IL-1- and TNF-α-mobilizing LC from the epidermis by abolishing the E-cadherin-mediated adhesion between LC and KC (10, 19, 20).

Contact allergens induce epidermal cells to produce not only proinflammatory cytokines but also anti-inflammatory cytokines, including IL-10 (21, 22). IL-10 was previously described as a product of Th2 cells (23). It has been demonstrated that IL-10 is also secreted by monocytes/macrophages, B cells, mast cells, eosinophils, APC, and KC (24). IL-10 inhibits Th1 cell cytokine production and the production of monocyte/macrophage cytokines such as IL-1, TNF-α, and IL-12 (23, 25–27), down-regulates costimulatory molecules such as

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³ Abbreviations used in this paper: DC, dendritic cells; LC, Langerhans cells; CHS, contact hypersensitivity; KC, keratinocytes; KO, knockout; wt, wild type; PE, phycoerythrin; m, mouse; h, human; IL-1Ra, IL-1R antagonist.
B7 expression (28), suppresses the Ag-presenting function of LC (29), and down-regulates CHS responses (30–32).

Since IL-10 inhibits the synthesis of proinflammatory cytokines and down-regulates CHS, we hypothesized that IL-10 might play a role in inhibiting LC migration. To test this hypothesis we have performed a contact allergen-induced LC migration assay in gene knockout (KO) mice deficient in IL-10 production.

Materials and Methods

Mice

Breeding pairs of IL-10 KO mice on the C57BL/10 background were provided by The Jackson Laboratory (Bar Harbor, ME) (33). Mice were housed and bred under specific pathogen-free conditions in the animal facility of Sunnybrook Health Science Center, University of Toronto (Toronto, Canada). Healthy 5- to 7-week-old mice were used for this study. For KC cultures, newborn mice were used. C57BL/10 mice were obtained from the Charles River Breeding Laboratories (Saint Constant, Canada) and used as a wild-type (wt) control. Each experimental group contained 10 mice. All animal protocols were approved by the institutional animal care and use committee.

Reagents

FITC (isomer), dibutyrylphthalate, and LPS (from Escherichia coli) were purchased from Sigma (St. Louis, MO). DNase I was purchased from Boehringer Mannheim (Laval, Canada). Anti-IA, F4/80 (macrophage marker), and anti-CD25 (IL-2R α-chain) were purchased from PharMingen (San Diego, CA). Goat anti-mouse IgG/biotin, goat anti-rat IgG/biotin, and streptavidin/FITC were purchased from Cedara Laboratories (Hongby, Canada). Neutralizing rabbit anti-mouse TNF-α Ab was purchased from Genzyme (Cambridge, MA). Human rHL-1R (rhl-1R) antagonist (IL-1Ra) was supplied by Dr. Charles A. Dinarello (Denver, CO).

Epidermal sheet preparation and immunolabeling

The density of epidermal LC was examined by anti-IA in situ immunolabeling. Mouse ears were excised, split, and floated dermal side down in 0.5 M ammonium thiocyanate for 30 min at 37°C. Epidermal sheets were separated from the dermis, fixed with acetone, and labeled with anti-IA in a three-step immunolabeling procedure as we have previously described (34). Briefly, the epidermal sheets were incubated with anti-IA, then reacted with goat anti-mouse IgG/biotin, and finally incubated with streptavidin/FITC. IAα cells (LC) were counted using a micrometer grid. Ten randomly selected fields were examined, and the IAα cells were expressed as cells per square millimeter (mean ± SEM). In addition, the epidermal sheets were immunolabeled with anti-CD25, anti-CD32, and F4/80 for phenotyping.

Assay for hapten-induced LC migration

Mice were painted on the dorsum of both ears with 25 μl of 1% FITC in acetone/dibutyrylphthalate (1/1), and 24 h later the draining LN, i.e., auricular LN were collected. As a control, auricular LN were taken from naive mice (0 h). LN were gently disrupted, and the stromal fragments were digested with a solution of collagenase IV (0.5 mg/ml)/DNase I (0.02 mg/ml) in PBS at 37°C. The epidermal sheets peeled from the skin samples were stirred in a solution of 0.05% dispase (Boehringer Mannheim) at 4°C overnight. The epidermal sheets were washed, fixed with acetone/dibutyrylphthalate (1/1), and 24 h later the draining LN, i.e., auricular LN were painted with an identical amount of vehicle (acetone/dibutyrylphthalate, 1/1). The CHS response was determined by measuring the degree of ear swelling of the FITC-exposed ear compared with that of the vehicle-treated contralateral ear at 12, 24, 48, and 72 h after challenge using a Peacock spring-loaded micrometer (Ozaki, Tokyo, Japan). The results were expressed as net ear swelling, which was calculated by subtracting the thickness of the vehicle-treated ear (left ear) from the thickness of the FITC-challenged ear (right ear). The percent response was calculated according to the following formula: response = (net ear swelling in KO mice/net ear swelling in wt mice) × 100%.

Cytokine mRNA expression in the epidermis following FITC painting

Ears were excised before FITC painting (0 h) or 2, 6, and 12 h after FITC painting. Epidermal sheets were prepared by 0.5 M ammonium thiocyanate and immediately stored in liquid nitrogen. Subsequently, frozen epidermal sheets were ground with a mortar and pestle. Total RNA was extracted by a single-step method using RNA STAT-60 (Tel-test “B.” Friendswood, TX). The synthesis of oligo(dT)-primed cDNA and PCR were conducted as described previously (43). Primer sets for murine IL-1α, TNF-α, and β-actin were purchased from Clontech (Palo Alto, CA). Primers for murine IL-1β and IL-10 were obtained from Dalton Chemical Laboratories (North York, Canada). The sequences for each primer were as follows: IL-1α primers: upstream, 5′-ATG GCC AAA GTC CCT GAT TGT TTG TT-3′; downstream, 5′-CTC TTT GAT TTC GAC CAC GAT TTC-3′; IL-1β primers: upstream, 5′-ATG GAA GAA GTA CAT GTC G-3′; downstream, 5′-ACA CAA ATT GCA TGG TGA AGT CAG TT-3′; TNF-α primers: upstream, 5′-ATG GGC ACC ACA GGC TTC TGC-3′; downstream, 5′-CCA AAG TAG ACC TGC CCG GAC TC-3′; IL-10 primers: upstream, 5′-GGG GAA GAC AAT TGG GC-3′; downstream, 5′-CAT TTC CGA TAA GGC TTG G-3′; and β-actin primers: upstream, 5′-GGT GGC CGC TTC TCT AGG CAT CAA-3′; downstream, 5′-CCT TTT GAT GTC ACG GAC GAT TTC-3′. PCR product sizes were size fractionated on an agarose gel and photographed under UV light. For relative quantitation, amounts of PCR products were determined by scanning of photo negatives using a laser densitometer, and then the densitometric value of each cytokine was normalized to that of the housekeeping gene, β-actin.

Preparation and stimulation of IL-10-deficient KC

Primary KC cultures were prepared from newborn IL-10 KO and wt mice. Skin samples were taken from the trunk, and connective tissue was trimmed and then placed in α-MEM with 10% heat-inactivated FCS and 1% dispase (Boehringer Mannheim) at 4°C overnight. The epidermal sheets peeled from the skin samples were stirred in a solution of 0.05% trypsin and 0.53 mM EDTA for 20 min at room temperature. Cell suspensions were filtered through a nylon mesh and centrifuged at 1200 rpm for 10 min. Cell pellets were resuspended in α-MEM containing 10% FCS, plated at 2 × 106 cells/10-cm dish, and cultured at 37°C in a humidified atmosphere of 5% CO2. Cultures were fed every 3 days. Before confluence, KC were subcultured by trypsinizing and then replating at a split ratio of 1:4. The KC were cultured to confluence, refed with α-MEM in the presence or the absence of LPS (100 μg/ml), and incubated for 24 h. Culture supernatants were collected and stored at −70°C.

To test whether exogenous IL-10 could reverse alterations in proinflammatory cytokine production in IL-10-deficient KC, cells were preincubated with 50 ng/ml rhl-1R for 20 min and then incubated with LPS for 24 h (44). Culture supernatants were collected and stored at −70°C.

Quantitation of in vitro cytokine production

The concentrations of TNF-α, IL-1α, and IL-10 were quantitated by a sandwich ELISA with Factor-Test-X Mouse ELISA Kits (Genzyme, Cambridge, MA). Absorbance was read at 450 nm. A standard curve was obtained by plotting the concentrations of mouse TNF-α, IL-1α, or IL-10.
FIGURE 1. Normal numbers of epidermal LC are present in IL-10 KO mice. Epidermal sheets were prepared from the ears of IL-10 KO and wt mice using 0.5 M ammonium thiocyanate and then were immunolabeled with anti-Ia/FITC as described in Materials and Methods. Ia⁺ cells (LC) were counted using a micrometer grid and are expressed as cells per square millimeter (mean ± SEM).

FIGURE 2. A higher frequency of hapten-bearing cells are detected from the draining LN of IL-10 KO mice. IL-10 KO and wt mice were painted on the dorsum of both ears with 25 μl of 1% FITC in acetone/dibutylphosphate (1/1); and 24 h later auricular LN were collected. As a control, auricular LN were taken from naive mice (0 h). Single-cell suspensions were prepared, immunolabeled with anti-Ia/PE, and then analyzed on a FACScan.

Results

Normal numbers of epidermal LC are present in IL-10 KO mice

Before performing the LC migration assay on the IL-10 KO mice, it was necessary to determine whether deletion of the IL-10 gene could alter the density of epidermal LC. Epidermal sheets were obtained from IL-10 KO and wt mice and then in situ immunolabeled with anti-Ia. A normal number of Ia⁺ cells (LC) was found in the epidermis in IL-10 KO mice (458 ± 26 cells/mm²), compared with wt mice (475 ± 29 cells/mm²). The level of MHC class II Ag (Ia) expression on LC was similar in the mutant mice and wt mice (Fig. 1). Moreover, the epidermal LC were IL-2Rα-chain⁻, FcyRII⁻, F4/80⁻ in both IL-10 KO mice and wt mice (data not shown). This suggests that the resident epidermal LC of IL-10 mutant mice have maintained an immature phenotype (3).

Greater numbers of hapten-bearing cells are detected from draining LN of IL-10 KO mice

To induce epidermal LC migration to the draining LN, IL-10 KO and wt mice were painted with the hapten, FITC. FITC is not only a contact allergen but is also a fluorescent marker for the migratory LC and has been used in the in vivo LC migration assay since the 1980s (4). The uptake and transport of FITC by LC can be easily traced by fluorescence. Twenty-four hours after FITC painting, the draining LN were collected, and cell suspensions were prepared. LN cells were immunolabeled with anti-Ia/PE and then analyzed by two-color FACS analysis. As shown in Fig. 2, the frequency of Ia⁺, FITC-bearing cells in the draining LN was significantly higher in IL-10 KO mice (4.5%) than in wt mice (2.2%).

To further quantitate FITC-bearing cells, LN cell suspensions were centrifuged on a metrizamide gradient. The cell fraction at the interface consisted of a higher percentage of FITC-bearing cells. As shown in Fig. 3, FITC-bearing cells contained FITC in the cytoplasm, which appeared as a bright granular pattern (Fig. 3A). When FITC-bearing cells were quantitated, their number in the draining LN was significantly greater in IL-10 KO mice than in wt mice (2920 ± 138 vs 1683 ± 124 cells/LN, mean ± SEM; p < 0.005; Fig. 3B).

Pretreatment with TNF-α Ab or IL-1Ra diminishes the enhanced LC migration in IL-10 KO mice

Since IL-10 down-regulates the production of TNF-α and IL-1, both of which promote epidermal LC migration, we examined whether TNF-α and/or IL-1 are involved in the mechanisms responsible for alterations in LC migration in IL-10 KO mice. To investigate the possible role of TNF-α, mice were pretreated with a neutralizing polyclonal rabbit anti-mouse TNF-α Ab before epicutaneous application of FITC. The number of FITC-bearing cells in draining LN was significantly diminished in TNF-α-pretreated mice compared with mice pretreated with rabbit serum (2066 ± 104 vs 2857 ± 118 cells/LN; p < 0.01; Fig. 4).

To examine the possible role of IL-1, we used rhIL-1Ra. IL-1Ra specifically inhibits IL-1 (both IL-1α and IL-1β) bioactivities by blocking the binding of IL-1 to its receptor. Pretreatment with IL-1Ra also decreased the number of FITC-bearing cells compared with pretreatment with PBS (1996 ± 188 vs 2718 ± 106 cells/LN; p < 0.05). These data suggest that the exaggerated migration of LC may be associated with the dysregulation of TNF-α and IL-1 production in IL-10 KO mice.

IL-10 KO mice demonstrate an exaggerated CHS to FITC

To determine whether the CHS response to FITC was altered in IL-10 KO mice, mice were sensitized by applying FITC onto the
shaved trunk and 6 days later were challenged with FITC on the ear. Ear thickness was measured at 12, 24, 48, and 72 h after challenge. As shown in Fig. 5, the CHS response to FITC in IL-10 KO mice was significantly higher than that in wt mice at various time points. The response was increased by 75% at 24 h in IL-10 KO mice compared with that in wt mice (net ear swelling, 17.8 ± 1.7 vs 10.2 ± 1.2 × 10^{-2} mm, mean ± SEM; p < 0.01).

Greater increases in TNF-α, IL-1α and IL-1β mRNAs in the epidermis of IL-10 KO mice painted with FITC

To determine whether expression of proinflammatory cytokine genes was dysregulated in the epidermis of IL-10 KO mice after FITC painting, mRNA levels of TNF-α, IL-1α, and IL-1β were analyzed by RT-PCR. As shown in Fig. 6, before sensitization TNF-α mRNA was below the level of detection in the epidermis of wt mice, but was detectable in IL-10 KO mice. A low level of mRNA for IL-1α was detected from the epidermis of both IL-10 KO mice and wt mice, while IL-1β mRNA was below the level of detection in both genotypes. Two hours following FITC painting, mRNAs for TNF-α, IL-1α, and IL-1β were markedly up-regulated in both KO and wt mice. However, the levels of TNF-α, IL-1α, and IL-1β mRNAs were significantly higher in the IL-10 KO mice at each time point, suggesting that there is an inhibitory effect of endogenous IL-10 on proinflammatory cytokine expression in epidermal cells.

As expected, no IL-10 mRNA was detected in the epidermis of mutant mice at the various time points. IL-10 mRNA was undetectable from the epidermis of wt mice before and 2 h after FITC painting. However, it was significantly up-regulated 6 h after FITC painting.

TNF-α and IL-1α proteins are overproduced by IL-10-deficient KC

To determine whether a deficiency of IL-10 affected proinflammatory cytokine production in KC, IL-10-deficient KC were generated from newborn IL-10 KO mice and then stimulated with

![FIGURE 3. FITC-bearing cells in the draining LN. FITC-bearing cells were enriched from LN cells by performing metrizamide gradient centrifugation as described in Materials and Methods and then examined under a fluorescent microscope. Most FITC was localized in the cytoplasm and appeared as a bright granular pattern (A). The number of FITC-bearing cells was significantly greater in IL-10 KO mice than in wt mice (B).](image1)

![FIGURE 4. TNF-α Ab or IL-1Ra pretreatment reverses the enhanced LC migration in IL-10 KO mice. IL-10 KO mice were injected i.p. with 100 μl of rabbit anti-mouse TNF-α Ab 2 h before FITC painting (A) or were injected intradermally with 10 μg of rhIL-ra into the dorsum of the ear 5 h before FITC painting (B). The auricular LN were collected 24 h after FITC painting. FITC-bearing cells were enriched and then examined by fluorescence microscopy.](image2)

![FIGURE 5. IL-10 KO mice demonstrate an increased responsiveness to FITC in CHS. Mice were sensitized with 400 μl of 0.5% FITC on the shaved trunk and 6 days later were challenged by applying 20 μl of 0.5% FITC on the right ear. The left ear was painted with vehicle as a control. Ear swelling responses were determined using a micrometer at various time points. The results were expressed as the difference in ear thickness between the right and left ears. The CHS responses were significantly higher in IL-10 KO mice compared with wt mice (*, p < 0.05; **, p < 0.01).](image3)
Without LPS stimulation, IL-1α was below the level of detection in the supernatants of IL-10-deficient and wt KC (Fig. 7). No TNF-α was detected in wt KC, but a very low level of TNF-α was detected in IL-10 KO and wt mice were painted on the ear with 1% FITC. Epidermal sheets were prepared from the ear, and RNA was extracted. RT-PCR was performed as described in Materials and Methods. PCR products were resolved on an agarose gel and photographed under UV light (A). For relative quantitation, amounts of PCR products were determined by scanning of negative films, and then the densitometric value of each cytokine was normalized to that of β-actin (B). The data represent the mean ± SEM of three different experiments.

FIGURE 7. IL-10-deficient KC in vitro overproduce TNF-α and IL-1α. Primary KC cultures were generated from newborn IL-10 KO and wt mice. The second generation of KC was incubated in α-MEM in the presence or the absence of LPS (100 µg/ml) and incubated for 24 h. Culture supernatants were collected, and the concentrations of cytokines were quantitated by ELISA. The data represent the mean ± SEM of three experiments performed on duplicate samples.

Exogenous IL-10 abrogates TNF-α and IL-1α overproduction in IL-10-deficient KC

To investigate whether the overproduction of IL-1α and TNF-α in IL-10-deficient KC was caused by deficiency of endogenous IL-10, we preincubated IL-10-deficient KC with rmIL-10 and then stimulated them with LPS. As shown in Fig. 8, preincubation with 50 ng/ml rmIL-10 resulted in a significant reduction of TNF-α and IL-1α production in IL-10-deficient KC, with levels comparable to those observed for wt KC.

Discussion

The two major cellular constituents in the epidermis, KC and LC, and their soluble products, i.e., cytokines, constitute a unique immunologic microenvironment (3). Following exposure of the skin to allergens, epidermal cells are induced to produce a variety of cytokines (45). The complex interactions between these cytokines may either up-regulate or down-regulate CHS responses.

Several studies have shown that epidermal proinflammatory cytokines, such as IL-1 and TNF-α, play an important role in the activation and mobilization of LC. IL-1β plays an essential role in the initiation of CHS responses (12, 21). IL-1β mRNA in LC is up-regulated within 15 min after epicutaneous application of contact allergens. Local injection of IL-1β results in the activation and migration of epidermal LC. Systemic administration of neutralizing Ab directed against IL-1β markedly inhibits contact allergen-induced LC migration (46). Application of contact allergens on
human skin organ cultures induces IL-1β synthesis and LC migration out of the epidermis, and this hapten-induced LC migration can be prevented by preincubation of skin explants with a neutralizing IL-1β Ab (47). Moreover, incubation of skin explants with rIL-1β induces LC migration out of the epidermis. IL-1α is probably also involved in LC/DC migration, since systemic administration of IL-1α in mice results in epidermal LC activation and dermal "cord" formation (16).

Another important proinflammatory cytokine involved in LC migration is TNF-α. In the epidermis, KC are the main source of TNF-α, although other cell types may also produce small amounts of this cytokine (18). Systemic or intradermal administration of TNF-α in mice results in LC migration (13, 16). The migration of epidermal LC induced by hapten or LPS can be inhibited by neutralizing Ab directed against TNF-α (13, 16). TNF-α has been demonstrated to be able to induce a complete rearrangement of the actin-based cytoskeleton in DC, including depolymerization of F-actin and loss of vinculin-containing adhesive structures. As a result, DC acquire high cell motility (48). Recently, we examined the role of each TNF receptor in LC migration using gene KO mice deficient in TNF receptor p55 or p75. We have demonstrated that while LC migration in receptor p55-deficient mice is normal, receptor p75-deficient mice demonstrate a markedly depressed migration of LC, suggesting a crucial role of TNF receptor p75 signaling in such an event (49, 50).

Since LC migration is a necessary step for the initiation of CHS, we examined whether the suppressive role of IL-10 in CHS is partly due to inhibition of LC migration. In the present study we performed hapten-induced LC migration experiments in IL-10 KO mice to address this issue. The FITC-induced LC migration assay has been used for the in vivo study since the 1980s (4, 5, 50, 51). This system allows us to investigate the entire pathway of LC migration, including the dermal "cord" formation (52).

We propose that in the epidermal microenvironment, a balance between pro- and anti-inflammatory cytokines may control LC mobility. In the normal state, resting epidermal cells do not produce significant amounts of cytokines. Homeostatic balance of pro- and anti-inflammatory cytokines keeps LC in the epidermis. Certain stimuli, such as contact allergens, induce epidermal cells to produce significant amounts of proinflammatory cytokines and thus promote LC migration. At a later stage, epidermal cells produce anti-inflammatory cytokines, thus inhibiting LC migration, and the epidermal cytokine milieu is rebalanced and finally returns to homeostatic balance of cytokines.
to basal levels. Understanding the molecular mechanisms of epidermal LC migration would have important clinical implications in immunologic and inflammatory diseases of the skin.

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