Role of the Parasite-derived Prostaglandin D$_2$ in the Inhibition of Epidermal Langerhans Cell Migration during Schistosomiasis Infection

By Véronique Angeli,* Christelle Faveeuw,* Olivier Roye,‡ Josette Fontaine,* Elisabeth Teissier,§ André Capron,* Isabelle Wolowczuk,‡ Monique Capron,* and François Trottein*

From the *Centre d’Immunologie et de Biologie Parasitaire, Institut National de la Santé et de la Recherche Médicale (INSERM) U547, ‡Centre National de la Recherche Scientifique 8527, Institut de Biologie de Lille, and §INSERM U545, Institut Pasteur de Lille, Lille 59019, France

Abstract

Epidermal Langerhans cells (LCs) play a key role in immune defense mechanisms and in numerous immunological disorders. In this report, we show that percutaneous infection of C57BL/6 mice with the helminth parasite *Schistosoma mansoni* leads to the activation of LCs but, surprisingly, to their retention in the epidermis. Moreover, using an experimental model of LC migration induced by tumor necrosis factor (TNF)-α, we show that parasites transiently impair the departure of LCs from the epidermis and their subsequent accumulation as dendritic cells in the draining lymph nodes. The inhibitory effect is mediated by soluble lipophilic factors released by the parasites and not by host-derived antiinflammatory cytokines, such as interleukin-10. We find that prostaglandin (PG)D$_2$, but not the other major eicosanoids produced by the parasites, specifically impedes the TNF-α–triggered migration of LCs through the adenylate cyclase–coupled PGD$_2$ receptor (DP receptor). Moreover, the potent DP receptor antagonist BW A868C restores LC migration in infected mice. Finally, in a model of contact allergen-induced LC migration, we show that activation of the DP receptor not only inhibits LC migration but also dramatically reduces the contact hypersensitivity responses after challenge. Taken together, we propose that the inhibition of LC migration could represent an additional stratagem for the schistosomes to escape the host immune system and that PGD$_2$ may play a key role in the control of cutaneous immune responses.

Key words: dendritic cells • migration • *Schistosoma* • eicosanoids • cAMP

Introduction

Dendritic cells (DCs)$^1$ are professional APCs that initiate primary immune responses in lymphoid tissues (1). Among them, epidermal Langerhans cells (LCs) play a key role in the establishment of cutaneous immunity. Under normal, noninflammatory conditions, LCs reside in the epidermis anchored to neighboring keratinocytes (KCs) via homotypic E-cadherin interactions (2). In this environment, they display an immature phenotype characterized by high antigen uptake and processing abilities and poor T cell stimulatory function. However, in response to stimulation occurring during infection or topical application of allergens, LCs activate, and a proportion of them migrates via afferent lymphatics to regional LNs where they accumulate as immunostimulatory DCs (1, 3). Similarly, emigration of LCs from the epidermis may be initiated via an antigen-independent manner, for instance by skin irritants, ultraviolet irradiation, or microbial CpG motifs (4, 5). During their migration, LCs undergo a complex process of maturation, becoming less effective in capturing and processing antigens but more specialized in stimulating naive T lymphocytes. This latter property is partly mediated by an increased expression of MHC class I and class II and...
costimulatory molecules including intracellular adhesion molecule (ICAM)-1 (CD54), CD40, B7-1 (CD80), and B7-2 (CD86) (6).

The molecular mechanisms that govern LC migration have been the purpose of extensive research in the past few years. Accumulating evidence suggests that the synthesis of inflammatory cytokines, particularly TNF-α and IL-1β, is one of the first events in the multistep cascade leading to LC departure from the epidermis (7, 8). These cytokines are respectively produced by KCs and LCs in response to skin-penetrating pathogens or to contact allergens, and affect the interactions between KCs and LCs by diminishing the expression of E-cadherin (9) and by stimulating actin-dependent movements of LCs (10). Other adhesion molecules such as ICAM-1, very late antigen (VLA)-6 (CD49d), CD40, and CD44 also play a role in the migratory properties of LCs. In addition, the importance of the seven transmembrane-spanning G protein-coupled receptor family in driving LC motility has also been reported (11-14). Among them, the CC chemokine receptor (CCR)7 is sharply upregulated during LC maturation and is crucial to attracting LCs into the LNs (11, 12). On the other hand, LC emigration is associated with a rapid decrease in the expression of receptors for inflammatory chemokines such as CCR1 (13).

Mechanisms controlling the emigration of epidermal LCs after activation have been reported. In this phenomenon, the antiinflammatory cytokine IL-1ra has been shown to block the binding of IL-1β to its receptor (15). Similarly, IL-4 and IL-10 may act as negative regulators of LC migration. Takayama et al. (16) recently showed that IL-4 interferes with the TNF-α–induced mobilization of LCs by downregulating the expression of TNFR-II on LCs. In a model of contact allergen-induced LC migration, Wang et al. (17) suggested that IL-10 impedes LC emigration, at least in part, by downregulating the synthesis of IL-1β and TNF-α by epidermal cells. Although other components may also be involved (18, 19), these studies suggest that LC motility is tightly controlled by the homeostatic balance between pro- and antiinflammatory cytokines produced early in the skin, and that this balance quantitatively and qualitatively affects the resulting adaptive immune response (20). During cutaneous infections, skin-penetrating pathogens may directly or indirectly influence such events. For instance, the intracellular parasite Leishmania major favors the rapid production of inflammatory cytokines in the skin and provokes LC migration to the skin-draining LNs (SLNs; reference 21).

In this report, we have analyzed the effects of the helminth parasite Schistosoma mansoni, the causative agent of schistosomiasis, on the activation state and migratory abilities of LCs. Indeed recent demonstrations that schistosomes interfere with some inflammatory pathways in host cells (22-24) and that certain pathogens, particularly viruses and intracellular parasites, can profoundly alter the functions of DCs (25-29), prompted us to initiate this study. Schistosoma mansoni has a complex migratory route within its vertebrate host that is initiated by the penetration of the larvae (termed cercariae) through the skin. In the cutaneous environment, transformation of cercaria into schistosomulum is accompanied by the release of a wide range of proteases and fatty acid derivatives which facilitate parasite migration through the skin (30). Additionally, parasite larvae closely interact with cutaneous immunocompetent cells, while remaining in the skin for 3 to 4 d. The nature and immunological consequences of these interactions have not yet been fully studied. Here, we show that, after murine infection, schistosomula activate LCs but, surprisingly, impede their migration to the SLNs. This inhibitory effect, which also occurs in a TNF-α–induced model of LC migration, is mediated by excreted/secreted (ES) lipophilic factors produced by parasite larvae, particularly by PGD2. We speculate that schistosomes may utilize this stratagem to limit and/or orientate the host immune response. We also propose a new function for PGD2 in skin homeostasis and in the regulation of the cutaneous immune response.

Materials and Methods

Reagents and Abs. All reagents were purchased from Sigma-Aldrich unless otherwise notified. PGD2, PGE2, PGF2α, 5-hydroxyeicosatetraenoic acid (HETE), 15-HETE, leukotriene (LT)B4, LTC4, and BW245C were from Cayman Chemical. BW A868C was donated by Dr. S. Lister (Glaxo Wellcome, Greenford, UK). The anti-I-A^d/I-E^d mAbs (clone M5/114, rat IgG2b) and the anti-DEC-205 (NLDC-145, rat IgG2a) were provided by Drs. A. Ager (National Institute for Medical Research, London, UK) and D. Sacks (National Institutes of Health, Bethesda, MD), respectively. The FITC-conjugated anti-CD80 (hamster IgG), anti-CD86 (rat IgG2a), and biotin-conjugated anti-CD11c (hamster IgG) mAbs were purchased from BD PharMingen. The neutralizing anti–IL-10 mAb (clone JES052A5, rat IgG1) was from R&D Systems and the iso- type control mAb from Caltag Laboratories.

Cell Lines. The LC line XS52 has been established from mouse epidermis and presents the phenotypic and functional features of LC (31). XS52 was cultured in RPMI containing 10% (vol/vol) heat-inactivated FCS in the presence of 2 ng/ml GM-CSF (Biosource International) and 10% (vol/vol) NS47 fibroblast supernatant as described (32). Mouse Pami212 KCs were cultured in Eagle’s MEM complemented with 10% FCS and 0.05 mM CaCl2 (31).

Mice, Parasites and Infection Protocols. Young adult wild-type (WT) and IL-10–deficient (knockout [KO]) C57BL/6 mice (6- to 8-wk old) were purchased from Iffa-Credo. The S. mansoni (Puerto Rican strain) life cycle was maintained in Biomphalaria glabrata snails as the intermediate host and the hamster Mesocricetus auratus as the definitive host. Skin schistosomula and schistosoma ES products (SESP; the supernatant of a 4-h culture containing 10^3 parasites/ml) were prepared as described (23, 24). The methanol/chloroform-extracted fraction from the SESP (termed the lipophilic fraction) was obtained by a modified Folch extraction protocol (24). The organic phase was dried under a stream of nitrogen and resuspended in DMSO (for biological studies) or methanol (for HPLC analysis) (50 μl/50 ml parasite culture). For S. mansoni infection, mice were anesthetized with pentobarbital

1136  PGD2 and Langerhans Cell Migration
(30 mg/kg; Sanoﬁ) and exposed to 250 cercariae by immersion of the ears for 25 min.

Identification and Quantification of Eicosanoids. Eicosanoids recovered from the schistosomula culture medium were extracted as described above and analyzed by HPLC on a 3.9 × 150 mm Novapack C-18 reverse phase column (Waters). Elution was carried out at a rate of 0.5 ml/min with acetonitrile/water (40:60 vol/vol) plus 0.01% (vol/vol) trisuforoacetic acid. Peak elution was monitored at 195 nm for PGs, 230 nm for conjugated dienes, and 270 nm for LTs. Identification and quantification of various HPLC peaks were performed by injecting known quantities of eicosanoid standards. Enzyme immunoassay (ELIA) was also used to quantify PGF$_2$α, PGE$_2$, PGD$_2$, LTB$_4$, and LTC$_4$ directly from the parasite culture supernatant with kits provided by Cayman. Results in Table II represent the concentration of individual eicosanoid detected per ml of culture (10$^3$ parasites/ml).

Cytokine and Ab Administration. Recombinant murine TNF-α (specific activity ≥ 5 × 10$^9$ U/mg) (R&D Systems) was reconstituted in sterile PBS containing 0.1% (wt/vol) BSA as a carrier protein. Mice were intradermally injected with 50 ng TNF-α in sterile PBS (final volume: 30 µl). Neutralizing anti–IL-10 or isotype-matched control mAb diluted to inhibit 50% IL-10 activity was injected intraperitoneally at 30 min before treatment and fixed in a formaldehyde-free zinc fixative (ImmunoHistoFix; Interstiles sprl) for 7 d at 4°C. After dehydration in graded alcohol baths, embedding was performed by three successive immersions in ImmunoHistoWax (Interstiles sprl) at 37°C. Sections of 5 µm thickness were dewaxed in acetone for 5 min and immunostained with anti-CD11c Ab as described above. For immunohistochemical analysis, sections were counterstained with hematoxylin and mounted in Immumount.

Induction and Elicitation of Contact Hypersensitivity Responses. Mice were sensitized by painting 10 µl of a 0.5% solution of FITC in acetone/dibutylphtalate (1:1, vol/vol; vehicle) on the total surface of the left ear. 30 µl of BW245 (100 nM) or DMSO (as a control) was injected intradermally 15 min before and 5 h after sensitization. Contact hypersensitivity (CHS) was elicited 5 d after the sensitization by painting the dorsal and ventral surface of the right ear with 10 µl of 0.5% FITC (35). Ear thickness was measured 8 h after elicitation.

Table I. Sequences of Primers Used for PCR Amplification of cDNA, Product Sizes, and PCR Cycle Numbers

| Gene          | Primer | Sequence                        | Size (bp) | Cycle |
|---------------|--------|---------------------------------|-----------|-------|
| β-actin       | 5’     | 5’-CTCGGGCGCAGCGAGCAGCA         | 539       | 28    |
|               | 3’     | 5’-CTCCTTAATGTCACCAGATTTTC      |           |       |
| TNF-α         | 5’     | 5’-AACCCACAACTGGAGGCCAGGC       | 312       | 36    |
|               | 3’     | 5’-TGACCTAGCGCCTGAGTTGTTGCCC   |           |       |
| IL-1β         | 5’     | 5’-TGAAGGGGTGCTTCCGAAAACCTTTGACC | 322       | 36    |
|               | 3’     | 5’-TCTCATTGAGTTGAGGGATCATCGG   |           |       |
| IL-1ra        | 5’     | 5’-CTGCAAGATGCGAGGCTTACAGG     | 353       | 38    |
|               | 3’     | 5’-GAGTGATACTGAGGCTTACATC      |           |       |
| IL-4          | 5’     | 5’-GATGCTACAGGAGGCCAGCT        | 384       | 35    |
|               | 3’     | 5’-GATGCTACAGGAGGCCAGCT        |           |       |
| TNFR-II       | 5’     | 5’-GTACGGCGCTGAGGCAAGAGGACCT   | 312       | 35    |
|               | 3’     | 5’-GTACGGCGCTGAGGCAAGAGGACCT   |           |       |
| IL-10         | 5’     | 5’-TCTTATAAGCGAGTAAAAGGGTTAC   | 246       | 38    |
|               | 3’     | 5’-GACACCTTGCTTGGAGGCTTAAA     |           |       |
| DP receptor   | 5’     | 5’-GAAGTTCTGCGAGGTAGTTGAGG    | 435       | 35    |
|               | 3’     | 5’-TCCACTATGGAATTCAGACAG       |           |       |

Skin Explant Assay. After treatment, ears were rinsed in 70% ethanol and split into dorsal and ventral halves with forceps (34). Four sheets were floated, dermal side down, on 4 ml RPMI supplemented with 25 mM Heps, 10% FCS, and gentamycin (50 µg/ml). After 24 h incubation at 37°C in a 5% CO$_2$ incubator, epidermal sheets were prepared and LCs were enumerated as described above.
measured using an engineers’ micrometer (Mitutoyo) 24 h after challenge. Results are expressed as ear swelling, which was calculated by subtracting the thickness of the ear before the challenge from the thickness of the ear after the challenge. In experiments where elicitations were not required, mice were killed 18 h (for the determination of epidermal LC density) or 24 h after sensitization. To determine the number of migrating FITC-positive DCs in the SLNs, single cell suspensions were prepared from auricular LNs and DCs were enriched by centrifugation on a 14.5% (wt/vol) metrizamide gradient. DCs were then stained with the biotin–conjugated anti-CD11c mAb followed by phycoerythrin–streptavidin. The percentage of CD11c 

\( ^{+} \)FITC 

\( ^{+} \)LN cells was determined on a FACSCalibur™ flow cytometer (Becton Dickinson). Data were analyzed using CELLQuest™ software.

**mRNA Extraction and Reverse Transcription PCR Amplification.** Ears from mice were excised, the epidermis were separated from the dermis, and total RNA was isolated using TRizol reagent (Life Technologies). RNA from resting XS52 and Pam212 cells were isolated as described above. cDNA was synthesized from 1 

\( \mu g \) of total RNA with random hexamer primers and Superscript reverse transcriptase (Life Technologies) using standard procedures. PCR amplifications were performed with the primer pairs indicated in Table I. Amplified products were subjected to 1% agarose gel electrophoresis and visualized by ethidium bromide staining.

**Results**

* S. mansoni Induces LC Activation In Vivo. The distribution of epidermal LCs was visualized by immunohistochemical staining with anti-MHC class II molecule Abs in noninfected or *S. mansoni*–infected skins (Fig. 1, A and B). In a kinetic study (1 to 120 h), the morphology of epidermal LCs dramatically differed in infected sheets compared with controls at all time points examined. In infected skins, LCs markedly increased in size and exhibited a more dendritic morphology with typical interdigitating cellular processes. Moreover, the MHC class II staining on LCs from infected epidermis was more intense. Immunolabeling with the LC-specific Ab NLDC-145 confirmed the activated phenotype of LCs in infected skins (not shown).

We next analyzed other surface markers known to be expressed by mature LCs. Although we could barely detect CD86-positive cells in control epidermis (Fig. 1 C), the expression of CD86 on LCs was strongly upregulated in infected skins, 12 h (Fig. 1 D) to 120 h (not shown) after parasite penetration. Interestingly, most of the CD86-positive cells were located in the vicinity of the parasite or around

![Figure 1](image-url). Immunohistochemical staining of murine epidermal sheets after transcutaneous infection by *S. mansoni*. Epidermal sheets were prepared either from noninfected or from *S. mansoni*-infected mice and LCs were stained for MHC class II (A and B) or for CD86 (C and D). The arrow indicates the “ghost” of parasite. The isotype control mAb did not reveal any reactivity (not shown). Original magnification: X400.
its “ghost.” In contrast, we were unable to detect any CD80-positive cells either in noninfected or infected skins whatever time after infection (not shown). Taken together, our data suggest that, early after S. mansoni infection, LCs display clear signs of activation, exhibiting a more dendritic appearance and expressing higher amounts of MHC class II and CD86 molecules.

*S. mansoni* Induces Retention of Activated LCs in the Epidermis *In Vivo* and *Ex Vivo*. As after activation, LCs normally migrate from the epidermal site of antigen capture to the SLNs after *S. mansoni* penetration. To this end, the frequency of MHC class II-positive cells was determined in the epidermis at different times after infection (1 to 120 h). As shown in Fig. 2 A, the density of MHC class II-positive epidermal cells in naive and noninfected mice ranged between 450 to 470 LCs/mm². Surprisingly, in comparison to control epidermis, the number of LCs/mm² remaining in the epidermis was not reduced 1 to 120 h after *S. mansoni* infection. To confirm this data, we used a complementary approach based on the spontaneous migration of LCs from skin explants cultured in vitro for 24 h (34; Fig. 2 B). In noninfected mice, compared with a freshly isolated epidermis, the number of LC/mm² remaining in the epidermis after 24 h culture dramatically decreased to 188 ± 22 (vs. 452 ± 32, 58% reduction). In contrast, in infected animals, the density of LCs remained constant 6 to 48 h after infection and decreased significantly to 225 ± 11 (50% reduction) 120 h after infection, a period that coincides with the departure of the parasites from the skin. Together, both in vivo and ex vivo approaches suggest that parasite infection causes the activation of epidermal LCs but prevents their migration from the epidermis.

*S. mansoni* Inhibits the TNF-α-induced Migration of LCs. We next investigated whether *S. mansoni* infection could alter LC migration in a system known to promote a strong LC departure to the SLNs (7). For this purpose, mice were injected into ear pinnae with TNF-α and the capacity of LCs to emigrate from the skin was then assessed 1 h after injection. As shown in Fig. 3 A, in noninfected mice, TNF-α caused ~54% reduction in LC frequency compared with control mice (carrier). By contrast, infection by *S. mansoni* inhibits the TNF-α-induced LC migration 6 and 24 h after infection. Interestingly, the migratory ability of LCs was restored in mice infected 120 h before TNF-α treatment. Taken together, these results show that schistosomula transiently interfere with TNF-α to inhibit LC migration from the epidermis to the SLNs. To further confirm this, we visualized the accumulation of DCs in the SLNs isolated from TNF-α-treated mice previously infected (24 h before TNF-α treatment) or not with *S. mansoni*. As observed in Fig. 3 B, in noninfected mice, TNF-α administration resulted in increased number of CD11c-positive cells in the T cell areas of the SLNs whereas few CD11c-positive cells were detected in the SLNs from TNF-α-treated *S. mansoni*-infected mice.

**IL-10 Is Not Sufficient to Inhibit LC Migration in *S. mansoni*-infected Mice.** To determine the mechanism by which *S. mansoni* inhibits LC migration, we first investigated by reverse transcription (RT)-PCR the presence of mRNAs for cytokines known to control LC mobility at various times after infection (1 to 120 h). As shown in Fig. 4 A, compared with noninfected mice (0 h), we observed a rapid and sustained increase in TNF-α and IL-1β mRNA levels in the epidermis of infected mice suggesting that the signals required for LC departure may be present. We then tested the hypothesis that the observed inhibitory effect could be associated with the expression of antiinflammatory cytokines. Interestingly, we detected a marked up-regulation of IL-10 mRNA, particularly between 6 and 24 h after infection. In contrast, we found that infection did not significantly affect the basal level of IL-1ra mRNA expression. In infected mice, the level of IL-4 mRNA increased progressively between 1 to 120 h after infection and this change was accompanied by a gradual decrease in mRNA.
levels of TNFR-II, but not by a total disappearance of the signal, in infected mice. Altogether, based on recent findings (17), our data suggest that IL-10 might be involved in the control of LC migration after *S. mansoni* infection. To test this hypothesis, IL-10 KO mice were infected and the density of LCs on epidermal sheets was assessed by immunohistochemistry 24 h after infection. As shown in Fig. 4B, the number of LC/mm² was identical in the epidermis of noninfected and infected IL-10–deficient mice, whereas TNF-α dramatically depleted the population of LCs by >60%. It is worth mentioning that in IL-10 KO mice, LCs exhibited an activated phenotype after parasite infection. Similar results were obtained in WT mice by using anti–IL-10 neutralizing mAbs injected intradermally before infection (Fig. 4B). These data indicate that the inhibition of LC migration in *S. mansoni–*infected skin probably involve other factors than antiinflammatory host-derived cytokines.

**ES Lipophilic Substances from Schistosomula Inhibit the TNF-α–induced LC Migration In Vivo.** We then investigated the possibility that factors released by parasites themselves may directly affect LC migration. To this end, SESP were intradermally injected into the ear pinnae and the density of LCs remaining in the epidermis was determined 1 h after TNF-α injection. As seen in Fig. 5A, SESP had a strong inhibitory effect on the TNF-α–induced LC mobility. We have previously demonstrated that the SESP contain bioactive lipophilic compounds able to activate host cells (24). We therefore tested the effect of the lipophilic...
fraction from the SESP, obtained by a modified Folch extraction protocol, on the TNF-α-induced LC departure. Compared with the control (DMSO), we found that the lipophilic fraction dose dependently abrogates the departure of LCs from the epidermis (Fig. 5 B). Previous studies revealed that parasite larvae secrete various arachidonic acid-derived eicosanoids and that PGD₂, PGE₂, 5-HETE, 15-HETE, LTB₄, and LTC₄ are the major compounds (30, 36). Using different chromatographic systems, such as thin-layer chromatography (not shown) and HPLC, we confirmed these data except for LTs (detected in low amounts in the lipophilic fraction) and PGF₂α (present in a detectable level) (Fig. 6). As represented in Table II, quantification by HPLC and/or EIA revealed that, in our culture conditions, the parasite culture supernatant contains micromolar concentrations of PGF₂α, PGE₂, PGD₂, 5-HETE, 15-HETE, and nanomolar concentrations of LTB₄ and LTC₄.

**PGD₂ Specifically Inhibits the TNF-α–induced LC Migration through a cAMP-dependent Pathway.** We therefore tested each of these molecules in our in vivo system of TNF-α–induced LC departure. As shown in Fig. 7 A, intradermal administration of increasing amounts of PGD₂ significantly inhibits LC migration in a dose-dependent manner. In contrast, PGF₂α, PGE₂, 5-HETE, and 15-HETE did not prevent the mobility of LCs after TNF-α treatment, although at 100 nM, 5- and 15-HETE partially increased the LC density compared with animals that received DMSO alone. Similarly, LTB₄ and LTC₄ had no effect (not shown). Although we do not exclude the possibility that other lipophilic compounds may also be involved, this strongly suggests that schistosomula may exert its inhibitory effect on LC mobility through the production of PGD₂. To confirm this, we used a synthetic analogue of PGD₂ that is...
highly specific for the PGD₂ receptor (DP receptor; reference 37). As seen in Fig. 7 B, compared with DMSO-treated animals, BW245C (10 nM) also abrogates the migration of LCs induced by TNF-α. As PGD₂, and particularly BW245C, are known to increase the level of intracellular cAMP via its specific binding to the adenylate cyclase (AC)-coupled DP receptor (37), we hypothesized that cAMP may be the major signaling pathway involved in LC blockade. To this end, we tested the effect of the AC activator forskolin. As represented in Fig. 7 C, we found that forskolin (10 μM) abrogates the TNF-α–induced emigration of LCs. Taken together, our data suggest that the retention of LCs in the epidermis is likely mediated by a cAMP-dependent mechanism specifically triggered by PGD₂.

**The DP Receptor Mediates the Inhibition of LC Migration Induced by Schistosoma.** We next verified by RT-PCR that the DP receptor is expressed on murine epidermal cells. For this purpose, as purification of freshly isolated LCs and KCs from mouse epidermis is extremely difficult to realize, we used the LC (XS52) and KC (Pam212) lines. As depicted in Fig. 8 A, we detected mRNA for the DP receptor in total epidermal cells, in the LC and, to a lesser extent, in the KC line. To demonstrate that the parasite-induced inhibitory effect on LC migration is due to the specific binding of PGD₂ to the DP receptor, we treated mice with the highly specific DP receptor antagonist BW A868C 15 min before infection. 6 h later, the LC frequency was established in DMSO- and in BW A686C-treated animals. As shown in Fig. 8 B, BW A868C dose dependently restores the ability of LCs to leave the epidermis in infected mice. Altogether, these results show that targeting of PGD₂ to the KC- and/or to the LC-expressed DP receptor is responsible for the blockade of LC emigration from the epidermis during infection by *Schistosoma*.

**BW245C Inhibits CHS Responses Elicited by FITC.** To confirm our finding, we tested the effect of the DP receptor agonist BW245C in a model of contact sensitization induced by the hapten FITC (35). Compared with unsensitized mice, the number of LCs was reduced in the epidermis of sensitized mice 18 h after FITC painting (Fig. 9 A). In contrast, we found that LC migration is significantly impaired in BW245C-treated mice compared with sensitized control mice (DMSO/FITC). As assessed by flow cytometry, this defect in LC departure was associated with a dramatic reduction of the number of CD11c, FITC double-positive cells in the SLNs 24 h after sensitization (Fig. 9 B). Finally, to investigate whether the activation of the DP receptor during the sensitization phase results in an altered development of LC-dependent immune response, we measured the CHS response 5 d after FITC challenge. As expressed as ear swelling, BW245C-treated mice developed a profoundly reduced CHS responses (80% inhibition) compared with controls.

**Discussion**

Migration of LCs from the epidermis to the SLNs is a tightly regulated multistep process requiring inflammatory cytokines, chemokines, and adhesion molecules. However, very few studies have been devoted to investigate the molecular mechanisms which negatively regulate LC departure from the skin, especially during infections. In this re-
port, we show for the first time that PGD$_2$ directly inhibits the migration of LCs from the epidermis. This finding suggests a novel and unexpected function for this PG member in the control of LC homeostasis in the skin.

During cutaneous infections, skin-penetrating pathogens (in)directly activate LCs to migrate to the SLNs and may eventually use them to invade the hosts (21, 38). During schistosomiasis, little is known about the immunological consequences of the interactions between schistosomes and cutaneous cells. Sato et al. suggested that resident LCs may participate in the initiation of the primary immune response in *S. mansoni*-infected guinea pigs, but the major APCs involved are rather newly recruited blood-born skin LCs/DCs (39, 40). In another study, Riengrojpitak et al. hypothesized that infiltrating dermal APCs are important in the T cell priming in the SLNs (41). In this report, we investigated the possibility that *S. mansoni* may affect LC functions in vivo during murine infection. After checking that LCs did not undergo apoptosis after parasite penetration (data not shown), we showed that LCs exhibited evident signs of activation characterized by modifications of both LC morphology and phenotype, particularly for cells in the vicinity of the parasites or of their “ghosts”. Furthermore, we assessed the frequency of LCs in epidermal sheets from freshly isolated or from explanted infected skins. In both cases, we found that LCs are retained in the epidermis at all time points after infection (except at 120 h for the explants). Moreover, immunohistochemical analysis revealed no detectable DC accumulation in the SLNs from infected mice (1 to 10 d after infection, not shown). These results do not support those of Sato et al. who reported a significant LC depletion 12 h after infection in the guinea pig model (39). This may be attributed to differences in the animal models, in the schistosome species, or in the protocol of infection used. Interestingly enough, our observation was confirmed in a model of LC migration provoked by TNF-α. Indeed, we show that parasites transiently inhibit the TNF-α–induced release of LCs from the epidermis and the subsequent accumulation of DCs in the SLNs. Although not yet understood, this phenomenon has recently been described as a possible mechanism that could prevent and/or control the activation of immune responses against skin tumors (42) or retroviruses (43). Consequently, therapeutics able to antagonize the inhibitory effects which block LC migration (caused by abnormal cells or by pathogens) would be of great value. Whether or not *Schistosoma* used this stratagem to control and/or to orientate the cutaneous immune response is an open question which deserves further investigation. Indeed, despite the herein described inhibitory effect, we cannot exclude the possibility that some antigen-bearing LCs may migrate to the SLNs to initiate the response. Similarly, other cutaneous APCs (der-
is strongly increased in recent report (45), we showed that IL-10–specific mRNA expression of cytokines known to be involved in LC migration, favor the priming of type 2 and nonpolarized T cells, also be important in initiating the immune response during schistosomiasis. Moreover, by transiently affecting the migration of LCs, Schistosoma may not only delay the induction of the immune response but also, by “exhausting” LCs, favor the priming of type 2 and nonpolarized T cells, as recently suggested by Langenkamp et al. (44).

We next explored the mechanisms that lead to the retention of LCs in the epidermis and attempted to identify the responsible factor(s). We first assessed by RT-PCR the expression of cytokines known to be involved in LC migration. We hypothesized that the antiinflammatory cytokine IL-10 may be implicated. Indeed, and in accordance with a recent report (45), we showed that IL-10–specific mRNA is strongly increased in S. mansoni–infected skin. Despite this, using IL-10 KO mice or WT mice treated with neutralizing anti–IL-10 Abs, we still observed the inhibition of LC migration in infected epidermis. Similarly, RT-PCR analysis suggest that the chemokine receptors CCR–1 and CCR–7 do not appear to be implicated in the herein described inhibitory effects (not shown). We then proceeded to the hypothesis that factors released by parasites while penetrating the skin may inhibit the TNF–α–induced signals involved in LC migration. We found that injection of lipopholic factors from the SESP mimicked the inhibitory effects observed during infection. We then tested the effects of the major cyclooxygenase and lipoxygenase products found in the SESP on the TNF–α–triggered migration of LC. Among them, we found that PGD₂ specifically induces the retention of LCs in the skin after TNF–α treatment. We propose that PGD₂ activates LCs by interacting with the AC–coupled DP receptor and that the resulting signaling pathway interferes with the TNF–α–induced signals implicated in LC departure. This later assumption is important as PGD₂, as well as its metabolite 15d-PGJ₂, can also activate the peroxisome proliferative-activated receptors, a family of nuclear receptors recently shown to inhibit the chemoattractant-induced migration of various cells (46, 47). In the same manner, a novel receptor for PGD₂ has recently been described (48). Interestingly, this seven transmembrane G protein–coupled receptor (termed CRTH2) is expressed on human T helper type 2 lymphocytes, eosinophils, and basophils and is involved in their recruitment to allergic inflammatory sites. We eliminate the possibility that CRTH2 may be involved in the inhibition of LC migration as (a) we did not find mRNA CRTH2 expression in the LC line XS52 (not shown), (b) BW245C is a poor agonist for CRTH2 (48), and (c) CRTH2 is not coupled to an AC system but, on the contrary, induces Ca²⁺ mobilization in activated cells. Therefore, the dual action of PGD₂ in either favoring or inhibiting cell migration is probably due to a selective expression of CRTH2 or DP receptor on target cells.

To our knowledge, PGD₂ is the first molecule described to impair LC migration in vivo by directly affecting LC motility. Indeed, although other mechanisms may also take place (18, 19), compounds known to block LC migration act rather by diminishing the synthesis or the release of inflammatory cytokines (49–51) or by interfering with their activities (52). For instance, in UVB- or enterotoxin-treated mice, agents that block protein kinase C or G protein–associated kinases inhibit LC departure in part by preventing the release of TNF–α or IL–1α in the epidermis (50, 53, 54). In this report, we show that, in a TNF–α–induced model of LC depletion, activation of the cAMP-mediated pathway inhibits LC departure from the skin. In this model, the exact mechanisms by which cAMP inhibits the TNF–α–induced migratory abilities of LCs are not elucidated but probably involve remodelling of the actin network and the reinforcement of contact between LCs and KCs. In these processes, different protein targets for cAMP may be involved including the small GTP-binding proteins rho, tyrosine kinases, or adhesion molecules (55). For instance, the sustained E-cadherin expression on LCs in S. mansoni–infected skins (even after TNF–α treatment; not shown), may be one of these.

In addition to its role in the development and/or the modulation of acute and chronic inflammation (56–58), PGD₂ has multiple effects on the immune system. It enhances the release of mediators by eosinophils and mast
cells, reduces the production of superoxide in neutrophils, and suppresses T cell mitogenesis (59, 60). Here, we describe a novel function for PGD2 in that, during immune/inflammatory reactions, it may also control the migration of APCs from the site of antigen capture to the LNs. In the skin, PGD2 is among the major arachidonic acid metabolites produced (together with PGE2 and HETEs), particularly in the epidermis. Besides its role in KC proliferation and differentiation and in inflammatory responses (61), we propose that PGD2 may also act as an upstream component in a cascade of events that regulate the emigration of LCs from the skin, by a feedback mechanism. This hypothesis is supported by data showing that increased production of PGD2 is observed in the skin after UVB irradiation or antigen challenge (62). During infections, host- as well as pathogen-derived PGD2 synthase (the enzyme which transforms PGH2 to PGD2) may therefore play a key role in the maintenance of LC homeostasis in the skin. In our model, we have recently identified the parasite enzyme responsible for PGD2 synthesis in schistosomes. This PGD2 synthase is massively excreted by parasites while penetrating through the skin (unpublished data). Consequently, in addition to the endogenously produced PGD2, it is likely that *Schistosoma* may exploit the lipid metabolism of the host to convert fatty acid precursors into PGD2.

Our findings may have important consequences in the improvement of therapeutic treatments which aim to control skin diseases. Indeed, using a CHS model system, we have confirmed the potent ability of DP receptor agonists to inhibit LC migration out of the skin and to impair DC accumulation in the LNs. Furthermore, this defect in LC migration after hapten sensitization was associated with defective CHS responses after challenge. At present, we are testing the efficiency of PGD2 analogues (agonists and/or antagonists of the DP receptor) as well as modulators of the PGD2 synthase activity in diseases where reduction of immune cutaneous response is sought, such as eczematous and atopic dermatitis or, conversely, in diseases where stimulation of LC migration would be beneficial, such as in certain skin cancers (carcinomas) and infectious pathologies.

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References

1. Banchereau, J., and R.M. Steinman. 1998. Dendritic cells and the control of immunity. *Nature.* 392:245–252.
2. Tang, A., M. Amagai, L.G. Granger, J.R. Stanley, and M.C. Udey. 1993. Adhesion of epidermal Langerhans cells to keratinocytes is mediated by E-cadherin. *Nature.* 361:82–85.
3. Kimber, I., R.J. Dearman, M. Cumberbatch, and B.J. Huby. 1998. Langerhans cells and chemical allergy. *Curr. Opin. Immunol.* 10:614–619.
4. Moodycliffe, A.M., I. Kimber, and M. Norval. 1992. Role of TNFα in ultraviolet B light-induced dendritic cell migration and suppression of contact hypersensitivity. *Immunology.* 81:79–84.
5. Ban, E., I. Dupre, E. Hermann, W. Rohn, C. Vendeville, B. Quatannens, P. Ricciardi-Castagnoli, A. Capron, and G. Riveau. 2000. CpG motifs induce Langerhans cell migration *in vivo.* *Int. Immunol.* 12:737–744.
6. Weinlich, G., M. Heine, H. Stossel, M. Zanella, P. Stoitzener, U. Ortner, J. Smolle, F. Koch, N.T. Sepp, G. Schuler, and N. Romani. 1998. Entry into afferent lymphatics and maturation *in situ* of migrating murine cutaneous dendritic cells. *J. Invest. Dermatol.* 110:441–448.
7. Kimber, I., and M. Cumberbatch. 1992. Stimulation of Langerhans cell migration by TNFα. *J. Invest. Dermatol.* 99:48–50.
8. Enk, A.H., V.L. Angeloni, M.C. Udey, and S.I. Katz. 1993. An essential role for Langerhans cell–derived IL-1β in the initiation of primary immune responses in skin. *J. Immunol.* 150:3698–3704.
9. Schwarzenberger, K., and M.C. Udey. 1996. Contact allergens and epidermal proinflammatory cytokines modulate Langerhans cell E-cadherin expression *in situ.* *J. Invest. Dermatol.* 106:553–558.
10. Winzler, C., P. Rovere, M. Rescigno, F. Granucci, G. Penna, L. Adorini, V.S. Zimmermann, J. Davoust, and P. Ricciardi-Castagnoli. 1997. Maturation stages of mouse dendritic cells in growth factor–dependent long-term culture. *J. Exp. Med.* 185:317–328.
11. Sacki, H., A.M. Moore, M.J. Brown, and S.T. Hwang. 1999. Secondary lymphoid-tissue chemokine (SLC) and CC chemokine receptor 7 (CCR7) participate in the emigration pathway of mature dendritic cells from the skin to regional lymph nodes. *J. Immunol.* 162:2472–2475.
12. Gunn, M.D., S. Kyuwa, C. Tani, T. Kakiuchi, A. Matsuzawa, L.T. Williams, and H. Nakano. 1999. Mice lacking expression of secondary lymphoid organ chemokine have defects in lymphocyte homing and dendritic cell localization. *J. Exp. Med.* 189:451–460.
13. Sállustro, F., P. Scarcher, P. Loetscher, C. Schaniel, D. Lenig, C.R. Mackay, S. Qin, and A. Lanzavecchia. 1998. Rapid and coordinated switch in chemokine receptor expression during dendritic cell maturation. *Eur. J. Immunol.* 28:2760–2769.
14. Maestroni, G.J. 2000. Dendritic cell migration controlled by α9/α10-adrenergic receptors. *J. Immunol.* 165:6743–6747.
15. Kondo, S., S. Pastore, H. Fujisawa, G.N. Shivi, R.C. McKenzie, C.A. Dinarello, and D.N. Sauder. 1995. Interleukin-1 receptor antagonist suppresses contact hypersensitivity. *J. Invest. Dermatol.* 105:334–338.
16. Takayama, K., H. Yokozeki, M. Ghoreschi, T. Sato, I. Katayama, T. Umeda, and K. Nishioka. 1999. IL-4 inhibits the migration of human Langerhans cells through the down-regulation of TNF receptor II expression. *J. Invest. Dermatol.* 113:541–546.
17. Wang, B., L. Zhuang, H. Fujisawa, G.A. Shinder, C. Feliciani, G.M. Shivy, H. Suzuki, P. Amerio, P. Toto, and D.N. Sauder. 1999. Enhanced epidermal Langerhans cell migration in IL-10 knockout mice. J. Immunol. 162:277–283.
18. Kobayashi, Y., M. Matsumoto, M. Kotani, T. Makino, Y. Kobayashi, M. Matsumoto, M. Kotani, and T. Makino. 1999. Possible involvement of matrix metalloproteinase-9 in Langerhans cell migration and maturation. J. Immunol. 163: 5989–5993.
19. Randolph, G.J., S. Beaulieu, M. Pope, I. Sugawara, L. Hoffmann, R. Steinman, and W.A. Muller. 1998. A physiologic function for p-glycoprotein (MDR-1) during the migration of dendritic cells from skin via afferent lymphatic vessels. Proc. Natl. Acad. Sci. USA. 95:6924–6929.
20. Wang, B., O. Aumero, and D. Sauder. 1999. Role of cytokines in epidermal Langerhans cell migration. J. Leukoc. Biol. 66:33–39.
21. Arnoldi, J., and H. Moll. 1998. Langerhans cell migration in murine cutaneous leishmaniasis: regulation by TNFα, IL-1β, and MIPα. Dev. Immunol. 6:3–11.
22. Ramaswamy, K., B. Salafsky, S. Poturi, Y.X. He, J.W. Li, and T. Shibuya. 1995. Secretion of an anti-inflammatory, immunomodulatory factor by schistosomules of Schistosoma mansoni. J. Inflamm. 46:13–22.
23. Trottein, F., L. Descamps, S. Nutten, M.P. Dehouck, V. Angeli, A. Capron, R. Cecchi, and M. Capron. 1999. Schistosoma mansoni activates host microvascular endothelial cells to acquire an anti-inflammatory phenotype. Infect. Immun. 67: 3403–3409.
24. Trottein, F., S. Nutten, V. Angeli, P. Delerive, E. Teissier, A. Capron, B. Staels, and M. Capron. 1999. Schistosoma mansoni schistosomula reduce E-selectin and VCAM-1 expression in TNFα-stimulated lung microvascular endothelial cells by interfering with the NF-κB pathway. Eur. J. Immunol. 29: 3691–3701.
25. Macaronesia, S.E., S. Patterson, and S.C. Knight. 1989. Suppression of immune responses by dendritic cells infected with HIV. Immunology. 67:285–289.
26. Van Overtvelt, L., N. Vanderheyde, V. Verhasselt, J. Ismaili, L. De Vos, M. Goldman, F. Willems, and B. Vray. 1999. Trypanosoma cruzi infects human dendritic cells and prevents their maturation: inhibition of cytokines, HLA-DR, and co-stimulatory molecules. Infect. Immun. 67:4033–4040.
27. Salio, M., M. Celli, M. Suter, and A. Lanzavecchia. 1999. Inhibition of dendritic cell maturation by herpes simplex virus. Eur. J. Immunol. 29:3245–3253.
28. Reis e Sousa, C., G. Yap, O. Schulz, N. Rogers, M. Schito, J. Aliberti, S. Hieny, and A. Sher. 1999. Paralysis of dendritic cell IL-12 production by microbial products prevents infection-induced immunopathology. Immunity. 11:637–647.
29. Urban, B.C., D.J. Ferguson, A. Pain, N. Willcox, M. Plebanek, J.M. Austyn, and D.J. Roberts. 1999. Plasmodium falciparum-infected erythrocytes modulate the maturation of dendritic cells. Nature. 400:73–77.
30. Fusco, A.C., B. Salafsky, and M.B. Kevin. 1985. Schistosoma mansoni: eicosanoid production by cercariae. Exp. Parasitol. 59:44–50.
31. Xu, S., P.R. Bergstresser, and A. Takashima. 1995. Phenotypic and functional heterogeneity among murine epidermal-derived dendritic cell clones. J. Invest. Dermatol. 105:831–836.
32. Roop, D.R., P. Hawley–Nelson, C. Cheng, and S.H. Huspa. 1983. Expression of keratin genes in mouse epidermis and normal and malignantly transformed epidermal cells in culture. J. Invest. Dermatol. 81:144–149.
33. Shelley, W.B., and L. Juhrin. 1977. Selective uptake of contact allergens by the Langerhans cell. Arch. Dermatol. 113: 187–192.
34. Larsen, C.P., R.M. Steinman, M. Witmer–Pack, D.F. Hankins, P.J. Morris, and J.M. Austyn. 1990. Migration and maturation of Langerhans cells in skin transplants and explants. J. Exp. Med. 172:1483–1493.
35. Kripke, M.L., C.G. Munn, A. Jeevan, J.M. Tang, and C. Bucana. 1990. Evidence that cutaneous antigen-presenting cells migrate to regional lymph nodes during contact sensitization. J. Immunol. 145:2833–2838.
36. Salafsky, B., and A.C. Fusco. 1987. Schistosoma mansoni: a comparison of secreted vs. non-secreted eicosanoids in developing schistosomulae and adults. Exp. Parasitol. 64:361–367.
37. Narumiya, S., Y. Sugimoto, and F. Ushikubi. 1999. Prostanoid receptors: structures, properties, and functions. Physiol. Rev. 79:1193–1226.
38. Wu, S.J., G. Grouard-Vogel, W. Sun, J.R. Mascola, E. Brachet, R. Puttvakan, M.K. Louder, L. Filgueira, M.A. Marovich, H.K. Wong, et al. 2000. Human skin Langerhans cells are targets of dengue virus infection. Nat. Med. 6:816–820.
39. Sato, H., and H. Kamiya. 1995. Role of epidermal Langerhans cells in the induction of protective immunity to Schistosoma mansoni in guinea-pigs. Immunology. 84:233–240.
40. Sato, H., and H. Kamiya. 1998. Accelerated influx of dendritic cells into the lymph nodes draining skin sites exposed to attenuated cercariae of Schistosoma mansoni in guinea-pigs. Parasite Immunol. 20:337–343.
41. Riegrojpitak, S., S. Anderson, and R.A. Wilson. 1998. Induction of immunity to Schistosoma mansoni: interaction of schistosomula with accessory leukocytes in murine skin and draining lymph nodes. Parasitology. 117:301–309.
42. Lucas, A.D., and G.M. Halliday. 1999. Progressor but not regressor skin tumors inhibit Langerhans cell migration from epidermis to local lymph nodes. Immunology. 97:130–137.
43. Gabrilovich, D.I., G.M. Woods, S. Patterson, J.J. Harvey, and S.C. Knight. 1994. Retrovirus-induced immunosuppression via blocking of dendritic cell migration and down-regulation of adhesion molecules. Immunology. 82:82–87.
44. Langenkamp, A., M. Messi, A. Lanzavecchia, and F. Sallustio. 2000. Kinetics of dendritic cell activation: impact on priming of Th1, Th2 and nonpolarized T cells. Nat. Immunol. 1:311–316.
45. Ramaswamy, K., P. Kumar, and Y.X. He. 2000. A role for parasite-induced PGE2 in IL-10-mediated host immunoregulation by skin stage schistosomula of Schistosoma mansoni. J. Immunol. 165:4567–4574.
46. Goetzé, S., X.P. Xi, H. Kawano, T. Gotlibowski, E. Fleck, W.A. Hsueh, and R.E. Law. 1999. PPARγ-ligands inhibit migration mediated by multiple chemoattractants in vascular smooth muscle cells. J. Cardiovasc. Pharmacol. 33:798–806.
47. Kintscher, U., S. Goetze, S. Wakino, S. Kim, S. Nagpal, R.A. Chandraratna, K. Graf, E. Fleck, W.A. Hsueh, and R.E. Law. 2000. Peroxisome proliferator-activated receptor and retinoid X receptor ligands inhibit monocyte chemotactic protein-1-directed migration of monocytes. Eur. J. Pharmacol. 401:259–270.
basophils via seven-transmembrane receptor CRTH2. *J. Exp. Med.* 193:255–261.

49. Cumberbatch, M., R.J. Dearman, and I. Kimber. 1999. Inhibition by dexamethasone of Langerhans cell migration: influence of epidermal cytokine signals. *Immunopharmacology.* 41:235–243.

50. Shankar, G., J. Johnson, L. Kuschel, M. Richins, and K. Burnham. 1999. Protein-kinase-specific inhibitors block Langerhans cell migration by inhibiting IL-1α release. *Immunology.* 96:230–235.

51. Cumberbatch, M., R.J. Dearman, S. Uribe-Luna, D.R. Headon, P.P. Ward, O.M. Conneely, and I. Kimber. 2000. Regulation of epidermal Langerhans cell migration by lactoferrin. *Immunology.* 100:21–28.

52. Halliday, G.M., and A.D. Lucas. 1993. Protein kinase C transduces the signal for Langerhans cell migration from the epidermis. *Immunology.* 79:621–626.

53. Burnham, K., S. Pickard, J. Hudson, and T. Voss. 1993. Requirements for Langerhans cell depletion following *in vitro* exposure of murine skin to ultraviolet-B. *Immunology.* 79:627–632.

54. Pickard, S., G. Shankar, and K. Burnham. 1994. Langerhans cell depletion by staphylococcal superantigens. *Immunology.* 83:568–572.

55. Ydrenius, L., M. Majeed, B.J. Rasmusson, O. Stendahl, and E. Sarndahl. 2000. Activation of cAMP-dependent protein kinase is necessary for actin rearrangements in human neutrophils during phagocytosis. *J. Leukoc. Biol.* 67:520–528.

56. Gilroy, D.W., P.R. Colville-Nash, D. Willis, J. Chivers, M.J. Paul-Clark, and D.A. Willoughby. 1999. Inducible cyclooxygenase may have anti-inflammatory properties. *Nat. Med.* 5:698–701.

57. Ajuebor, M.N., A. Singh, and J.L. Wallace. 2000. Cyclooxygenase-2-derived prostaglandin D2 is an early anti-inflammatory signal in experimental colitis. *Am. J. Physiol. Gastrointest. Liver Physiol.* 279:238–244.

58. Matsuoka, T., M. Hirata, H. Tanaka, Y. Takahashi, T. Murata, K. Kabashima, Y. Sugimoto, T. Kobayashi, F. Ushikubi, Y. Aze, et al. 2000. Prostaglandin D2 as a mediator of allergic asthma. *Science.* 287:2013–2017.

59. Raible, D.G., E.S. Schulman, J. DiMuzio, R. Cardillo, and T.J. Post. 1992. Mast cell mediators prostaglandin D2 and histamine activate human eosinophils. *J. Immunol.* 148:3536–3542.

60. Kanamori, Y., M. Niwa, K. Kohno, L.Y. Al-Essa, H. Matsuno, O. Kozawa, and T. Uematsu. 1997. Migration of neutrophils from blood to tissue: alteration of modulatory effects of prostanoid on superoxide generation in rabbits and humans. *Life Sci.* 60:1407–1417.

61. Ikai, K., and S. Imamura. 1988. Prostaglandin D2 in the skin. *Int. J. Dermatol.* 27:141–149.

62. Massey, W.A., W.C. Hubbard, M.C. Liu, A. Kagey-Sobotka, P. Cooper, and L.M. Lichtenstein. 1991. Profile of prostanoid release following antigen challenge *in vivo* in the skin of man. *Br. J. Dermatol.* 125:529–534.