Role of the Parasite-derived Prostaglandin D_2 in the Inhibition of Epidermal Langerhans Cell Migration during Schistosomiasis Infection

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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 emigration 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

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[1]*Abbreviations used in this paper:* AC, adenylate cyclase; CHS, contact hypersensitivity; CCR, CC chemokine receptor; DC, dendritic cell; DP receptor, PGD_2 receptor; EIA, enzyme immunoassay; ES, excreted/secreted; HETE, 5-hydroxyeicosatetranoic acid; KC, keratinocyte; KO, knockout; LC, Langerhans cell; LT, leukotriene; RT, reverse transcription; SESP, schistosomula ES products; SLN, skin-draining LN; WT, wild-type.

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 (CD49f), 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 PGD_2. We speculate that schistosomes may utilize this stratagem to limit and/or orientate the host immune response. We also propose a new function for PGD_2 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. PGD_2, PGE_2, $PGF_{2\alpha}$, 5-hydroxyeicosatetranoic acid (HETE), 15-HETE, leukotriene $(LT)B_4$, LTC_4, and BW245C were from Cayman Chemical. BW A868C was donated by Dr. S. Lister (Glaxo Wellcome, Greenford, UK). The anti-$I\text{-}A^d$/$I\text{-}E^d$ 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 following were used as secondary Abs: biotin-conjugated anti–rat and anti-FITC peroxidase-conjugated (Boehringer). The biotinylated reagents were detected using ABC complex horseradish peroxidase (HRP; Dako). The neutralizing anti–IL-10 mAb (clone JES052A5, rat IgG1) was from R&D Systems and the isotype 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 Pam212 KCs were cultured in Eagle's MEM complemented with 10% FCS and 0.05 mM $CaCl_2$ (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 schistosomula 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

(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) trifluoroacetic 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 (EIA) was also used to quantify $PGF_2\alpha$, 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 ≥ 5×10^7 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 (30 μl) into both ear pinnae with 27 3/4-gauge stainless steel needles. Epidermal sheets were analyzed 1 h after injection, a time previously shown to be optimal for TNF-α–induced LC emigration (7). Treatment of mice with Abs was as follows: 1 h before infection, C57BL/6 mice were injected intradermally with 40 μg of neutralizing anti–IL-10 or isotype-matched control mAb diluted in sterile PBS (final volume: 30 μl).

Preparation and Analysis of Epidermal Sheets. The epidermis was separated from the dermis by means of ammoniumthiocyanate as described previously (33). Epidermal sheets were fixed in paraformaldehyde (PFA; 2% in PBS) for 10 min at room temperature, and washed three times with PBS. For immunohistochemical staining, sheets were placed for 15 min in 3% H_2O_2 to inhibit endogenous peroxidase, washed three times with PBS, and incubated for 30 min with PBS plus 1% (wt/vol) blocking reagent (Boehringer). Epidermal sheets were then incubated with primary Abs for 90 min and washed in PBS before adding either biotinylated conjugated goat anti–rat Ig or peroxidase-conjugated anti-FITC for an additional 30 min. In the final step, sheets were developed with 3-amino 9-ethyl carbazol, washed three times in PBS, and mounted onto glass slides in Immumount (Shandon) for immunohistochemical analysis. LCs were enumerated by counting MHC class II–positive cells. Epidermal sheets were prepared from each experimental group and for each sheet 10 random fields were examined. Cell frequency was converted to LC/mm^2 and results were expressed as mean ± SD. The statistical significance of differences between experimental groups was calculated using the Student's *t* test.

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 Hepes, 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.

Immunochemical Analysis of SLNs. SLNs were removed 18 h after TNF-α 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 prepared 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

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′-GTCGGGCGCCCCAGGCACCA	539	28
	3′	5′-CTCCTTAATGTCACGCACGATTTC		
TNF-α	5′	5′-AACCACCAAGTGGAGGAGCAGC	312	36
	3′	5′-TGACCTCAGCGCTGAGTTGGTCC		
IL-1β	5′	5′-TGAAGGGCTGCTTCCAAACCTTTGACC	322	36
	3′	5′-TGTCCATTGAGGTGGAGAGCTTTCAGC		
IL-1ra	5′	5′-CCTGCAAGATGCAAGCCTTCAGG	353	35
	3′	5′-CAGCCTCTAGTGTTGTGCAGAGG		
IL-4	5′	5′-GAATGTACCAGGAGCCATATC	384	38
	3′	5′-CTCAGTACTACGAGTAATCCA		
TNFR-II	5′	5′-GTAGGCCTTGAGCAGCAGCACCT	312	35
	3′	5′-GTGTCTCTGTAGTCTCACACGG		
IL-10	5′	5′-TCCTTAATGCAGGACTTTAAGGGTTAC	246	38
	3′	5′-GACACCTTGGTCTTGGAGCTTATTAAA		
DP receptor	5′	5′-GAAGTTCGTGCAGTACTGTCCAG	435	35
	3′	5′-TCCACTATGGAAATCACAGACAG		

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 determinate 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 μg 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 interdigiting 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

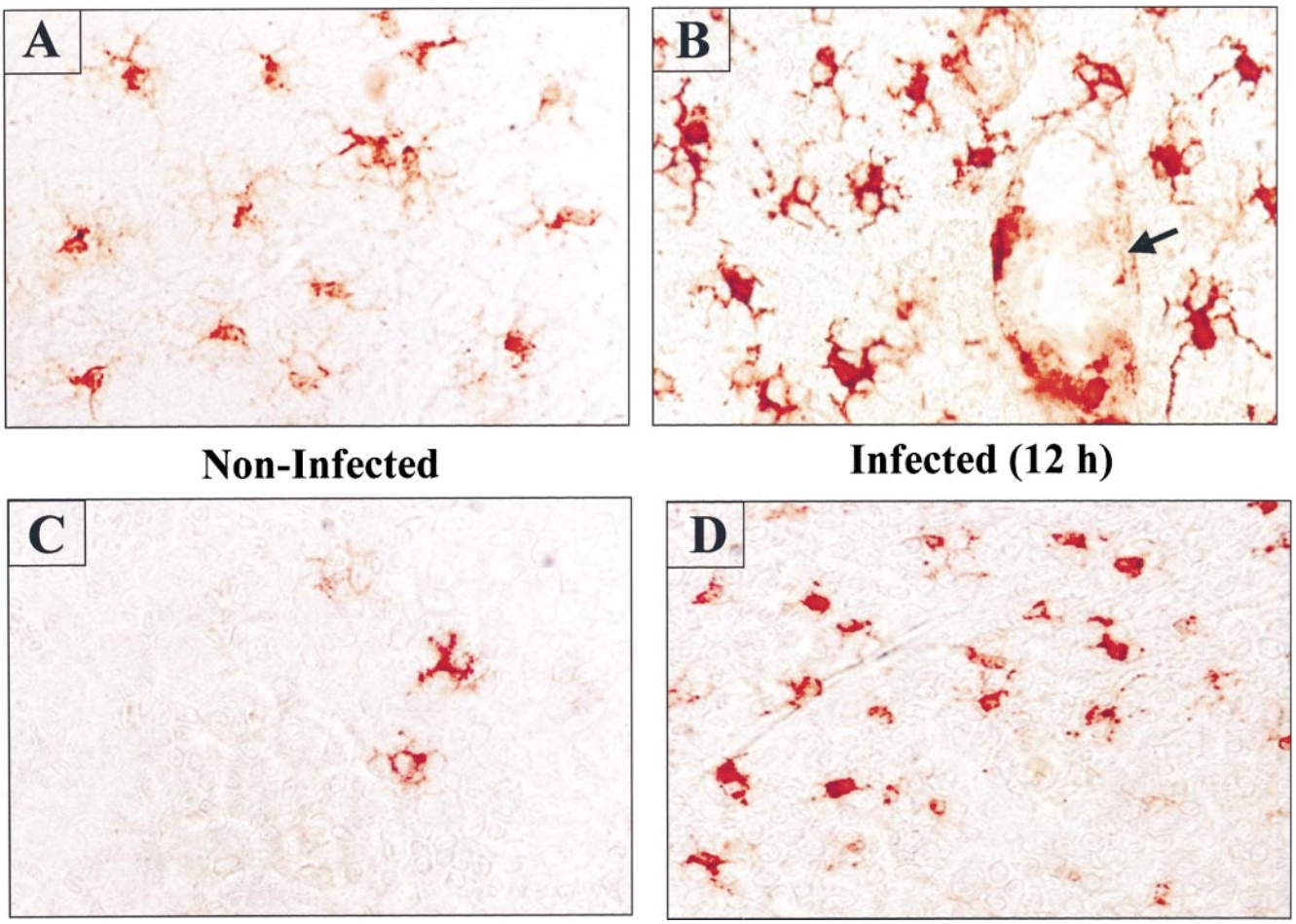

Figure 1. Immunohistochemical staining of murine epidermal sheets after transcutaneous infection by *S. mansoni*. Epidermal sheets were prepared either from noninfected or from *S. mansoni*–infected mice and LCs were stained for MHC class II (A and B) or for CD86 (C and D). The arrow indicates the "ghost" of parasite. The isotype control mAb did not reveal any reactivity (not shown). Original magnification: ×400.

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, we attempted to study the migratory behavior of LCs 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/mm^2. Surprisingly, in comparison to control epidermis, the number of LCs/mm^2 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/mm^2 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.

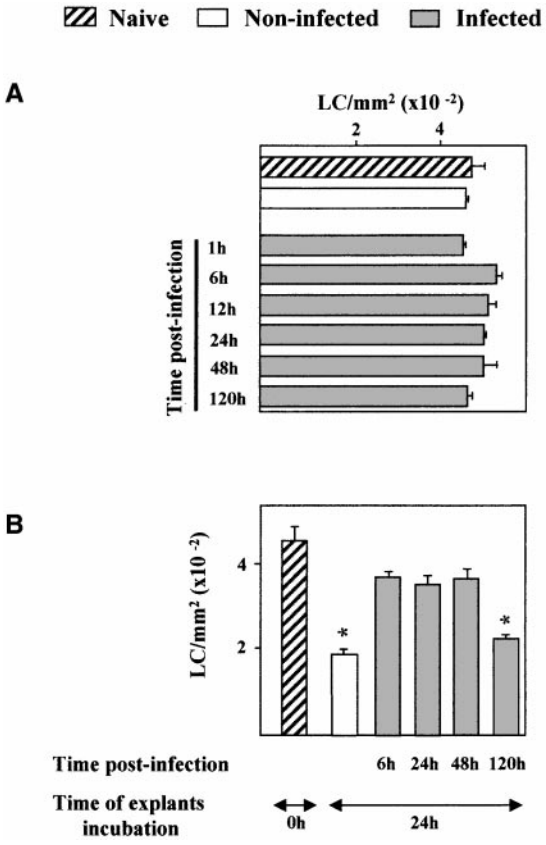

Figure 2. Effect of *S. mansoni* infection on the migration of epidermal LCs in vivo (A) and ex vivo (B). (A) Epidermal sheets were prepared at different times after the infection (1 to 120 h) and the number of LC/mm^2 was determined after anti-MHC class II staining. Controls included epidermal sheets from naive mice and from mice exposed to water without parasites (Non-infected). Results are expressed as means ± SD and are representative of four independent experiments ($n = 7$). (B) Skin explants were obtained from ears of noninfected or *S. mansoni*–infected mice (6, 24, 48, and 120 h). The number of LC/mm^2 was determined in the epidermis from the explants after 24 h of culture and compared with the epidermis from fresh skin (naive). Results are expressed as means ± SD and are representative of three independent experiments ($n = 4$). Significant differences are designated by * ($P < 0.001$).

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

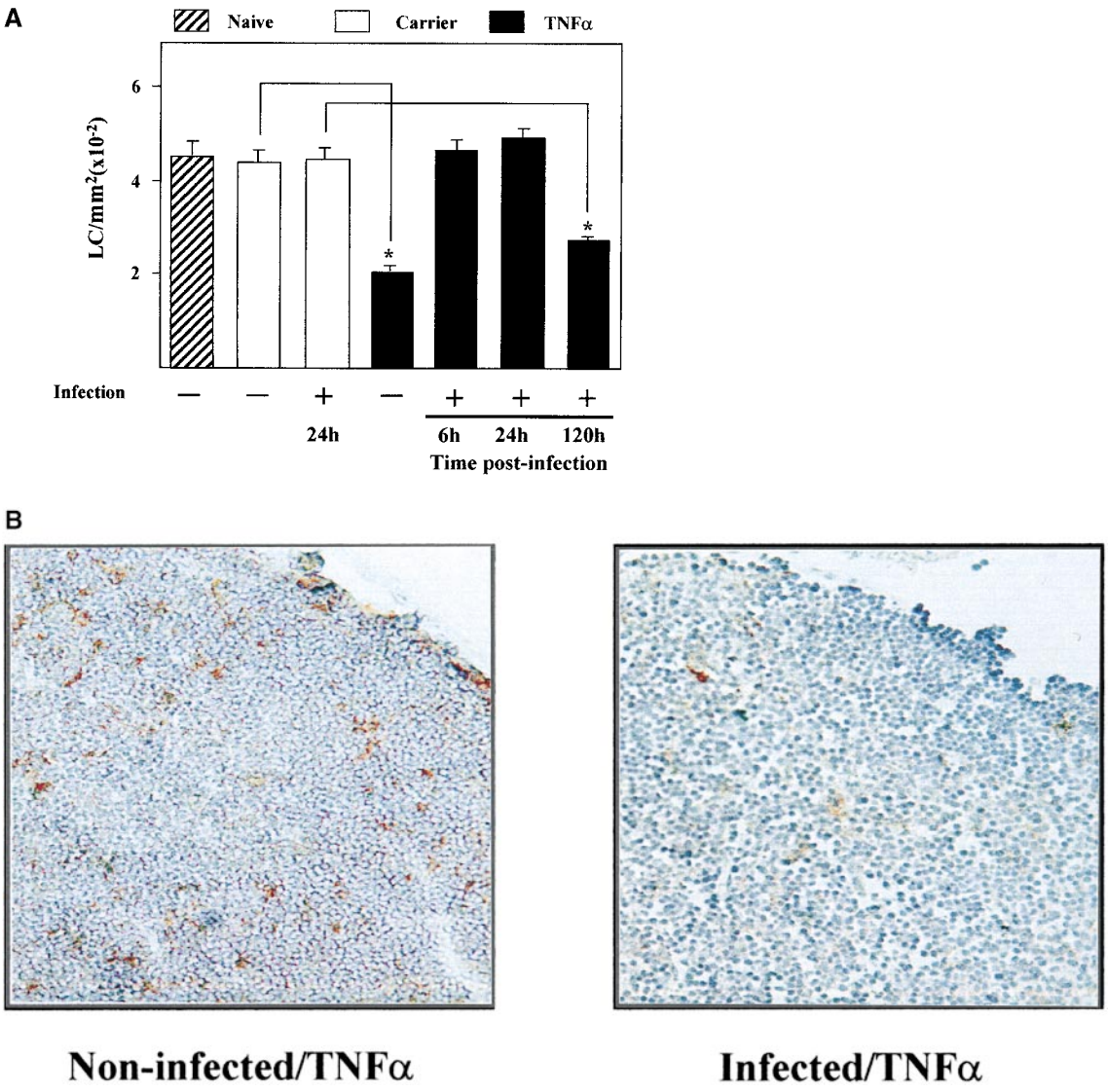

Figure 3. Effect of *S. mansoni* infection on the TNF-α–induced LC migration in vivo. (A) 6, 24, and 120 h after infection, mice (four mice/time point) were intradermally injected with 30 μl of PBS/BSA (carrier) containing or not containing 50 ng TNF-α into both ear pinnae. Ears were removed 1 h later, epidermal sheets were prepared, and the number of LC/mm^2 was determined by immunohistochemistry. In TNF-α–treated *S. mansoni*-infected mice, we noted that LCs remained interdigitated among surrounding KCs and still expressed E-cadherin (not shown). The experiment shown is representative of five experiments ($n = 8$) and values are means ± SD. (B) Detection of CD11c-expressing cells in SLNs from TNF-α–treated mice previously (24 h), or not, infected with *S. mansoni* (original magnification: ×200).

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. 4 B, the number of LC/mm^2 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. 4 B). 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. 5 A, 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

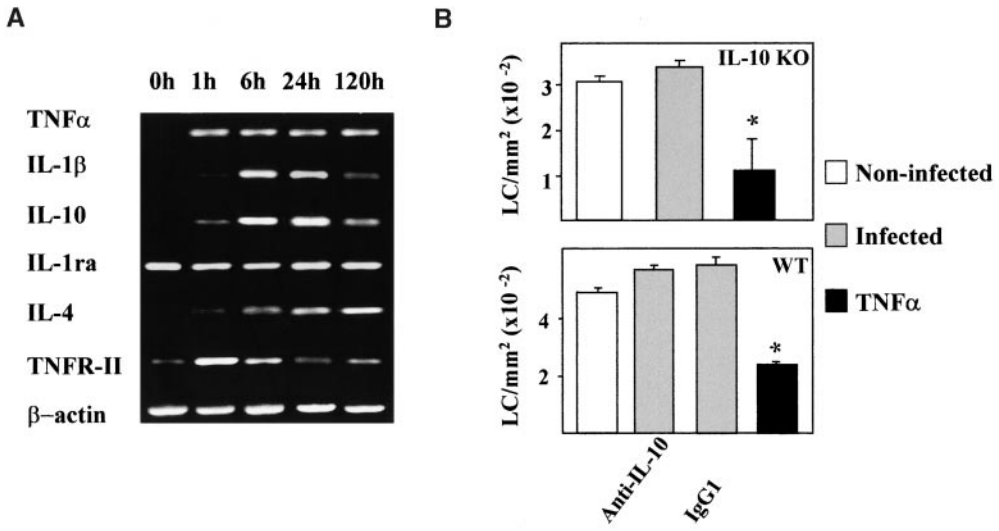

Figure 4. (A) RT-PCR analysis of mRNAs specific for pro- and antiinflammatory cytokines in the epidermis of *S. mansoni*–infected mice. Epidermal sheets were prepared from noninfected (0 h) or infected (1, 6, 24, and 120 h) mice, total RNA extracted, and RT-PCR was carried out using the primers shown in Table I. Representative results of three independent experiments are shown. (B) Role of IL-10 in the inhibition of LC migration. IL-10 KO or WT mice were infected (or not) and 24 h after infection, epidermal sheets were prepared and the number of LCs/mm^2 determined by immunohistochemistry. Before infection, WT mice were treated with neutralizing anti–IL-10 or isotype-matched mAbs (IgG1). As a positive control, TNF-α was intradermally injected 1 h before the analysis. Significant differences are designated by * ($P < 0.001$).

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 PGD_2, PGE_2, 5-HETE, 15-HETE, LTB_4, and LTC_4 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 $PGF_{2\alpha}$ (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 $PGF_{2\alpha}$, PGE_2, PGD_2, 5-HETE, 15-HETE, and nanomolar concentrations of LTB_4 and LTC_4.

PGD_2 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 PGD_2 significantly inhibits LC migration in a dose-dependent manner. In contrast, $PGF_2\alpha$, PGE_2, 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, LTB_4 and LTC_4 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 PGD_2. To confirm this, we used a synthetic analogue of PGD_2 that is

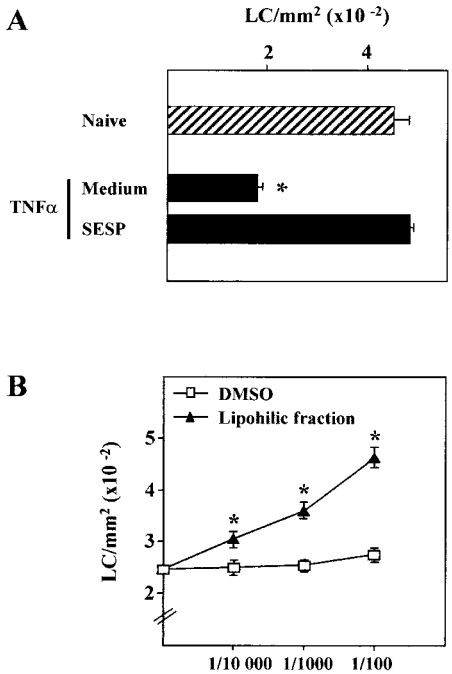

Figure 5. Effect of SESP on the TNF-α–induced LC migration in vivo. (A) The supernatant of a 4-h culture of schistosomula (SESP) or (B) increasing amounts of the lipophilic fraction from the SESP (diluted in DMSO) were intradermally injected to mice. After 20 min, mice were treated with 50 ng of TNF-α and the epidermal sheets were analyzed 1 h after for the determination of LC density. These data are representative of three experiments ($n = 4$). Significant differences are designated by * ($P < 0.001$).

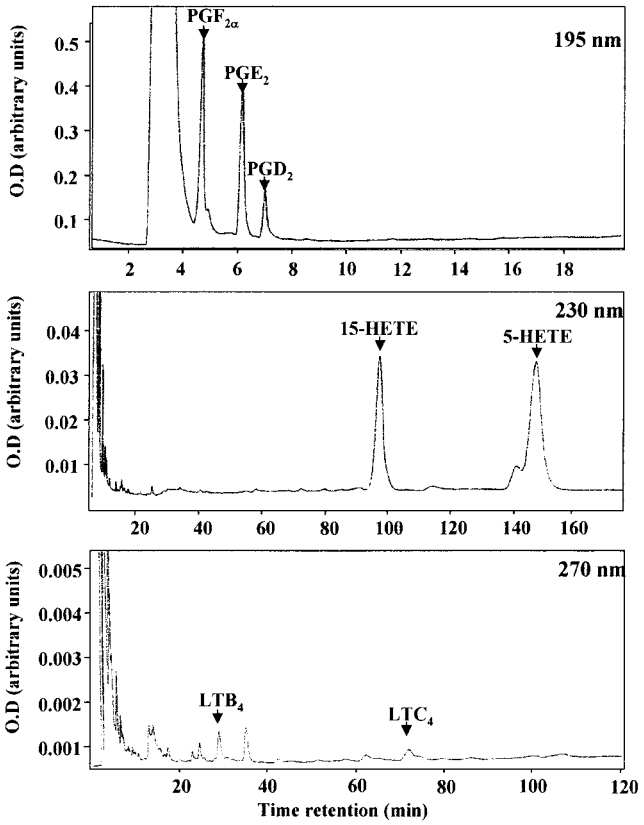

Figure 6. HPLC analysis of *S. mansoni* schistosomula eicosanoid production. 1 μl of the lipophilic fraction was injected, fractions were collected every 30 s, and monitored using a densitometer (wavelength: 195, 230, and 270 nm). The elution position of external standards are indicated. Note that the scales of the arbitrary values are different in each panel.

highly specific for the PGD_2 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 PGD_2, 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 PGD_2.

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 PGD_2 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 PGD_2 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.*

Table II. *Schistosomula Eicosanoid Production as Determined by HPLC and/or EIA*

Eicosanoid	Retention time	Wavelength	Concentration		Concentration*	
	min	*nm*	*pg/ml*	*μm*	*pg/ml*	*μm*
$PGF_{2\alpha}$	4.32	195	2,359	6.53	1,841	5.09
PGE_2	5.68	195	2,273	6.43	2,589	7.32
PGD_2	6.57	195	691	1.96	752	2.13
15-HETE	98	230	263	0.82	nd	nd
5-HETE	150	230	218	0.68	nd	nd
LTB_4	26	270	nd	nd	18	0.05
LTC_4	66	270	nd	nd	14	0.04

Shown is one representative experiment out of four. nd, not determined.
*Concentration determined by EIA.

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-

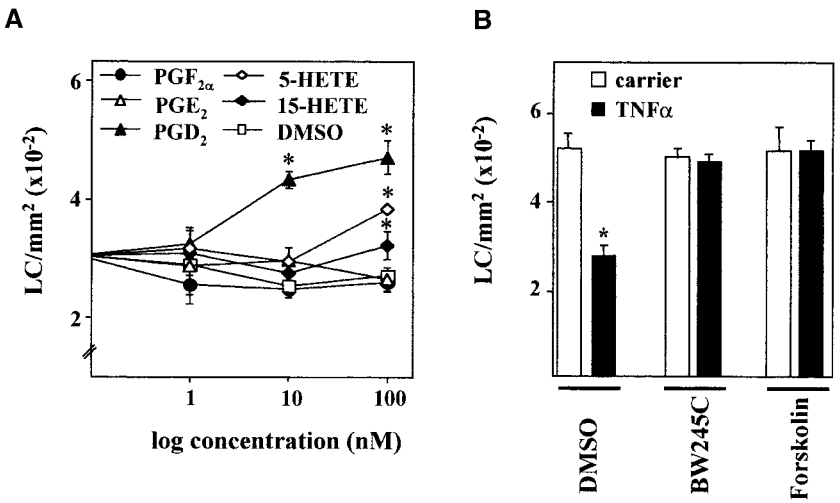

Figure 7. Effect of (A) the major schistosomula ES eicosanoids and (B) of the PGD_2 analogue BW245C and the cAMP-elevating agent forskolin on the TNF-α–induced LC migration in vivo. (B) Mice received increasing concentrations of $PGF_{2\alpha}$, PGE_2, PGD_2, 15-HETE, 5-HETE, or vehicle alone (DMSO), or (B) they received BW245C (10 nM), forskolin (10 μM), or DMSO. After 20 min, mice were treated with 50 ng of TNF-α and epidermal sheets were prepared 1 h later. The number of LC/mm^2 was determined by immunohistochemistry. This data is representative of three experiments ($n = 4$). Significant differences are designated by * ($P < 0.001$).

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-

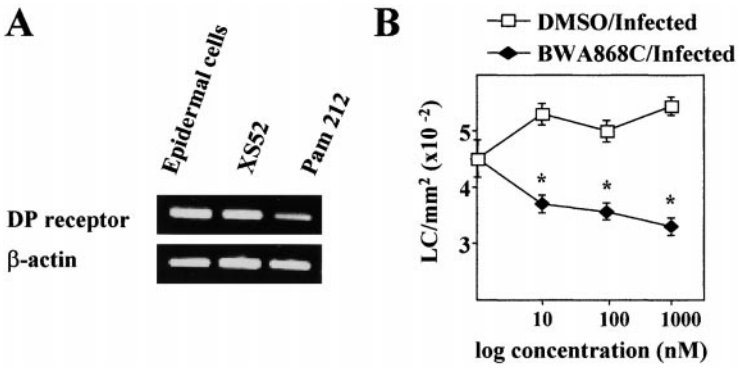

Figure 8. (A) Expression of mRNA for the DP receptor in total epidermal cells and in the LC (XS52) and KC (Pam212) lines as assessed by RT-PCR. (B) Effect of the DP receptor antagonist BW A868C on LC emigration after infection with *S. mansoni*. 15 min before the infection, mice were injected intradermally with increasing amounts of BW A868C. 6 h later, epidermis were stained using anti-MHC class II Abs. This data is representative of three experiments ($n = 4$). Significant differences are designated by * ($P < 0.001$).

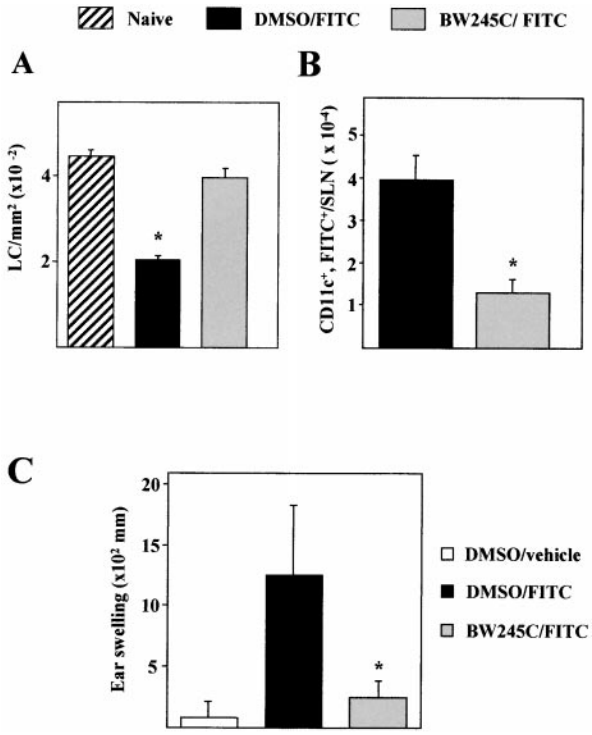

Figure 9. Effect of the PGD_2 analogue BW245C on the FITC-induced migration of LCs and on CHS responses. Mice were injected intradermally into ear pinnae with BW245C (100 nM) 15 min before and 5 h after FITC topical application. (A) Epidermal LC density was analyzed 18 h after FITC painting and (B) the number of $CD11c^+FITC^+$ cells present in the SLNs determined 24 h after FITC application. (C) 5 d after sensitization, mice were challenged and 24 h later, ear thickness was measured. Results are expressed as means ± SD and are representative of three independent experiments ($n = 7$). Significant differences are designated by * ($P < 0.001$) for A and C and ($P < 0.05$) for B.

mal DCs; reference 39) or SLN resident APCs (41) may 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 lipophilic 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 PGD_2 specifically induces the retention of LCs in the skin after TNF-α treatment. We propose that PGD_2 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 PGD_2, as well as its metabolite 15d-PGJ_2, 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 PGD_2 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 Ca^{2+} mobilization in activated cells. Therefore, the dual action of PGD_2 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, PGD_2 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), PGD_2 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 PGD_2 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, PGD_2 is among the major arachidonic acid metabolites produced (together with PGE_2 and HETEs), particularly in the epidermis. Besides its role in KC proliferation and differentiation and in inflammatory responses (61), we propose that PGD_2 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 PGD_2 is observed in the skin after UVB irradiation or antigen challenge (62). During infections, host- as well as pathogen-derived PGD_2 synthase (the enzyme which transforms PGH_2 to PGD_2) 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 PGD_2 synthesis in schistosomes. This PGD_2 synthase is massively excreted by parasites while penetrating through the skin (unpublished data). Consequently, in addition to the endogenously produced PGD_2, it is likely that *Schistosoma* may exploit the lipid metabolism of the host to convert fatty acid precursors into PGD_2.

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 PGD_2 analogues (agonists and/or antagonists of the DP receptor) as well as modulators of the PGD_2 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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