A Role for Parasite-Induced PGE\(_2\) in IL-10-Mediated Host Immunoregulation by Skin Stage Schistosomula of \textit{Schistosoma mansoni}

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\textit{J Immunol} 2000; 165:4567-4574; doi: 10.4049/jimmunol.165.8.4567
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A Role for Parasite-Induced PGE$_2$ in IL-10-Mediated Host Immunoregulation by Skin Stage Schistosomula of Schistosoma mansoni$^1$

Kalyanasundaram Ramaswamy,$^2$ Pawan Kumar, and Yi-Xun He

Significant quantities of PGE$_2$ were produced by cercariae of Schistosoma mansoni following incubation with linoleic acid, a free fatty acid found on the surface of the skin. Cyclooxygenase (COX) 2 inhibitors failed to block this PGE$_2$ production, suggesting that a different biochemical pathway may be involved in the production of PGE$_2$ by the parasite. In addition, the parasites were also able to induce PGE$_2$ and IL-10 from human and mouse keratinocytes. Analysis of mouse skin during skin migratory phases of infection confirmed these in vitro observations. COX2 inhibitors blocked the parasite-induced PGE$_2$ and IL-10 from keratinocytes. Further analysis of the parasite secretions showed that the PGE$_2$/IL-10-inducing effect was associated with a fraction $<$30 kDa molecular size. Addition of this fraction or parasite-stimulated keratinocyte culture supernatant to Con A-stimulated spleen cells resulted in the suppression of cell proliferation. This effect could be blocked by anti-IL-10 treatment. In sharp contrast, attenuation of the parasites with $\gamma$-irradiation significantly abrogated their ability to induce PGE$_2$ or IL-10 from skin cells. Significance of IL-10 in host immunoregulation by skin stage schistosomula of S. mansoni was further confirmed by using IL-10-deficient mice. In these mice the normal subdued cutaneous reaction to the parasite was absent. Instead, a prominent cellular reaction occurred around the parasite, and there was considerable delay in parasitic migration through the skin. Thus these results suggest a key role for parasite-induced PGE$_2$ in IL-10-dependent down-regulation of host immune responses in the skin. *The Journal of Immunology*, 2000, 165: 4567–4574.

Human Schistosomiasis mansoni infections are acquired through the skin (1). Larval stages of the parasite (schistosomula) after gaining entry into the body remain in the skin for about 48–72 h before migrating to the lungs (1–3). This stay in the skin potentially provides ample opportunity for the host immune system to mount an effective immune response against the migrating parasite. Yet the host fails to elicit any tissue response against the skin-residing schistosomula (3). Conversely, infection with $\gamma$-irradiation-attenuated parasites, which are known to confer significant immunity against this infection, stimulated a marked inflammatory response in the skin, resulting in delayed migration of the parasite through the skin (2–4). This delayed migration correlated with the development of IFN-$\gamma$-producing putative effector cells in the skin and draining lymph nodes (5, 6). Interestingly, a challenge infection with normal parasites down-regulated the IFN-$\gamma$ response in the skin and draining lymph nodes (5, 7). This suggested that a normal infection could potentially hamper the generation of a protective immune response against the parasite in immune individuals. The mechanism of this down-regulation is poorly understood. Cytokine analysis in the skin and draining lymph nodes of mice infected with normal parasites showed predominantly a Th2-type cytokine response (5, 7). Specifically, mRNA for IL-10 and IL-4 were increased in the skin within 16 h after infection (5). It is baffling, especially in immunized animals, how the skin-stage schistosomula are able to induce an overbearing IL-10 response in a milieu predominated by IFN-$\gamma$-producing cells (4–7). IL-10 has been implicated in the down-regulation of various immune responses against S. mansoni in both mice (8, 9) and humans (10, 11). Therefore, it is possible that this early IL-10 response in the skin and draining lymph nodes may play a pivotal role in parasite establishment by modulating the Th1-type responses (11, 12).

Previous studies showed that the infective stages of S. mansoni (cercariae) has the capacity to produce a wide range of eicosanoids, including the arachidonic acid metabolite, PGE$_2$ (13–14), which is a potent regulator of immune responses (15). Picomolar concentrations of PGE$_2$ can up-regulate IL-10 production and down-regulate IL-12 production from various cell types including skin cells (16, 17). In turn, IL-10 can potentially influence the local and systemic immune responses by modulating the Th1 type cytokine production in the skin (17–19). PGE$_2$ is also known to be produced by other helminth and protozoan parasites (20). However, the role of PGE$_2$ in parasite-induced immunomodulation has not been fully understood. This study focuses on the role of parasite-induced PGE$_2$ in the host immunoregulation via IL-10.

Materials and Methods

Parasite and parasite secretary products

Collection of cercariae, in vitro transformation, and collection of secretory products from schistosomula were as described before (3). Biomphalaria glabrata snails infected with S. mansoni were obtained from Dr. Fred Lewis (Biomedical Research Institute, Rockville, MD). Normal or irradiated cercariae (exposed to 20 kR $\gamma$-irradiation) suspended in MEM (at a concentration of 10,000 cercariae/ml) were transformed into schistosomula.
by incubating them in sterile MEM for 3–5 h in linoleic acid (2 µg/ml)-coated culture flasks as described previously (21). These culture supernatants will contain both secretions produced at the time of transformation (transformation fluid) and material secreted during the 3- to 5-h incubation period. This preparation is called, collectively, the “secretory products of schistosomula”. Following incubation, the culture supernatant was collected, concentrated, and sterile filtered (0.2 µm; Costar, Cambridge, MA), and protein concentration was estimated using a micro BCA protein assay kit purchased from Pierce (Rockford, IL). In some studies cercariae were mechanically transformed into schistosomula in the absence of linoleic acid by a syringe passage (22). Briefly, cercariae were concentrated after shedding by centrifugation at 600 × g for 15 min at 4°C and resuspended in MEM (at a concentration of 10,000 cercariae/ml). Using a 5-ml syringe, the cercarial suspension was passed 10–15 times. This procedure induces transformation of cercariae into schistosomula. The mechanically transformed schistosomula were then incubated for 3–5 h in the same medium, and the supernatant was collected, concentrated, and sterile filtered, and protein concentration was estimated as above. This preparation is called “secretory products of mechanically transformed schistosomula”. In some studies PGE2 present in the secretory products was affinity depleted by incubating with PGE2 affinity sorbent beads (mouse anti-PGE2 linked to 50 kDa), fraction II (30 kDa), fraction III (30 kDa), and fraction IV (<30 kDa) using Centriprep concentrators (Amicon, Beverly, MA) having membrane with respective m. w. cut-off.

In Vitro culture of keratinocytes
Neonatal human keratinocytes (clone NHEK P131; Clonetics, San Diego, CA) were grown in keratinocyte growth medium (KGM2; Clonetics) and used in their second passage in all experiments. Mice epidermal keratinocytes were separated from C57BL/6 mouse ear skin as described previously (23). Briefly, ear skin was split in half. After removing the s. c. tissue, ears were floated on 0.05% trypsin (diphenyl carbamyl chloride-treated; Sigma, St. Louis, MO) and 160 µg/ml DNase I (Sigma) for 30 min at 37°C. Single cell suspensions were prepared by mechanical disruption, and keratinocytes were isolated by density gradient centrifugation (200 × g for 10 min). Dead cells were removed by pipetting using sterile pipette tips, and cells were washed and used at 37°C until use. In some studies the secretory products depleted off PGE2 were separated into four fractions. Fraction I (>100 kDa), fraction II (50–500 kDa), fraction III (>30 kDa), and fraction IV (<30 kDa) using Centriprep concentrators (Amicon, Beverly, MA) having membrane with respective m. w. cut-off.

Quantitation of PGE2 and IL-10 levels
Levels of PGE2 present in the secretory products of linoleic acid or mechanically transformed schistosomula, culture supernatant of keratinocytes, homogenate of cercariae, and homogenate of adult worms were all quantitated using a colorimetric enzyme immunoassay kit (catalog no. DE0100h; R&D Systems, Minneapolis, MN) per manufacturer instructions. This assay is based on the competitive binding of PGE2 present in the sample to a mouse mAb against PGE2, bound to goat anti-mouse Abs immobilized to a microtiter plate. A fixed amount of alkaline phosphatase-labeled PGE2 was used as the competitor. The intensity of color developed is inversely proportional to the concentration of PGE2 present in the sample. Media control homogenates in the presence of linoleic acid and had no PGE2 (<0.0002 ng/ml; shown). Indomethacin (Sigma), aspirin (Sigma), or nimesulide (Sigma) was used as inhibitor of PGE2. In blocking studies, the samples were incubated with linoleic acid (0.2 µg/ml in PBS) in the presence or absence of respective inhibitors at three different concentrations (50, 25, and 12.5 µg/ml) for 3 h at 37°C. Following incubation, PGE2 levels in the supernatant were measured as described above. Wells with no linoleic acid served as negative controls. Levels of IL-10 in the culture supernatant of keratinocytes were quantitated using a sandwich ELISA kit purchased from Endogen (Woburn, MA) per manufacturer instructions.

T cell proliferation assay
Single cell suspension of spleen cells was prepared from naive C57BL/6 mice in complete RPMI medium containing 10% FCS. A total of 2 × 106 cells was plated in each well of a 96-well plate and stimulated with Con A (0.1 µg/ml; Sigma). Use of animals in this study was approved by the Biological Resources Committee of the University of Illinois at Rockford. Different concentrations of secretory products (1, 5, and 10 µg/ml), their fractions (14–20 µg/ml), or keratinocyte culture supernatant stimulated with normal cercariae (100 µg/ml) were then added to these cultures to measure their effect on Con A-induced cell proliferation. After 72 h in culture, cells were pulsed with 1 µCi of [3H]thymidine (Amersham, Arlington Heights, IL) for an additional 18 h. Following incubation, cells were harvested (Nalge Nunc, Naperville, IL) and incorporated [3H]thymidine was measured in a β counter (Beckman, Palo Alto, CA). In some experiments, monoclonal rat anti-mouse IL-10 Abs (10 µg/ml, azide free; Endogen) was added to the wells at the start of the culture to neutralize IL-10 in the cultures.

Delayed-type hypersensitivity (DTH)3 response
C57BL/6 mice were infected with 500 cercariae via the abdominal skin as described previously (3). On days 2 and 5 after infection, each mouse received 100 µg of monoclonal rat anti-mouse IL-10 (Endogen) or control rat IgG (Pierce) injected into the ear. On day 5 after infection, mice were immunized with 2 × 107 BALB/c spleen cells injected s.c. into each flank. Six days later, that is, on day 11 after infection, thickness of hind footpads of each mouse was first measured using a plastic dial caliper (General Hardware, New York, NY) and then challenged with 107 BALB/c cells (in 50 µl) into each hind footpad. At 20–24 h after the challenge, footpad thickness was again measured and values were recorded.

Cutaneous response in IL-10-deficient mice
C57BL/10J mice with an IL-10 gene-targeted mutation (Il10<sup>fl/fl</sup>C<sup>ex</sup>) were purchased from The Jackson Laboratory (Bar Harbor, ME). C57BL/10J wild-type mice served as controls. Mice were infected with 150 normal cercariae of S. mansoni via the abdominal skin, and the skin sites were collected 72 h later for histological analysis as described earlier (3). Migration of schistosomula from the skin to the lungs was evaluated by a compressed organ autoradiography technique using [35S]methionine-labeled cercariae as described previously (3). Briefly, S. mansoni-infected snails were labeled with Tran<sup>3</sup>S-label (25 µCi of [35S]methionine/milliliter of water; sp. act. 1047 Ci/mmol, catalog no. 51006; ICN Pharmaceuticals, Irvine, CA) and cercariae released 72 h after labeling were collected, counted, and used in infections. To determine the presence of the parasite, skin and lungs were collected from each animal and pressed in a tissue press for 24 h. The pressed tissue was then exposed to x-ray film at ~70°C for 5–7 days. Following autoradiographic development, the spots (which represent reduced silver foci on the film) were counted, and the percentage of worm establishment was calculated (number of spots/total number of cercariae used for infection × 100).

Statistical analysis
All the values are expressed as mean ± SD. Statistical difference between the different groups was determined by one way ANOVA using Student-Newman-Keuls method, with p < 0.05 considered significant.

Results
PGE2 levels in the culture supernatant of cercariae/schistosomula of S. mansoni
Significant amounts of PGE2 were present in the secretory products of normal schistosomula of S. mansoni after exposure to linoleic acid-coated plates (Fig. 1). PGE2 was also present in the culture supernatant of irradiated schistosomula. However, on a comparative basis, the levels of PGE2 produced by normal schistosomula were 2.35-fold higher (p < 0.01) than those produced by irradiated schistosomula. In the absence of linoleic acid, no transformation of cercariae to schistosomula occurred and no PGE2 was detected in the culture supernatant. Cercariae were also mechanically transformed into schistosomula by syringe passage in the absence of linoleic acid, and PGE2 levels were measured in the

Abbreviations used in this paper: DTH, delayed-type hypersensitivity; COX, cyclooxygenase.
culture supernatant. These studies showed that significant amounts of PGE2 are present in the secretory products of mechanically transformed schistosomula in the absence of linoleic acid (Fig. 1). However, the levels of PGE2 in these samples were significantly lower than those exposed to linoleic acid. Nearly identical levels of PGE2 were present in the secretory products of mechanically transformed normal or irradiated schistosomula. When secretory products of mechanically transformed normal schistosomula were added to linoleic acid-coated plates, significant amounts of PGE2 were produced in the culture supernatant. These results are representative of 1 of 5 separate experiments with 10 different measurements from each sample.

Levels of PGE2 in the parasite tissue at different stages of its life cycle

Although the levels were low, the presence of PGE2 in the secretory products of mechanically transformed schistosomula in the absence of linoleic acid (Fig. 1). However, the levels of PGE2 in these samples were significantly lower than those exposed to linoleic acid. Nearly identical levels of PGE2 were present in the secretory products of mechanically transformed normal or irradiated schistosomula. When secretory products of mechanically transformed normal schistosomula were added to linoleic acid-coated plates, significant amounts of PGE2 were present in the culture supernatant, suggesting that these secretions contain activities that can produce PGE2 from linoleic acid.

Effect of cyclooxygenase (COX) inhibitors on PGE2 production by the parasite

In mammalian system COX enzymes are involved in the production of PGE2 from arachidonic acid (24). Therefore, the above results suggested that worm homogenate may potentially contain COX-like enzymes. To test this, we added COX2 inhibitors such as indomethacin, aspirin, and nimesulide to parasite cultures before incubation with linoleic acid. These studies showed that addition of mammalian COX2 inhibitors had no effect on PGE2 production by normal cercariae/schistosomula (Fig. 3).

PGE2 levels in the skin

We measured PGE2 levels in the skin during the course of infection. These studies showed a significant increase in PGE2 levels in the skin initially following infection; thereafter, the levels dropped as parasites migrated out of the skin, especially 5 days after infection (Fig. 4). A similar analysis after infection with radiation-attenuated cercariae did not show an increase in PGE2 levels in the skin.
Parasite-induced PGE₂ production by human and mouse keratinocytes

Keratinocytes constitute >90% of cells in the epidermal layer of skin where the parasite resides during the initial 2–3 days after infection (25). Among other factors, keratinocytes can produce PGE₂ in response to specific stimuli (24). Therefore, we wanted to analyze whether cercariae or their secreted products can induce PGE₂ production from human keratinocytes.

Addition of normal cercariae to human or mouse keratinocyte cultures resulted in a significant increase in PGE₂ levels in culture supernatant within 24 h (Fig. 5A). Within a few minutes after culture, the majority of the cercariae were transformed into schistosomula. However, a similar addition of irradiated cercariae to human keratinocyte cultures produced significantly lower amounts of PGE₂ in the culture supernatant. Interestingly, there was a slower transformation of irradiated cercariae to schistosomula. Even after 24 h of culture, a substantial population of irradiated cercariae did not transform into schistosomula. Therefore, we repeated the above experiment with equal quantities (10 µg protein/ml) of secretory products depleted of PGE₂ from either normal or irradiated parasites (Fig. 5A). These studies confirmed that normal cercariae (or their secretory products) are potent inducers of PGE₂ from human keratinocytes.

The PGE₂-inducing activity in the secretory products of normal schistosomula were then narrowed down by separating the secretory products into four different fractions and testing each fraction for its ability to induce PGE₂ in human keratinocytes (Fig. 5A). The PGE₂-inducing activity in the secretory products of normal schistosomula were then narrowed down by separating the secretory products into four different fractions and testing each fraction for its ability to induce PGE₂ in human keratinocytes. These studies showed that only the fraction below 30 kDa (fraction IV) retained the ability to induce PGE₂ from human keratinocytes (Fig. 5B).

Parasite secretion-induced IL-10 production by human and mouse keratinocytes

Significant amounts of IL-10 were present in the culture supernatant of human keratinocytes incubated with normal cercariae or their fraction IV secretory product, whereas incubation of cells with irradiated cercariae or their fraction IV secretory product induced only low levels of IL-10 (Fig. 6). Addition of the COX2 inhibitor indomethacin to these cultures blocked the secretory product-induced PGE₂ production from human keratinocytes (Fig. 6). Similar results were obtained when mouse keratinocytes were used (data not shown).

Cutaneous response to schistosomula in IL-10-deficient mice

Migration of schistosomula from skin to the lungs was traced in IL-10-deficient C57BL/10 mice using ³⁵S-labeled cercariae. These studies showed that there was a delayed migration of schistosomula from the skin to the lungs of IL-10-deficient mice compared with wild-type mice (Table I). Histological analysis of the skin showed massive accumulation of inflammatory cells around the parasite in the skin of IL-10-deficient mice, whereas fewer inflammatory cells were seen in the skin of wild-type mice (Fig. 7).
Infection with cells showed a significant swelling of the foot pad (DTH response) C57BL/6 mice sensitized and challenged with BALB/c spleen injection of neutralizing anti-IL-10 Abs (100 \( \mu \)g/mouse) there was a significant reduction in the DTH response (Fig. 8). Injection of a control IgG had no effect on DTH response. Postinfection reversed the parasite-induced suppression of proliferation as with the secretory products (Table II). This inhibition could be reversed with a neutralizing Abs against murine IL-10 (Table II). Suppression of DTH response in IL-10 (Table II). Addition of secretory products from normal schistosomula to Con A-stimulated spleen cells resulted in a significant dose-dependent inhibition of cell proliferation (Table II). This activity appeared to be associated with fraction IV of the secretory product. The secretory product-induced inhibition could be reversed by adding neutralizing Abs against murine IL-10 to these cultures. We observed a similar inhibition of cell proliferation as with the secretory products (Table II). This inhibition could be reversed with a neutralizing Abs against murine IL-10 (Table II). Suppression of DTH response in S. mansoni-infected animals C57BL/6 mice sensitized and challenged with BALB/c spleen cells showed a significant swelling of the foot pad (DTH response) compared with media controls (Fig. 8). However, when concurrent infection with S. mansoni was present at the time of sensitization, there was a significant reduction in the DTH response (Fig. 8). Injection of neutralizing anti-IL-10 Abs (100 \( \mu \)g/mouse) on days 2 and 5 postinfection reversed the parasite-induced suppression of DTH response (Fig. 8). Injection of a control IgG had no effect on the parasite-induced DTH suppression. When the above experiment was repeated in IL-10-deficient mice, S. mansoni infection had no suppressive effect on DTH reaction (Fig. 8). Discussion Results from this study suggest a plausible role for PGE2- and/or PGE2-dependent IL-10 in the host immunoregulation by skin stage schistosomula of S. mansoni. Larval stages of the parasite S. mansoni are notorious for evading host cellular immune responses in their natural hosts (26, 27). Schistosomula of S. mansoni can down-regulate IFN-\( \gamma \)-response and produce an IL-4 and IL-10 response in the skin and draining lymph nodes soon after entry into the host (5, 7). This transient switch in the cytokine pattern from Th1 to a more Th2 type response initially after infection is believed to be an evasive mechanism by the parasite to establish in the host. In this study, we show that the parasite may achieve this by inducing large quantities of PGE3 in the microenvironment, which in turn may be responsible for the predominant IL-10 response. Previous studies showed that polyunsaturated essential free fatty acids, such as linoleic acid, present on the surface of the skin can act as potent penetration stimuli for the cercariae of S. mansoni (28). Upon exposure to free fatty acids such as 3.0 mM of linoleate, the cercariae transform into schistosomula, and detectable levels of PGE1, PGE2, 5-HETE, and 15-HETE appear in the culture medium (13, 14). This suggested that the skin stage schistosomula of S. mansoni have the ability to synthesize eicosanoids from free fatty acids (29). However, because only a small amount of PGE2 is produced by the parasite, the biological significance of parasite-produced PGE2 may be trivial compared with the substantial amounts of PGE2 induced in the host cells. Nevertheless, significant amounts of PGE2 were present in the culture supernatant of mechanically transformed schistosomula in the absence of any free fatty acids (Fig. 1), suggesting that the parasites can constitutively produce PGE2 in their tissue. Among the various life cycle stages, the tissue migratory stages of the parasite appeared to produce substantial amounts of PGE2. In the mammalian tissue, biosynthesis of PGE2 is a complex multistep pathway mediated by COX enzymes. There are two COX isoenzymes, COX1 and COX2. COX1 is constitutively expressed in mammalian cells, whereas COX2 is inducible (24). In the presence of COX2, arachidonic acid is metabolized to PGE2 (24). Because PGE2 is produced by several larval stages of S. mansoni (Fig. 2), it is apparent that these parasites may possess enzymes similar to mammalian COX2. However, an immunoblot analysis using a polyclonal Ab against mammalian COX2 failed to detect the presence of COX2 in the parasite tissue or in their secretions (data not shown). This finding was further confirmed by the fact that COX2 inhibitors such as indomethacin, aspirin, and nimesulide failed to block PGE2 production by cercariae or schistosomula. In addition, we also searched the schistosome expressed sequence tag database and found no DNA or amino acid sequence information. The Journal of Immunology

![Figure 6](http://www.jimmunol.org/) Parasite-induced IL-10 production by human keratinocytes. IL-10 levels in the culture supernatant of human keratinocytes were measured after incubation with normal or irradiated cercariae and their respective fraction IV secretory products. Significant (\( p < 0.001 \)) levels of IL-10 were present in the culture supernatant of keratinocytes incubated with normal cercariae or their fraction IV compared with those of irradiated cercariae. Addition of indomethacin to these cultures significantly (\( p < 0.001 \)) decreased the amount of IL-10. These results are representative of one of three separate experiments using five separate analyses per sample. **Effect on spleen cell proliferation**

Addition of secretory products from normal schistosomula to Con A-stimulated spleen cells resulted in a significant dose-dependent inhibition of cell proliferation (Table II). This activity appeared to be associated with fraction IV of the secretory product. The secretory product-induced inhibition could be reversed by adding neutralizing Abs against murine IL-10 to these cultures. In the above studies, we showed that normal parasites induce IL-10 production by human keratinocytes. Therefore, we cocultured mouse keratinocytes with normal cercariae and added the culture supernatant to spleen cell cultures. We observed a similar inhibition of cell proliferation as with the secretory products (Table II). This inhibition could be reversed with a neutralizing Abs against murine IL-10 (Table II).

**Suppression of DTH response in S. mansoni-infected animals**

C57BL/6 mice sensitized and challenged with BALB/c spleen cells showed a significant swelling of the foot pad (DTH response) compared with media controls (Fig. 8). However, when concurrent infection with S. mansoni was present at the time of sensitization, there was a significant reduction in the DTH response (Fig. 8). Injection of neutralizing anti-IL-10 Abs (100 \( \mu \)g/mouse) on days 2 and 5 postinfection reversed the parasite-induced suppression of DTH response (Fig. 8). Injection of a control IgG had no effect on the parasite-induced DTH suppression. When the above experiment was repeated in IL-10-deficient mice, S. mansoni infection had no suppressive effect on DTH reaction (Fig. 8).

**Table I. Percentage of worm recovery in the skin and lungs of mice after infection with S. mansoni**

<table>
<thead>
<tr>
<th>Days After Infection</th>
<th>Wild-Type Mice</th>
<th>IL-10-Deficient Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Skin</td>
<td>Lungs</td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>97.3 ± 3.9</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>80.6 ± 1.4</td>
<td>22.6 ± 6.1</td>
</tr>
<tr>
<td>4</td>
<td>58.2 ± 6.8</td>
<td>39.5 ± 2.5</td>
</tr>
<tr>
<td>5</td>
<td>28.5 ± 3.5</td>
<td>68.9 ± 1.4</td>
</tr>
<tr>
<td>7</td>
<td>6.9 ± 3.5</td>
<td>89.5 ± 4.5</td>
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</tbody>
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**Discussion**

Results from this study suggest a plausible role for PGE2- and/or PGE2-dependent IL-10 in the host immunoregulation by skin stage schistosomula of S. mansoni. Larval stages of the parasite S. mansoni are notorious for evading host cellular immune responses in their natural hosts (26, 27). Schistosomula of S. mansoni can down-regulate IFN-γ response and produce an IL-4 and IL-10 response in the skin and draining lymph nodes soon after entry into the host (5, 7). This transient switch in the cytokine pattern from Th1 to a more Th2 type response initially after infection is believed to be an evasive mechanism by the parasite to establish in the host. In this study, we show that the parasite may achieve this by inducing large quantities of PGE3 in the microenvironment, which in turn may be responsible for the predominant IL-10 response.

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homology to human or mouse COX2 (data not shown). Based on these observations, we hypothesize that *S. mansoni* lack COX2 enzyme and may possess biochemical pathways different from their mammalian counterpart for their production of PGE$_2$.

Our studies show that in addition to producing substantial quantities of PGE$_2$ from free fatty acids, schistosomula of *S. mansoni* also has the ability to induce PGE$_2$ production in human keratinocytes. Although the molecular mechanism of this PGE$_2$ induction from host cells is not fully understood at this time, our findings tentatively suggest that the parasite secretory products contain a factor that can potentially induce PGE$_2$ production from human keratinocytes. Preliminary characterization studies suggest that the size of this factor may be $<$30 kDa and is not PGE$_2$. Further studies are in progress to characterize this molecule. Velupillai and Harn (30) reported the presence of an oligosaccharide Ag (LNFP-III) in the egg of *S. mansoni* that can induce IL-10 and PGE$_2$ from spleen cells. At this time we do not know whether the factor that we observed in the secretions of schistosomula is similar to LNFP-III.

Thus the skin-residing schistosomulum has the potential to generate substantial quantities of PGE$_2$ around their local milieu (29). This notion was confirmed when we analyzed the PGE$_2$ levels in the skin. A significant correlation was seen between the presence of the parasite in the skin and elevated PGE$_2$ levels. As parasites migrated out of the skin, the levels of PGE$_2$ also substantially decreased, suggesting that the majority of the PGE$_2$ produced in the skin is in response to the parasite. Among various other functions, PGE$_2$ is a potent vasodilator (24). Such a vasodilatation in the skin may help easy passage of the parasite into the circulation (29). Thus, production and/or induction of PGE$_2$ in the skin may be a devious adaptive mechanism developed by the parasite for its survival in the host.

After entry into the host, schistosomula remain in the skin for $>$3 days before migrating further into the lungs. Analysis of the cytokine response during this skin migratory phase of the parasite shows a significant increase in IL-10 within a few hours after entry of the parasite into the skin (5). Previously we reported that T cells are not a source for this early IL-10 in the skin (5). These studies suggest that the majority of the parasite-induced IL-10 in the skin may be produced by keratinocytes (Fig. 6). It is well established that keratinocytes are a major source of IL-10 in the epidermis (16). Using COX2 blockers we show that this parasite-induced IL-10 by keratinocytes is PGE$_2$ dependent. Previous studies have also shown that PGE$_2$ is a potent stimulator of IL-10 from a variety of cells including keratinocytes (17–19). Thus our finding potentially revealed an interesting pathway by which the parasites may be inducing a strong IL-10 response in the skin.

Previously we showed that the host mounts very little inflammatory response against the skin-residing parasite, despite the fact that the parasite stays in the skin for $>$72 h (3). IL-10 and IL-1ra are potent anti-inflammatory molecules that play a key role in orchestrating inflammatory reaction in the skin by limiting the production of a large spectrum of proinflammatory cytokines and mediators (18, 19). Cytokine analysis in the skin following infection with *S. mansoni* showed a significant reduction in the levels of IL-1$\alpha$ and IL-1$\beta$, whereas the levels of IL-1ra were substantially increased (21). IL-10 has been shown to inhibit IL-1$\alpha$ and IL-1$\beta$ (18, 19) and to up-regulate production of IL-1ra (31). Therefore, PGE$_2$-driven IL-10 production in the skin following infection may play a significant role in the immunomodulation of inflammatory responses in the skin of infected animals. These findings are similar to those reported by Hoffmann et al. (32), who state that a major consequence of schistosome-induced IL-10 production is to

### Table II. Inhibition of T cell proliferation by secretory products of normal schistosomula of *S. mansoni*

| Media control | 1,350.36 ± 268.3* |
| Con A (0.1 µg/ml) | 13,487.06 ± 748.2 |
| Con A + secretory product |  |
| 1 µg/ml | 9,520.40 ± 845.7* |
| 5 µg/ml | 6,922.30 ± 218.7* |
| 10 µg/ml | 3,277.40 ± 262.2* |
| Con A + fraction IV (14 µg/ml) | 3,125.50 ± 213.5* |
| Con A + keratinocyte supernatant (50 µg/ml) | 2,214.10 ± 102.4* |
| Con A + 14 µg/ml fraction IV + 10 µg/ml anti-IL-10 | 10,987.78 ± 321.8 |
| Con A + 50 µg/ml keratinocyte sup + 10 µg/ml anti-IL-10 | 12,596.14 ± 457.0 |

* Statistically significant ($p < 0.01$) compared to Con A alone treated groups. Values are presented in cpm.
down-regulate the immune responses of the host against the parasite. However, this subdued inflammation is seen only so long as live parasites are present in the skin (3), suggesting that the PGE$_2$/IL-10-inducing activity is present only in the secretions of the tissue-migrating parasites.

Previous studies showed that secretions of schistosomula contain an activity that inhibited lymphoproliferation (33). In this study we show that this inhibitory activity is potentially mediated by IL-10 inducted in the host cell via a PGE$_2$-dependent mechanism. Because parasite-stimulated keratinocyte supernatant can induce suppression of lymphoproliferation, it is possible that a similar mechanism may be operative in vivo in the skin of infected animals. Such a down-regulatory mechanism may be responsible for the parasite-induced suppression of IFN-γ-producing T cells in the skin (5) and draining lymph nodes (7). A role for IL-10 in the suppression of Th1 cytokine synthesis in murine and human schistosomiasis has already been reported previously (8–11). Our studies underscore the significance of parasite-induced PGE$_2$ in these IL-10-mediated mechanisms.

Parasite-induced suppression of DTH reaction has been reported previously in murine schistosomiasis (34), and a role for IL-10 in this down modulation has been well documented (35). In this study we show that this IL-10-induced DTH down modulation may be mediated by parasite-induced PGE$_2$, especially during the acute phase of infection (Fig. 8). This finding may be critical for a realistic goal of an antischistosome vaccine using candidate Ags, cautioning that the skin stage parasite can still modulate the immune responses in the host, irrespective of whether the host is immune or not.

Interestingly, exposure to γ-irradiation appears to interfere with the PGE$_2$ production by the parasite and its ability to induce PGE$_2$ production from human keratinocytes (Fig. 1). Because immunization with γ-irradiation-attenuated parasites confers significant immunity against challenge infection (4), it is possible that the initial PGE$_2$ production by the normal parasite is a survival mechanism to down-regulate host inflammatory and immune responses. Cytokine analysis of the skin following infection with radiation-attenuated parasites showed a predominately IFN-γ response, and there was very little IL-10 response in the skin (5). Because the PGE$_2$/IL-10-inducing factor appears to be absent in the secretions of irradiated parasites, it is possible that the harmful effects of irradiation could have damaged the ability of the parasite to produce this host immunomodulatory factor (36). A severe inflammatory reaction surrounds the γ-irradiation-attenuated parasites in the skin and potentially allows immune cells to come in close contact with the parasites (3, 4). This initial host response is believed to be critical for the generation of a protective immune response against this parasite (5–7).

A pivotal role for IL-10 in the parasite-induced immunoregulation in the skin was further reiterated by our studies using IL-10-deficient mice. Parasite-induced immunosuppression was less prominent in these mice, and the cutaneous responses resembled those in immune mice vaccinated with irradiated cercariae (3). Therefore, these findings complement those reported by Hoffmann et al. (32), who show vigorous expansion of Ag-specific cytokine-producing effector cells in IL-10-deficient mice in response to parasite Ags. In addition, we observed a severe inflammatory reaction around the parasite in the skin that potentially delayed the migration of the parasite through the skin (Fig. 7 and Table I). Similar results were reported by Hoffman et al. (32), who show increases in perivascular inflammatory responses in the lungs of IL-10-deficient mice following infection with S. mansoni.

Our results support the notion that a major consequence of schistosomiasp-induced IL-10 production is to down-regulate host immune responses against the parasite, especially during the early phase of infection. Furthermore, our studies suggest a central role for parasite-induced PGE$_2$ in the IL-10-dependent down-regulation. The study also suggests the presence of a low m.w. PGE$_2$/IL-10-inducing factor in the secretions of normal parasites. Neutralizing the effect of this factor and subverting the initial outburst of PGE$_2$ production in the skin by the parasite may contribute to decreased IL-10 responses, which in turn might prove an effective approach to increase the efficacy of some of the defined vaccine Ags against schistosomiasis.

References


