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*J Immunol* 1998; 161:4161-4168; ;
http://www.jimmunol.org/content/161/8/4161

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Dermal Endothelial Cells and Keratinocytes Produce IL-7 In Vivo After Human Schistosoma mansoni Percutaneous Infection

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The parasite Schistosoma mansoni infects its definitive mammalian host through an obligatory cutaneous penetration. In this work, we studied early immune response following migration of larvae through human skin, the first immunocompetent organ encountered by the parasite. For this purpose we used an experimental model of severe combined immunodeficient mice engrafted with human skin and injected with autologous PBL. Six days after percutaneous infection, we observed an infiltration of lymphocytes within the human skin, predominantly composed of CD4+ T cells. Moreover, among the cytokines potentially present in the infected skin, immunohistochemistry analysis revealed an in vivo expression of IL-7 in the epidermal layers and strikingly at the level of vascular endothelium. Using an in vitro coculture system, we showed that the S. mansoni larvae directly trigger IL-7 production by human dermal microvascular endothelial cells but not by keratinocytes. Finally, measurements of IL-7 concentrations in plasma of 187 S. mansoni-infected individuals showed that the youngest, which are also the most infected, displayed the highest IL-7 levels. Together, these findings describe dermal endothelial cells as a novel source of IL-7, a cytokine particularly important in schistosomiasis.


IL-7 is produced by the bone marrow, spleen, and thymus stromal epithelial cells, intestinal epithelial cells, keratinocytes, and monocytes (1). Its synthesis by endothelial cells has only been recently reported (2). This cytokine, initially described as a growth factor for B cell precursors (3), exerts pleiotropic effects, such as stimulation of thymocyte growth, promotion of NK/LAK activity, modulation of cytokine production (IL-4, IFN-γ), increased IL-2Rα expression on T lymphocytes, and activation of mature T lymphocytes and monocytes (see Refs. 1 and 4 for review). More recently, the pivotal role of IL-7 in the skin has been highlighted (5). Several authors described the involvement of IL-7 in human skin pathology. In Mycobacterium leprae infection, IL-7 produced at the site of lesion facilitates the cellular infectious processes in potentiating Ag-specific T cells. The authors postulated that the dysregulation of IL-7 production might contribute to the host’s inability to control infection (6). Bonifati et al. (7) described an increase in IL-7 concentrations in lesional skin and in the sera of patients with plaque-type psoriasis. Increased IL-7 mRNA synthesis was also demonstrated in skin of patients with atopic dermatitis. Indeed, IL-7 is supposed to amplify inflammation, acting principally as a growth factor for in vivo primed Ag-specific T cells and enhancing their proliferation in the skin (8).

Apart from these studies, the role of IL-7 produced in the skin of Schistosoma mansoni-infected mice has only been recently reported (9). Indeed, this parasite infects its definitive host by an obligatory penetration through the skin. The larvae that are responsible for schistosomiasis, a parasitic disease affecting over 200 million individuals worldwide (10), remain in cutaneous tissue for 3 to 4 days, almost exclusively in the deep epidermal layers in close contact with keratinocytes. Subsequently, they reach dermal vessels (11), then are passively carried by the bloodstream to the lungs where they remain a few days. Finally, via the systemic circulation, they enter the hepatic portal system, where they mature into egg-producing adult worms at the mesenteric vein level (12). During the penetration and shortly thereafter, the cutaneous cells could play an active role in the initiation of the immune response through processing and presentation of the Ag to the competent cells of skin draining lymphoid compartments. By itself, the skin presents all the characteristics which suggest that it can function as an autonomous immunologic organ (13). We recently reported the early immunologic cutaneous events occurring during murine S. mansoni infection and their importance to the fate of the late immune response. Indeed, we were able to show that IL-7 expression was detectable in the skin of infected mice, between days 1 and 21 following infection. In addition, intradermal injection of exogenous IL-7, before the penetration of the cercariae, led to a more severe liver pathology and to an increased number of surviving adult parasites, thus favoring the parasite rather than its host (9).

The growing body of information on the important role of IL-7 in immunologic and pathologic cutaneous reactions and...
our own demonstration of its participation in cutaneous reactions in the murine experimental system (9) prompted us to assess the involvement of this cytokine in human *S. mansoni*, particularly at cutaneous level, in the first days of infection. Recently, human skin grafts were performed on SCID mice. This system was extensively used to investigate fundamental and pathologic dermatology (14–18). Subsequent injection with human peripheral blood leukocytes led to the SCID-hu-PBL/skin mouse model, allowing human cutaneous immunologic assays (46). We thus decided to use SCID-hu-PBL/skin mice to infect human skin with *S. mansoni* and analyzed, on the one hand, the unknown localization of schistosomula in human skin and, on the other hand, the onset of early immunologic events (6 days after infection), such as human cell recruitment and human cytokine production. Interestingly, we observed a cutaneous infiltration of lymphocytes and mononuclear cell recruitment and human cytokine production. Interestingly, we observed a cutaneous infiltration of lymphocytes and mononuclear cell recruitment and human cytokine production. Interest-

### Materials and Methods

#### Animals

Six-week-old homozygous *scid/scid* C57BL/6 (SCID) mice were obtained from the specific pathogen-free animal breeding facility at the Pasteur Institute (Lille, France) and kept in isolators under barrier-sustained conditions, without prophylactic administration of antibiotic.

#### Skin transplantation and hu-SCID mice reconstitution

The protocol of skin transplantation was performed as previously described by Yan et al. (14). Animals were anesthetized by i.p. injection of a mixture of Imalgene 1000 (80 mg/kg; Rhône Mérieux, Lyon, France) and valium (2.5 mg/kg; Roche Products, Neuilly-sur-Seine, France). Human skin (1.5 x 2 x 0.2 cm) was grafted onto a full skin excisional field of the shaved back of the SCID mice and fixed with nonadsorbable 4-0 Ethibond Excel sutures (Ethicon, Neuilly-sur-Seine, France). After applying a sterile compressive dressing. Dressings were kept in place, and the wounds resolved spontaneously within 4 to 6 wk. Human skin consisted of biopsies of Imalgene 1000 (80 mg/kg; Rhône Mérieux, Lyon, France) for histologic observations.

#### Parasites and infection protocols

The *S. mansoni* (Puerto Rican strain) life cycle was maintained at the Pasteur Institute of Lille using the Biomphalaria glabrata snail as the intermediate host and the *Mesocricetus auratus* hamster as the definitive host. For infection of human skin-grafted SCID mice, animals were anesthesized with pentobarbital (30 mg/kg; Sanofi, Libourne, France) and valium (2.5 mg/kg; Roche Products, Neuilly-sur-Seine, France). Human skin (1.5 x 2 x 0.2 cm) was grafted onto a full skin excisional field of the shaved back of the SCID mice and fixed with nonadsorbable 4-0 Ethibond Excel sutures (Ethicon, Neuilly-sur-Seine, France). After applying a sterile Vaseline-impregnated gauze, the graft was protected from self injury with compressive dressing. Dressings were kept in place, and the wounds resolved spontaneously within 4 to 6 wk. Human skin consisted of biopsies of reconstructive surgery (Roger Salengro Hospital, Lille, France) after obtaining informed consent from the patient. Blood samples from skin donors were collected 4 wk after surgery. Mononuclear cells were isolated using Ficoll-Paque density gradient centrifugation (Pharmacia Biotech, Uppsala, Sweden). Skin-grafted SCID mice were injected i.v. with 23 x 10⁹ human peripheral blood leukocytes per mouse.

####Histologic analysis

**Histology.** Human skin graft infected with 1600 furcocercariae was aseptically harvested 1 h postinfection (p.i.) then fixed overnight in Bouin’s solution and paraffin embedded. Sections of 6 µm were dewaxed in xylene then, rehydrated, and stained with Masson’s trichrome (Sigma, St. Louis, MO) before being mounted with Entellan resin (Labonord, Villeneuve d’Ascq, France) for histologic observations.

1. Abbreviations used in this paper: p.i., postinfection; HMVEC-d, dermal human microvascular endothelial cells; EPG, number of eggs per gram of feces.
ELISA for IL-7 determination

IL-7 concentrations were determined in plasma from S. mansoni-infected or uninfected humans and in endothelial cell-schistosomulum coculture supernatants using a highly sensitive test (R & D Systems, Oxon, U.K.; detection level, 0.025 pg/ml for a 200-μl sample). Assays were performed strictly following the manufacturer’s instructions. A multichannel spectrophotometer, Labsystems, Helsinki, Finland were used to measure the absorbance at 492 nm.

Results

Human skin-grafted SCID mice is a suitable model to study early cutaneous events during S. mansoni infection

Five to six weeks after engraftment, macroscopic examination of the human skin revealed a classical aspect as defined by a normal pigmentation and by the absence of inflammation and skin breakdown. Histologic observations of biopsies corresponding to the connective zone between human and murine skins showed a continuous junction (Fig. 1). An usual aspect of the different cutaneous components, such as epidermis, dermis, annexa, and blood vessels, could be observed without cellular alterations. As the human engrafted skin appeared macroscopically and microscopically intact, we performed an infection with S. mansoni cercariae (as described in Materials and Methods).

When human skin was examined 1 h p.i., schistosomula were observed principally at the dermo-epidermal junction, (Malpighi layer; Fig. 2). In all cases, they were found within lacunae as described by Wilson et al. for rodent skin (24). Furthermore, we found parasites in the lungs 6 days p.i. (data not shown), as observed during the migration in the mouse model, thus demonstrating that vascularization of the human graft is perfectly connected with the host’s vascular system. Taken together, these observations presented no difference compared with the mouse model (12, 24) and validated this experimental system for the study of early immunologic events in human skin.

Lymphocytes infiltrate the infected human skin

The skin was described as a unique immunologic environment that contains many of the cellular constituents needed for the initiation, modulation, and elicitation of the immune response (25). Immunologic events occurring immediately after penetration of cercariae in human skin were not extensively studied in previous reports.
for obvious ethical reasons. In our model of SCID-hu-PBL/skin mice, a functional humanized immune response to schistosomulum transcutaneous penetration could potentially occur.

The anatomo-pathologic analysis of the infected human skin sections (6 days p.i.) showed a moderate intradermal infiltration of human lymphocytes. Immunohistochemistry analysis confirmed the infiltration of human CD4<sup>+</sup> Th lymphocytes, exclusively in the dermis with a perivascular predominance (Fig. 3A). No CD4<sup>+</sup> T cells were detected in control noninfected SCID-hu-PBL/skin mice (Fig. 3B). Few human CD8<sup>+</sup> T lymphocytes were present in the basal epidermis, but no differences could be observed between control and infected skin. No CD22<sup>+</sup> B lymphocytes were found in any biopsies whether infected or not (data not shown).

Keratinocytes and endothelial cells produce increased amounts of IL-7 in human infected skin

In earlier work we reported that IL-4, IL-7, and IFN-γ are produced in murine infected skin within the first days after parasite penetration (9). Thus, we investigated cytokine production following penetration of the parasite through the human skin graft. Immunohistochemical stainings were conducted on skin sections (6 days p.i.), and a strong increase of IL-7 was revealed in human

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**FIGURE 3.** Immunohistochemical distribution of CD4-positive T cells in skin from SCID-hu-PBL/skin mice on day 6 after *S. mansoni* infection (A) compared with that in uninfected skin (B). In A, the infected skin is characterized by a dermal infiltrate of CD4-positive T cells with a preferential perivascular localization. Notice the absence of such cells on uninfected skin sections (B). The epidermis from the control sample presented in B was thicker than the corresponding layers in the infected samples (A). This only reflects the conditions of skin regeneration after graft, since other control samples presented epidermis thickness equivalent to that of infected skin, and identical results were obtained with these samples. Avidin-biotin immunoperoxidase stain was used. Magnification, ×200.
skin following infection (Fig. 4A; noninfected control is shown in Fig. 4B). A diffuse staining in the total epidermis and at the vascular level in the dermis was observed. At higher magnification, a well-defined staining surrounding the endothelial cells was apparent (Fig. 4C), suggesting a possible synthesis of IL-7 by the dermal vascular cells. In contrast, neither IL-4 nor IFN-γ was detected by immunohistochemistry at the protein level (data not shown). These findings were confirmed using RT-PCR method. Indeed, IL-4 mRNA and IFN-γ mRNA were not detectable in either human infected skin or human uninfected skin (Fig. 5).

Schistosomula increase IL-7 production by human microvascular dermal endothelial cells, but not by keratinocytes, in vitro

To determine whether the parasite could be directly responsible for the IL-7 endothelial production and to exclude a possible diffusion from keratinocytes, we conducted cocultures of schistosomula with dermal human microvascular endothelial cells. At different time points, culture supernatants were harvested, and IL-7 accumulation was measured. As shown in Figure 6, a continuous constitutive synthesis of IL-7 by HMVEC-d was observed in the absence of parasite. Cocultures with schistosomula showed a significantly higher synthesis at 72 h compared with that in the control culture. This was confirmed after 120 h, with 2.5-fold increased production.

To determine the involvement of the parasite in IL-7 production in epidermis (observed on tissue sections), similar experiments using cocultures were performed to study the possible IL-7 production by human keratinocytes. In contrast to dermal endothelial cells, from 12 to 120 h in the absence or the presence of parasite, no significant production of IL-7 by keratinocytes could be measured in culture supernatants (data not shown).

Highly S. mansoni-infected patients show high IL-7 levels in plasma

Considering these results, we questioned whether the local (epidermal and endothelial) IL-7 production in S. mansoni-infected patients is increased.  

FIGURE 4. IL-7 expression in human infected skin on day 6 after infection (A) compared with that in uninfected skin (B). Note in A the diffuse staining in the epidermis and the strong staining of the vessels in the dermis. A higher magnification shows a well-defined staining surrounding the endothelial cells (C). IL-7 was not detectable in uninfected skin sections on either epidermis or dermal vessels (B). Avidin-biotin immunoperoxidase stain was used. Magnification: A and B, ×200; C, ×400.

FIGURE 5. IL-4 mRNA and IFN-γ mRNA are absent in human infected skin on day 6 after infection. β-Actin was used as an internal control for the integrity of the RNA. PCR with specific primers was then used to detect IL-4 and IFN-γ mRNA. Infected human skin (lane 2), noninfected human skin (lane 3), and cDNA positive for IL-4 (48-h Con A-stimulated Jurkat cells) and for IFN-γ (48-h PHA-stimulated PBMC; lane 1) are shown. Neither IL-4 nor IFN-γ mRNA was detected in infected or noninfected human skin.
human skin could be extended to detectable IL-7 levels in the plasma of infected individuals. The results presented in Figure 7B show the distribution of IL-7 concentrations in the different age classes in the infected population (with comparable numbers of individuals for each class). The youngest individuals exhibited the highest IL-7 concentrations, whereas the oldest displayed the lowest levels. Several population studies in major schistosomiasis endemic areas highlighted a negative correlation between age and infection intensity (determined by EPG) (26–29). It was then crucial to check whether this was the case in our chosen population and subsequently to test potential correlation between IL-7 and age. To validate statistical tests, we conducted logarithmic transformations of EPG values and IL-7 values to obtain a normal distribution of these parameters. Therefore, correlation coefficients \((r)\) were calculated between EPG and age and between IL-7 and age. On the one hand, the highly significant negative correlation \((r = 0.252; p < 0.0005)\) between EPG and age was confirmed. On the other hand, we showed a strong negative correlation \((r = 0.371; p < 0.0001)\) between IL-7 and age (Fig. 7C). Therefore, IL-7 concentrations and EPG are displaying the same age-dependent evolution (the highest IL-7 levels and EPG values in the lowest age class). Moreover, identical analysis of the African and European uninfected control populations revealed comparable IL-7 plasma concentrations in the whole populations (Fig. 7A). Any difference between the amount of IL-7 in each age group could be observed, and statistical analysis for each of these populations did not single out any significant correlation between IL-7 levels and age.

**Discussion**

The first description of skin as an immunocompetent organ was made by Streilein (30). Langerhans cells, keratinocytes, epidermotropic T cells, and skin-associated draining lymph nodes collectively form an immunologic unit that provides the skin with immune surveillance mechanisms (13). In addition, it is now obvious that inflammatory reactions and immune responses involve close interactions between immunocompetent cells and the vascular endothelium. By producing cytokines and chemokines (31) and by expressing specific adhesion molecules (32), endothelial cells play an important part in modulating selective cellular recruitment. Viral, bacterial, or protozoan infections directly or indirectly involve endothelial cells (33–35). In the present report we studied early events occurring in human skin grafted onto SCID-hu-PBL mice after *S. mansoni* infection, and we demonstrated an important involvement of the dermal endothelial cells. Indeed, these cells are able to produce IL-7 and to recruit T lymphocytes in the skin in response to schistosomula penetration.

*S. mansoni* is a topical endovascular parasite; from host penetration through the skin, to migration via the lungs to the liver and mesenteric vein, *S. mansoni* is in close contact with the endothelial compartment at each maturation step. In a previous work (9) we detected IL-7 mRNA synthesis in murine infected skin and demonstrated that parasite migration at least in part depends on this cytokine. Indeed, mice infected 12 h after intradermal injection of rIL-7 show a different migration and/or localization of the parasites at the very early stages of infection. In addition, this single injection before infection leads to an increased worm burden and to a more severe pathology associated with *S. mansoni* infection. Furthermore, in IL-7-deficient mice (36) infected with *S. mansoni*, the worm burden is decreased, and the clinical status of the infected host is ameliorated. In addition, and interestingly, worms never develop to full sexual maturity in infected IL-7-deficient mice, implying an important role of IL-7 in schistosome growth and maturation (I. Wolowczuk, manuscript in preparation).

Therefore, our demonstration of increased IL-7 endothelial production in human infected skin triggered by the parasite could be to its own benefit. Whether others auto-, para-, and/or intracrine molecular activities are involved is still unknown, and the molecular identity and the mechanism of action of parasitic factors involved in this stimulation remain to be determined. Among others cytokines potentially produced by endothelial cells, IL-7 appears to be uniquely expressed in response to the parasite (F. Trottein, unpublished observation). Beyond IL-7 production by dermal endothelial cells, IL-7 synthesis by lung endothelium was investigated in vitro subsequent to parasite entry, using human pulmonary endothelial cells. Cytokine assays showed high baseline levels of IL-7 production, which were not modulated by the presence of parasites (data not shown). Therefore, the parasite-dependent increase in IL-7 synthesis might be limited to the cutaneous environment. We also noticed increased production of IL-7 in the epidermis (in vivo) probably produced by keratinocytes (37). Moreover, IL-7 was not observed in the epidermis of SCID-hu-skin mice not injected with autologous PBL and was undetectable in keratinocyte-schistosomulum cocultures (data not shown). Taken together, these observations could mean that keratinocytes need additional factors present in the *S. mansoni*-infected skin to produce IL-7, in contrast to endothelial cells. We are investigating the potential increase in IL-7 production due to the parasite using cytokines added to coculture medium, such as IFN-γ, which is known to activate keratinocytes (6). Therefore, from these observations we could postulate that IL-7 would be available for the parasite, at least from skin to lung.

In our study we reported a perivascular recruitment of CD4⁺ T cells in the dermis of *S. mansoni*-infected human skin. That seems to be specific to parasite penetration, and the absence of CD8⁺ T lymphocytes could not be due to limitation of cellular reconstitution of the mice. Indeed, using the same experimental system, Delhem et al. observed a cutaneous infiltration of CD8⁺ T lymphocytes after injection of virus in human skin (see Footnote 3). Recently, IL-7 was described as an inducer of T cell adhesion to endothelial cell (38, 39). Particularly, this cytokine increase VLA-4 and LFA-1 expression on activated CD4⁺ CD23⁺ T cells subsets (40). Therefore, we could hypothesize that IL-7 produced by dermal endothelial cells may promote the observed CD4⁺ T
cell recruitment. In addition, IL-7 could probably act as a costimulus (coreceptor and/or cytokine) to induce human resting peripheral blood T lymphocytes to proliferate within the skin (41). In murine skin, IL-7 overexpression by keratinocytes from transgenic mice causes a large expansion of $\alpha \beta$ and $\gamma \delta$ TCR cells in the skin (42). Finally, IL-7 regulates TCR usage and T cell proliferation in HIV-1 infection (43). Therefore, it seems pertinent to hypothesize a role for endothelium- and/or keratinocyte-derived IL-7 not only for increasing T cell adhesiveness but also for final peripheral activation and proliferation of dermal recruited T cells. T cells of the cutaneous infiltrate are presently being cloned to determine their specificity, effector role, and particularly the presence of the cutaneous leukocyte antigen, a unique skin homing receptor expressed by memory T cells (44). Finally, double-graft experiments would be performed to determine whether T cell infiltration is restricted to the infection site or is systemic, within the uninfected fragment of human skin.

We have demonstrated that IL-7 is not only locally produced in the skin but that IL-7 concentrations are also increased in the plasma of the youngest infected patients. Monocytes (6) and platelets (45) from peripheral blood might be the principal sources of this secretion. Moreover, we established a significant negative correlation between IL-7 concentration (after logarithmic transformation) and age in the infected cohort is presented. The population was first sorted following increasing ages. We obtained a highly significant negative correlation ($r = 0.323; p < 0.0001$).

**FIGURE 7.** IL-7 concentration analysis in the plasma of 104 healthy individuals vs that in 187 S. mansoni-infected individuals. In A, African (□) and European (×) healthy populations were stratified in age groups with the indicated number (n) of individuals. In B, the cohort of S. mansoni-infected individuals (○) was also stratified in the same age groups. The mean (m) of IL-7 concentrations is represented by a continuous horizontal bar for each age group and by a dashed line by integrating the whole population. In C, the correlation between IL-7 concentration (after logarithmic transformation) and age in the infected cohort is presented. The population was first sorted following increasing ages.
sera re-enforces the validity of the observation made in the model of SCID-hu-PBL/skin mice and justifies further analysis of human infected populations.

IL-7 has a very pleiotropic action; it is thus difficult to hypothesise a single specific role in schistosomiasis or in other parasite diseases. Our results provide evidence that keratinocytes and dermal endothelial cells synthesize increased amounts of IL-7 upon penetration of S. mansoni larvae. These findings were relevant in the field, since high IL-7 levels could be measured in the plasma of infected individuals. Finally, this first demonstration of IL-7 production by dermal endothelial cells, the first cells encountered by infiltrating T cells, provides a new view of the T cell proliferation responsible for the most common cutaneous diseases, such as psoriasis (7) or atopic dermatitis (8). To conclude, the effect of IL-7 concomitantly exerted on the parasite and on the immune response could be very intricate and reflects the complexity relationships between host and parasite.

Acknowledgments

We thank M. Niang (ESPOIR Program), M.-C. Galisot, M. Marguerite, and the inhabitants of Guidakhar village. We are grateful to F. Richard, S. Danet, and J.-L. Neyrinck for statistical evaluation of our data; to F. Carpenter for pathologic anatomy examination; and to F. Hautecoeur for coordination with the Roger Salengro Hospital. Blood from healthy donors was purchased by P. Boutin and C. Derycke. Finally, we thank the patients who donated skin and blood to make these studies possible, and D. Dombovitz for critical review of the manuscript.

References


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