Epidermal Compromise in American Cutaneous Leishmaniasis

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In American cutaneous leishmaniasis (ACL), *Leishmania* parasites enter the epidermis of the host via the bite of infected sandflies. Immune responses against the parasite vary from "effective" in localized (LCL) to a state of "selective anergy" in diffuse (DCL) cutaneous leishmaniasis, whereas the intermediate muco-cutaneous form (MCL) is characterized by an exacerbated cell-mediated immunity. We have shown that in LCL epidermis, Langerhans cells (LC) are increased, HLA-DR is universally expressed and intercellular adhesion molecule-1 (ICAM-1) immunoreactivity is distributed in patches. In addition, mRNA for IL-1β, IL-8, TNFα, TNFβ, and INFγ may be detected in epidermal sheets by reverse transcriptase followed by polymerase chain reaction (RT-PCR). In contrast, DCL epidermis shows fewer LC than LCL epidermis, and expression of ICAM-1, HLA-DR, and IL-1β mRNA cannot be detected. MCL lesions show a mucosal epithelium lacking LC, but ICAM-1 is universally expressed. The clinical manifestations of ACL can be reproduced experimentally in different strains of inbred mice. In healthy mice, we have shown a positive correlation between LC and dendritic epidermal T cells (DETC) numbers. This correlation was not, however, observed in *L. mexicana*–infected mice, suggesting that infection alters the balance between the two cell types. In addition, agents that modulate LC and DETC cell densities change the development of experimental leishmaniasis. These results suggest that the epidermis is essential in determining the type of immune response that is developed against the *Leishmania* parasites. *J Invest Dermatol 99:95S–98S, 1992*

Leishmaniasis is produced by flagellated protozoa of the genus *Leishmania,* which are obligate intracellular parasites of phagocytic cells. The disease is transmitted by the bite of female sandflies of the *Phlebotominae* subfamily. In the New World, the multiple strains of *Leishmania* parasites can be distinguished biochemically and immunochemically, suggesting an active state of speciation, which, combined with the genetic background of the host, contribute to the wide range of clinical forms of the disease. American cutaneous leishmaniasis (ACL) is a chronic granulomatous disease with a spectrum of clinical manifestations. In localized cutaneous leishmaniasis (LCL), the most common form, an adequate cell-mediated immune response is mounted, and the disease is restricted to well-defined skin lesions. In contrast, diffuse cutaneous leishmaniasis (DCL), which occurs infrequently, is characterized by selective anergy in cell-mediated immunity, resulting in extensive involvement of the skin, naso-bucopharyngeal mucous tissue, and some lymph nodes [1–4]. Some ACL patients develop muco-cutaneous leishmaniasis (MCL), which is characterized by exacerbated cell-mediated immunity and destructive lesions of the oral and nasopharyngeal cavities [2–4]. These variations in the immune response to parasites in human hosts, and the existence of experimental models in different inbred strains of mice, have made leishmaniasis an excellent prototype for studying the immunoregulatory processes involved in infectious diseases.

Because the parasite is injected into the epidermis by the sandfly, it is reasonable to propose that epidermal immunocompetent cells play a role in eliminating the protozoan. In this respect, Langerhans cells (LC) may play a critical role in this local response as targets of the parasite, because they can be infected in vitro [5].

EPIDERMAL LANGERHANS CELLS IN ACL

Previous work by our group has shown that the numbers of epidermal LC are often increased in ACL lesions [6–9], although this varies significantly among its three clinical forms. Thus, epidermal LC are increased in LCL (Fig 1), and numerous large CD1a$^+$ cells are also found in the granulomas of these patients. In contrast, the density of LC in DCL is variable, with values that are higher than in normal skin but lower than in LCL [6,7]. Finally, LC (CD1a$^+$ cells) are absent from the mucosal epithelium in MCL lesions [9]. This latter finding may reflect the selective migration of antigen-primed LC from the epithelium to regional lymph nodes, or may be the result of a direct cytolytic effect of the parasite. In this respect, similar results have been observed in virally induced mucosal lesions [10].

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Abbreviations:

ACL: American cutaneous leishmaniasis
DCL: diffuse cutaneous leishmaniasis
DETC: dendritic epidermal T cells
ICAM-1: intercellular adhesion molecule-1
IL: interleukin
LC: Langerhans cells
LCL: localized cutaneous leishmaniasis
MCL: muco-cutaneous leishmaniasis
RT-PCR: reverse transcriptase followed by polymerase chain reaction
TCR: T-cell receptor

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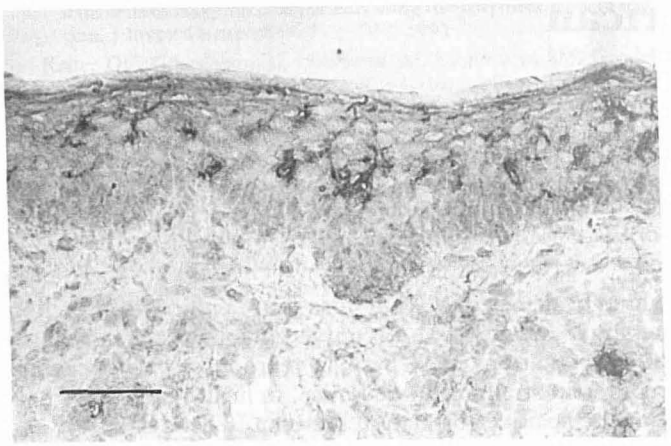

Figure 1. Langerhans cells in localized cutaneous leishmaniasis. Abundant $CD1a^+$ cells present in the epidermis of these lesions. Avidin-biotin immunoperoxidase staining. *Bar,* 20 μm.

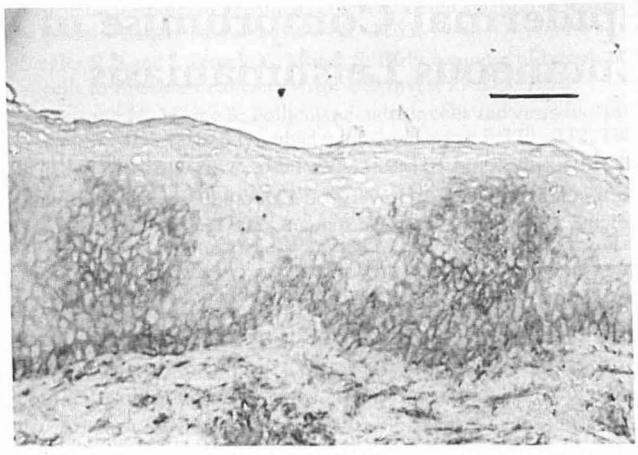

Figure 2. ICAM-1 immunostaining in the epidermis of localized cutaneous leishmaniasis. Positive cells are organized in patches through out the epidermis. *Bar,* 20 μm.

EPIDERMAL LANGERHANS CELLS IN MURINE CUTANEOUS LEISHMANIASIS

Murine models of cutaneous leishmaniasis have been used widely and, depending on the *Leishmania* strain, inoculum size, and mouse strain, it has been possible to reproduce the clinical forms observed in humans. Thus, BALB/c mice develop lesions similar to DCL; C57BL/6 mice are intermediately resistant, reproducing LCL-like lesions; and DBA mice show intermediate forms of the disease [11–13].

We have studied epidermal LC in *L. mexicana*–infected BALB/c and C57BL/6 mice using the NLDC-145 antibody and immunostaining techniques [7]. Healthy BALB/c mice ordinarily have more epidermal LC (1300 LC/mm^2) than C57BL/6 mice (700 LC/mm^2), the latter values being similar to those found in normal human skin. The high density of LC in susceptible BALB/c mice may contribute to their failure to eliminate the parasite, possibly due to excessive numbers of target cells. *L. mexicana*–infected BALB/c mice showed an increase in the numbers of epidermal LC, reaching maximal values on the third (2106 ± 31 cells/mm^2) and fifth (2196 ± 34 cells/mm^2) weeks after infection. These values then decreased after the ninth week and reached normal values by the eleventh week. In intermediate-resistant C57BL/6 mice, LC increased from the time of inoculation and for the first 5 weeks, reaching a maximal values (1284 ± 29 cells/mm^2) by the third week.

The observed increase in epidermal LC in both human and experimental leishmaniasis suggests the involvement of these cells, although whether they serve as antigen-presenting cells or target cells has yet to be determined.

HLA-DR, ICAM-1, AND CYTOKINE PROFILE IN THE EPIDERMIS OF ACL

LC, epidermal T cells (including $\gamma\delta$ T cells), and keratinocytes all participate in pathologic skin conditions. Interferon-γ (INFγ), major histocompatibility complex class II molecules (HLA-DR), and adhesion molecules, such as LFA-1 and its ligand, ICAM-1, are key participants in these processes. Not only are adhesion molecules also cited with immigration of lymphocytes into the epidermis, it is likely that once T cells and antigen-presenting cells (APC) interact, INFγ is produced. This lymphokine, in turn, induces the keratinocytes to express HLA-DR and ICAM-1 [14,15]. These two molecules, and cytokines such as interleukin (IL)-1, IL-3, IL-6, IL-8, TNFα, TNFβ, and granulocyte monocyte colony-stimulating factor (GM-CSF), presumably produced by LC and keratinocytes, would then promote the immune response.

We have studied the expression of ICAM-1 and HLA-DR in the epidermis of patients with ACL. In addition, we have determined lymphokine profiles in epidermal sheets for ACL patients, using a reverse transcriptase polymerase chain reaction (RT-PCR). In patients with LCL, HLA-DR was universally expressed throughout the epidermis, whereas ICAM-1 was distributed in patches (Fig 2), as has also been described for other cutaneous disorders [16]. In disseminated DCL, HLA-DR expression was restricted to LC, and ICAM-1 immunoreactivity was absent. In the hypereactive MCL epidermis, both HLA-DR and ICAM-1 were universally expressed. Using the RT-PCR, we have identified INFγ mRNA transcripts in epidermal sheets of LCL and DCL. IL-1β mRNA was expressed in LCL epidermis but absent in most DCL samples. INFγ has been identified as a potent macrophage-activating factor, involved in the killing of the *Leishmania* parasite, and as an important factor in selecting a TH1 response [17]. Thus, one would expect to find high levels of INFγ in LCL patients and resistant mice, and low levels of INFγ in patients with active visceral leishmaniasis or in DCL-susceptible mice [18,19]. The presence of INFγ in DCL lesions may contribute to the lack of message for IL-1β in these patients, because INFγ is known to downregulate IL-1 production [20]. In LCL epidermis, we have also identified mRNA transcripts for TNFα, TNFβ, and IL-8, but not for IL-6.* These results demonstrate that LCL epidermis has the majority of the components associated with an active inflammation.

T CELLS IN THE EPIDERMIS OF ACL

In LCL and MCL, we have observed a selective accumulation of T cells towards the basal layer of the epidermis. Phenotypically, these $CD3^+$ T cells are either $CD4^+$ or $CD8^+$. We have shown that most infiltrating T cells express the $\alpha\beta$ T-cell receptor (TCR) with only a few cells expressing the $\gamma\delta$ TCR (Fig 3). In DCL lesions, one observes more $\gamma\delta$ T cells in the granulomas than in LCL lesions; however, few of these cells were observed in the epidermis.

Dendritic epidermal T cells (DETC) are murine $\gamma\delta$ T lymphocytes that reside normally in epidermis [21], and the LC/DETC ratio has correlated with the intensity of contact hypersensitivity reaction in mice [22]. We have evaluated LC/DETC ratios in murine models of leishmaniasis, as a criterion for determining the epidermal participation in the immune response.† In healthy BALB/c

* Cáceres-Dittmar G, Sánchez MA, Tapia FJ: Cytokine profiles in the epidermis of American cutaneous leishmaniasis using polymerase chain reaction (unpublished).

† Sánchez MA, Cáceres-Dittmar G, Oriol O, Mosca W, Kraal G, Tapia FJ: Epidermal Langerhans cells and dendritic epidermal T Cells in murine cutaneous leishmaniasis (unpublished).

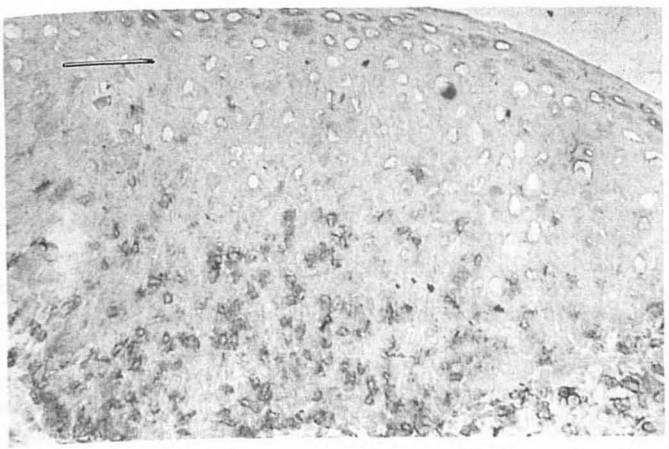

Figure 3. Infiltrating T lymphocytes in localized cutaneous leishmaniasis. A selective accumulation of $\alpha\beta$ T cells are observed toward the basal layer of the epidermis. Avidin-biotin immunoperoxidase and βF1 antibody. *Bar,* 20 μm.

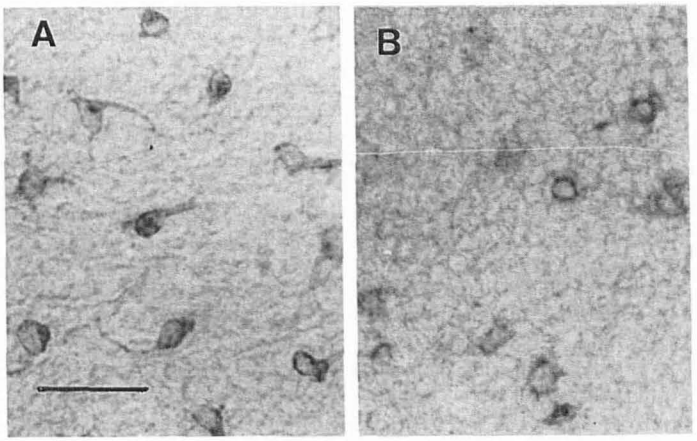

Figure 4. NLDC-145$^+$ Langerhans cells *(A),* and Thy-1.2$^+$ dendritic epidermal T cells *(B),* in murine cutaneous leishmaniasis. Avidin-biotin immunoperoxidase in EDTA-separated epidermis (9 weeks post-infection). *Bar,* 10 μm.

and C57BL/6 mice, the density of LC and DETC are positively correlated, and our results show that both cell types increase after *Leishmania* infection (Fig 4). However, maximal numbers of LC appear in the third week, whereas the maximal values for DETC appear in the fifth week. This difference in triggering suggests that LC may present parasite antigens to DETC (and other T cells), inducing them to proliferate several days later. In addition, although absolute numbers of LC were always higher than DETC, the increase associated with infection was for the latter, emphasizing their role in experimental leishmaniasis. These changes may result from direct involvement of LC and DETC, or as a consequence of the underlying granuloma. Giannini [23], using B10.129(10M) mice and *L. major* parasites, showed that low doses of UVB applied locally to the inoculation site suppressed the development of skin lesions. However, she observed that although UVB affected epidermal cells it did not alter the parasite load, concluding that local epidermal pertubation during initial phases of leishmanial infection influences both the immunologic response to the parasite and the subsequent development of clinical disease.

Work in our laboratory has also shown important changes in the development of skin lesions in *L. mexicana*-infected C57BL/6 mice after treatment with prednisolone acetate, tape-stripping, and monobenzyl ether of hydroquinone (MBEH).‡ After treatment with MBEH, which increases LC but not DETC, animals become more resistant, and lesions heal faster. The steroid depletes both epidermal LC and DETC and, although this exacerbates the disease, steroids probably affect other immunocompetent cells as well. Finally, tape-stripping, which depletes the epidermis of both cell types, leads to faster healing, suggesting that it may restore a lost balance between LC and DETC, which is necessary for protective immunity. An alternative interpretation is that the depletion of LC by tape-stripping eliminates target cells for the parasite.

In summary, the epidermal component of the skin immune system participates in the immunoregulatory mechanisms involved in human and experimental ACL. The cascade of events that occurs in inflammatory reactions involving LC, release of interacting cytokines, migration of T cells, and other inflammatory cells, and the subsequent expression of adhesion molecules, is altered in the disseminated form of leishmaniasis, whereas it is more appropriate in the localized forms. The understanding of the epidermal involvement in cutaneous leishmaniasis will help in the development of new therapeutic and prophylactic schemes.

‡ Oriol O, Sánchez MA, Cáceres-Dittmar G, Kraal G, Tapia FJ: Modulation of epidermal cell density alters the course of *Leishmania* infection in mice (unpublished).

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