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Immunocytochemical Characterization of Epidermal Cells in American Cutaneous Leishmaniasis

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ABSTRACT

The epidermal involvement in American cutaneous leishmaniasis (ACL) was evaluated by an **in situ** characterization of epidermal Langerhans cells (LC), the expression of the major histocompatibility complex molecule HLA-DR and the intercellular adhesion molecule ICAM-1. Differences were observed in the epidermis of the different clinical forms of ACL: localized (LCL), mucocutaneous (MCL) and diffuse (DCL) cutaneous leishmaniasis. LCL epidermis is characterized by a large number of LC, HLA-DR expression by all the keratinocytes, and ICAM-1 expression distributed in patches. In contrast, DCL epidermis lacks HLA-DR and ICAM-1 expression by the keratinocytes, and shows variable numbers of LC. The universal expression of HLA-DR and ICAM-1 in MCL epidermis is characteristic of the tissue-damaging hypersensitivity associated with this form of the disease. These results suggest that the epidermis is an important site for the induction of the immune response in ACL.

KEYWORDS

Cellular immunology, epidermis, keratinocytes, Langerhans cells, leishmaniasis.

Several studies have indicated the importance of the epidermis in the initiation of the inflammatory process [1,2]. After a cutaneous antigenic stimulus, immunocompetent cells are sequestered from peripheral blood circulation to the site by the participation of homing receptors present on the circulating leukocytes, and adhesion molecules present in the so-called high endothelial venules (HEV). Subsecuently, these immune cells are antigen-primed in the epidermis by direct contact with antigen presenting and accessory cells, such as Langerhans cells (LC) and keratinocytes (KC). Keratinocytes become immunlogically active in most inflammatory proccesess of the skin. In the epidermis, the major human histocompatibility class II molecule HLA-DR and the intercellular adhesion molecule ICAM-1 are essential in determining the type of immune response that is developed against a particular antigenic insult. In addition, a feedback control mechanism may be established between the epidermal response and dermal infiltrates or granulomas. For example interleukins such as interferon-γ(ACII 231), are known to induce epidermal cell activation [1]. Antigen-primed accessory cells also migrate from the dermal granulomas to regional lymph nodes, where they promote B cell differentiation and immunological memory.

Leishmaniasis is a disorder produced by protozoa of the genus **Leishmania,** which are obligate intracellular parasites of phagocytic cells. American cutaneous leishmaniasis (ACL) is a chronic granulomatous disease with a spectrum of clinical manifestations. In localized cutaneous leishmaniasis (LCL), an adequate cell-mediated immune response is generated against the parasite, and the presentation of the disease is restricted to well defined skin lesions. In contrast, diffuse cutaneous leishmaniasis (DCL), is characterized by a selective anergy of the specific cell-mediated immunity, resulting in extensive involvment of the skin, naso-bucopharyngeal mucous tissue and some lymph nodes, with abundant

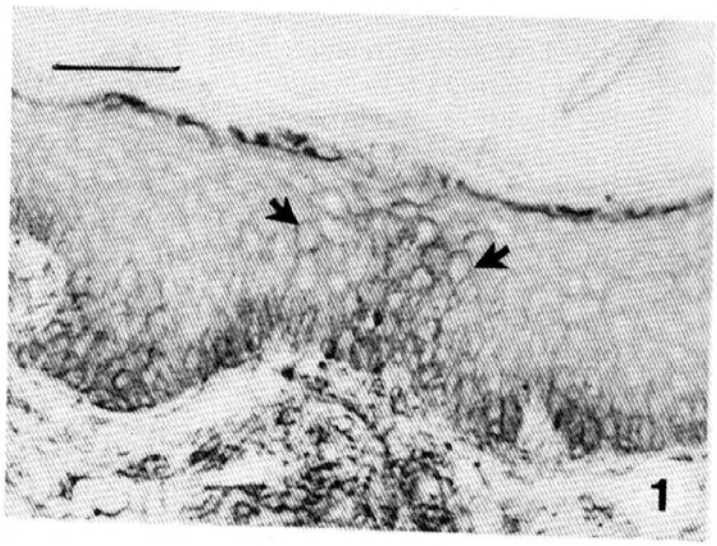
parasites [3-6]. Mucocutaneous leishmaniasis (MCL) is an intermediate form characterized by exacerbated cell-mediated immunity, and destructive lesions of the oral and nasopharyngeal cavities [4-6]. These variations in the immune response to the parasite in the human host have made leishmaniasis an excellent prototype for studying the immunoregulatory processes involved in infectious diseases.

Since the parasite is injected into the epidermis by a sandfly of the **Phlebotominae** subfamily, it is reasonable to propose that epidermal immunocompetent cells play a role in eliminating the protozoan. In the present study we used immunocytochemical techniques to characterize the expression of HLA-DR and ICAM-1, and the presence of CD1a+ Langerhans cells.

Skin biopsy specimens were embedded in OCT compound (Miles Scientific, U.S.A.), snap-frozen in liquid nitrogen and stored at -70°C until examination. Frozen sections (5μm) were cut with a cryostat and air-dried overnight before the immunostaining procedure. The antibodies used recognized the following mononuclear cell markers: HLA-DR (B .33. 1. 1 at 1:500), kindly donated by Dr. G. Trichieri (The Wistar Institute, Philadelphia, U.S.A.); CD1a (Langerhans cells at 1:100) Becton Dickenson, Inc, Mountain View, U.S.A. and ICAM-1 (RR1/1 at 1:3000), kindly donated by Dr. T. Springer (Harvard Medical School, Boston, U.S.A.). The immunoperoxidase staining was carried out as previously described [7,8]. Briefly, the sample were hydrated in phosphate-buffered saline (PBS) and sequentially incubated for 30 min with primary mouse monoclonal antibody, biotinylated goat anti-mouse IgG at 1:150 (BRL, Gaithersburg, U.S.A.) for 15 min, and streptavidin-horseradish peroxidase conjugate (BRL, U.S.A.) at 1:300, 30 min. Five minute washes with PBS were performed between incubations. The reactions were developed for 10 min with 90 μM H_2O_2 and 3-amino- 9-ethylcarbazole (AEC) (final concentration 0.88 mM), which was dissolved in 50 mM N,N-dimethylformamide in 0.1M acetate buffer, pH 5.2. The sections were then washed and counterstained with Mayer' s haematoxylin.

In patients with LCL, HLA-DR is universally expressed throughout the epidermis, whereas ICAM-1 showed a distribution in patches (Fig. 1). In the epidermis of the disseminated DCL form, HLA-DR expression is restricted to LC, and ICAM-1 immunoreactivity was absent. In the hypereactive MCL epidermis, both HLA-DR and ICAM-1 were universally expressed (Fig. 2). LC are increased in LCL epidermis (Fig. 3), and numerous large CD1a+ cells are also found in the granulomas of these patients. In contrast, the density of LC in DCL epidermis is variable, with values that are higher than in normal skin but lower, than in LCL epidermis. In contrast, LC (CD1a+) are absent from the mucosal epithelium in MCL lesions.

The present study corroborated our previous findings regarding the density of Langerhans cells in ACL [2,9,10], and confirmed similar observations in experimental mouse models [2,8]. The lack of LC in MCL lesions 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 on these cells. In this respect, similar results have been observed in virally-induced mucosal lesions [11]. The results concerning the expression of HLA-DR and ICAM-1 suggest that LCL epidermis has the appropriate effector mechanisms to eventually eliminate the parasite. The coexpression of HLA-DR and ICAM-1 by KC has been associated with the homing of T cells towards the epidermis, and the subsequent induction of interacting cytokines, which are necessary for the development of effective immunoregulatory processes [1,2]. In contrast, the lack of HLA-DR and ICAM-1 expression by DCL epidermis is highly suggestive of an impaired epidermal function in these cells. The universal expression of HLA-DR and ICAM-I by epidermal cells in MCL lesions confirmed the hypersensitivity state associated with this clinical form. These results suggest that the epidermis is an important site in the development of cutaneous leishmaniasis, and open new possibilities for the understanding of the immunoregulatory mechanisms associated with the disease.



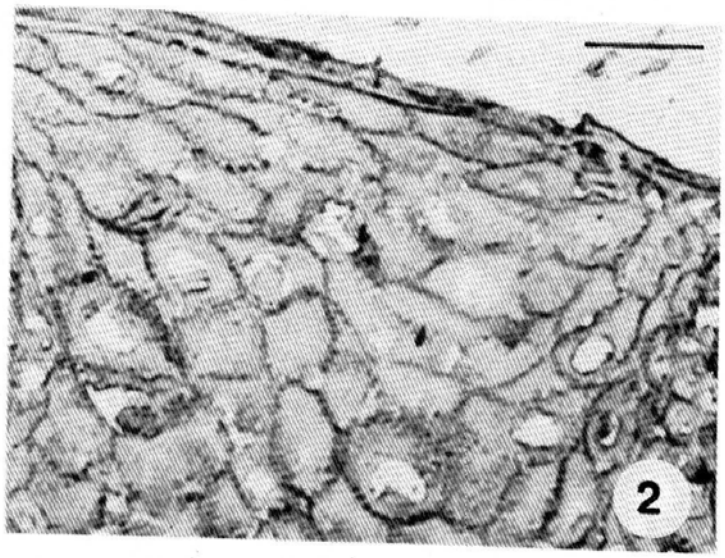
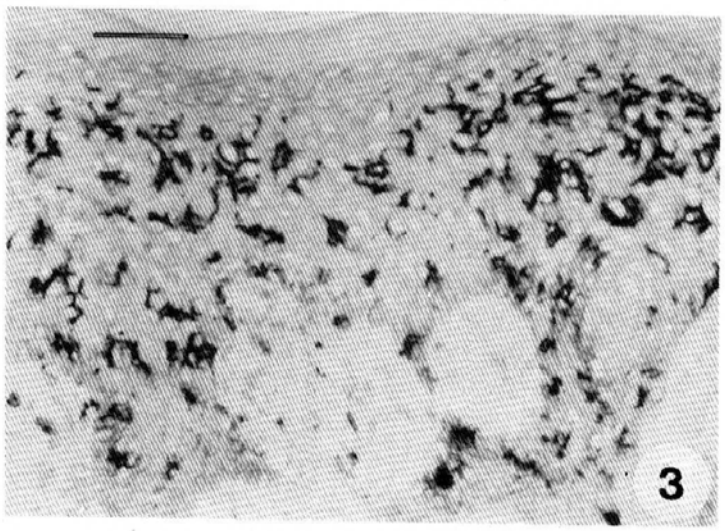


Fig . 1
ICAM-1 immunostaining in the epidermis of localized cutaneous leishmaniasis (arrows). Positive cells are organized in specific areas of the epidermis. Bar = 20 μm.
Fig . 2
ICAM-1 immunostaining in the epidermis of mucocutaneous leishmaniasis. Immunostaining is distributed throughout the epidermis. Bar = 10 μm.
Fig . 3
Langerhans cells in localized cutaneous leishmaniasis. Abundant CD1a+ cells present in the epidermis of these lesions. Avidin-biotin immunoperoxidase staining. Bar = 20 μm.



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