CHAPTER 8

EPIDERMAL IMMUNE PRIVILEGE IN AMERICAN CUTANEOUS LEISHMANIASIS

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THE SKIN IMMUNE SYSTEM: CUTANEOUS ROADSIGNS AND ROUTES

The skin is an important component of the immune system conforming a particular microenviroment referred to as the skin immune system (SIS).[1] The SIS includes immunocompetent cells such as Langerhans cells, keratinocytes, dermal cells and cutaneous-specific T cells, the dermal perivascular unit, and epidermal and dermal humoral constituents that involve interacting cytokines and chemokines.[2] The perivascular unit embraces the high endothelial venules (HEV), T cells, mast cells and dermal dendrocytes.[3] The Langerhans cells, which are members of the dendritic cell family, are the professional omnipresent antigen-presenting cells (APC) of the epidermis. In contrast, keratinocytes become active immunocompetent cells solely after a cutaneous stimulus.[4,5]

The immunoregulatory processes of the skin have been divided into three phases: recruitment, retention/proliferation and recirculation.[6] The recruitment phase involves the extravasation of leukocytes through the perivascular unit, and their subsequent attraction toward the epidermis. The retention/proliferation phase comprises the interaction between Langerhans cells, keratinocytes, epidermotropic T cells and cytokines, with subsequent T-cell proliferation, leading to the formation of a dermal infiltrate or granuloma. The recirculation phase is activated after the elimination of the cutaneous insult, and involves the downregulation of accessory signals by Langerhans cells and keratinocytes.

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The extravasation of leukocytes is a multistep process that requires the sequential interactions of leukocytes with the endothelium under the direction of an adhesion cascade.[7,8] These steps may be divided into primary adhesion (attachment and rolling), firm adhesion (activation and sticking) and diapedesis. In primary adhesion, free-flowing leukocytes within the venular lumen first slow down and roll on the endothelial cell surface under conditions of shear force and the selectin family of adhesion molecules.[9] L-selectin and P-selectin participate during attachment, P-selectin and E-selectin during rolling and E-selectin and the integrins intercellular adhesion molecule 1 (ICAM-1) and vascular adhesion molecule 1 (VCAM-1) during the firm adhesion phases. The interaction between various pairs of adhesion molecules and the participation of a series of cytokines that immobilizes the endothelia surfaces are necessary for firm adhesion. Among the adhesion molecules involved in firm adhesion are the β2 integrins CD11a/CD18 and CD11b/CD18 that interact with ICAM-1 and other ligands on the endothelium, and the β1 integrin very late antigen 4 (VLA-4, CD49d/CD29) that binds to VCAM-1 and fibronectin.[10,11] Chemokines produced by endothelial cells or surrounding epithelia such as the epidermis generate a gradient promoting diapedesis. Chemokines may be either located on the surface of endothelial cell or delivered to optimize the function of the integrins. The tethered lymphocyte responds to chemokine gradients by migrating first to the epidermis and later to the foci of inflammation. The additional adhesion provided by epidermal cells, which is also induced by antigen and microenvironmental factors, triggers the binding of leukocytes to epidermal cells and extracellular matrix. This binding is critical in determining the specificity of lymphocyte localization and homing necessary for the inflammatory response.[12]

In the epidermis, major histocompatibility complex (MHC) class II molecules and the intercellular adhesion molecule ICAM-1 are essential in guiding the immune response. In addition, a feedback control mechanism with the participation of cytokines may be established between the epidermal response and dermal infiltrates. Epidermal Langerhans cells and keratinocytes produce cytokines such as interleukin-1 (IL-1), IL-6, IL-8, granulocyte-macrophage colony stimulating factor (GM-CSF) and tumor necrosis factors TNF-α and TNF-β.[6] IL-1 is released by keratinocytes and Langerhans cells, and then acts in a paracrine and autocrine mode promoting the expression of IL-1 receptors by these cells.[13] IL-1α stimulates keratinocyte synthesis of IL-1α and transforming growth factor alpha (TGFα) and modulates the affinity of epidermal growth factor (EGF)/TGFα receptor for its ligand.[14] TGFα is known to enhance keratinocyte locomotion.[15] Subsequently, GM-CSF receptors will be upregulated and the cells respond to GM-CSF produced by activated keratinocytes.[16] TNFα and GM-CSF induce Langerhans cells to mature and migrate to regional lymph nodes, where they promote B cell differentiation and immunological memory.

Recently, it has been shown that the direction of Langerhans cell migration may be regulated by extracellular matrix components.[17] On their way to the lymph node and while passing through the basement membrane, Langerhans cells first come into contact with laminin and type IV collagen, and then with type I collagen in upper dermis and fibronectin in the afferent lymphatics.[18,19] Adhesion to fibronectin and laminin is mediated by $\alpha 5\beta 1$ and $\alpha 6\beta 1$ receptors on Langerhans cells, respectively.[20] Staquet et al[17] have shown that Langerhans cells can adhere to dermal extracellular matrix components after contact with basement membrane laminin or type IV collagen, whereas a first contact with dermal extracellular type I collagen reduces the binding capacity of the cells to laminin. The results suggest that this interaction may impede the return of Langerhans cells to the epidermis.

Activated Langerhans cells rapidly migrate to the draining lymph nodes and accumulate in the paracortical area where they participate in the immunostimulatory phase of the immune response by presenting antigen to naive T cells, which then become memory T cells.[21-24] Most antigen-bearing Langerhans cells remain in the epidermis for an ample period of time.[24] Memory T cells specifically home to the skin and participate in the retention/proliferation phase by interacting with epidermal keratinocytes and Langerhans cells through a series of accessory signals. Among these signals are the expressions of MHC-II and ICAM-1 molecules by epidermal cells and certain cytokines such as TNF α, and IL-10, which are induced by IFN-γ or IL-4.[25,26]

Most T cells of the skin express the glycoprotein cutaneous lymphocyte-associated antigen (CLA), recognized by the monoclonal antibody HECA-452.[27] CLA is a major T cell ligand to E-selectin, and in chronic inflammation CLA+ T cell infiltrates are closely associated to E-selectin+ HEV of the upper dermis.[8,28] About 40% of intraepidermal and perivascular T cells expressed CLA in normal human skin, however, this expression is not observed at areas distant from cutaneous vessels, suggesting the participation of other pairs of adhesion molecules in skin homing of lymphocytes.[29]

The immune response mounted against a cutaneous insult takes the form of a dermal infiltrate or granuloma, which have a particular configuration, with CD8+ cytotoxic T cells (CTL) and CD1a+ Langerhans cells arranged in the mantle surrounding the infiltrate, and CD4+ T helper cells and epithelioid cells located randomly. This organization has been observed in delayed-type hypersensitivity (DTH) responses and tuberculoid-type granulomas.[30-32] In addition, the differential cytokine production by T cells during an immune response plays an important role in regulating the nature of the response. Thus, Th1 cells secrete IL-2 and IFN-γ, and induce cell mediated immune responses, including DTH and macrophage activation, whereas Th2 cells secrete IL-4, IL-5, IL-6 and IL-10, and assist in antibody production for humoral immunity.[33,34] The cytokine milieu determines the type

of immune response, thus IFN-γ and IL-12 drive Th1, while IL-4 promotes a Th2 response.[35] Recent evidence shows that innate immunity may condition the type of acquired immune response, whereas activated macrophages and natural killer (NK) cells secrete IL-12 that primes IFN-γ production, and mast cells or basophils secrete IL-4.[35] Besides the local microenviroment of cytokine, the skewing towards a Th1 or a Th2 response may depend on the concentration and type of antigen and possibly on the type of APC that is present.[36]

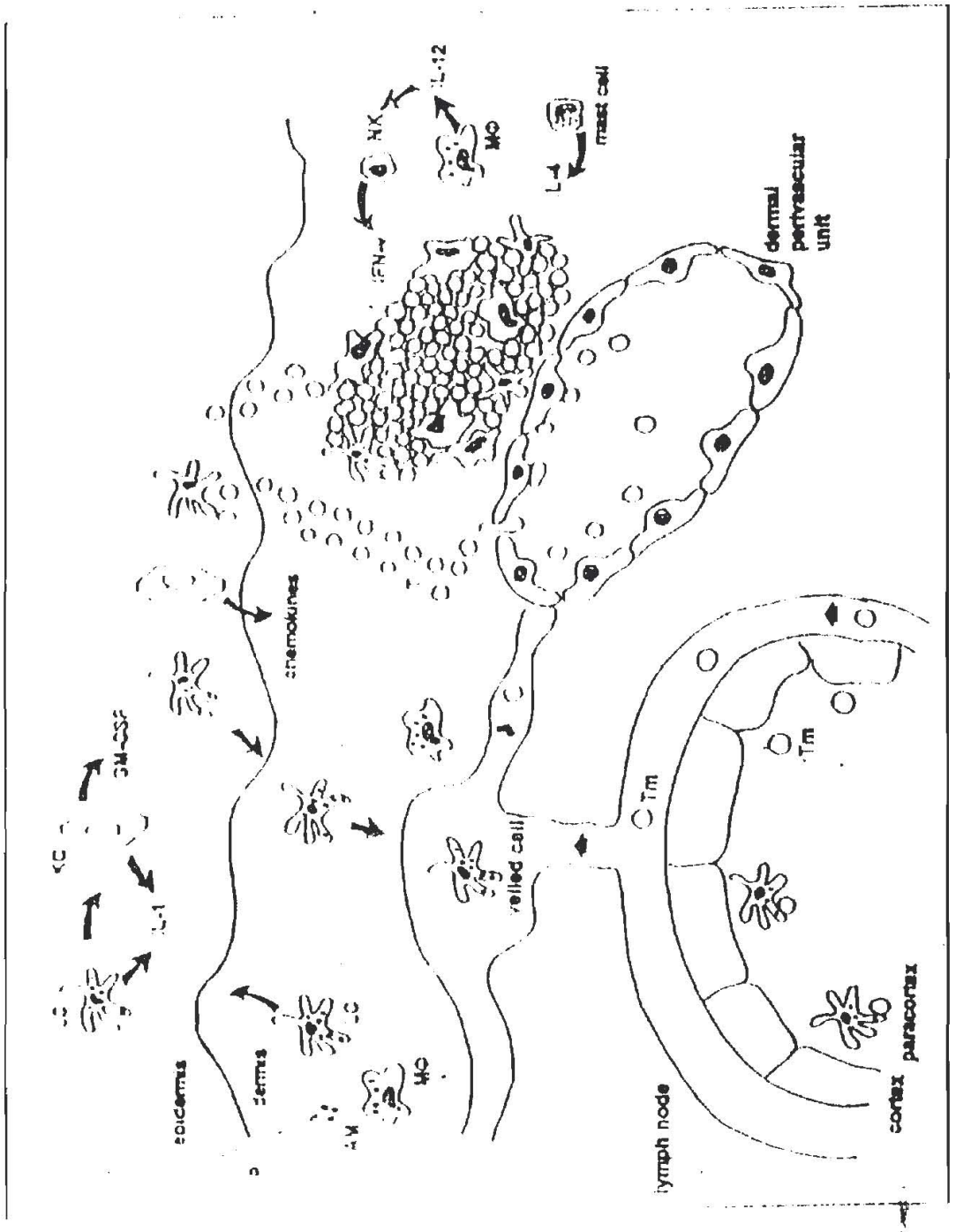
THE *LEISHMANIA* INTRUSION AND ITS LOCAL IMMUNE RESPONSE

During a blood meal, an infected *Phlebotominae* sandfly salivates into the skin of its vertebrate host, inoculating *Leishmania* in the epidermis or the upper layer of the dermis. Vasodilator peptides, such as maxadilan found in the saliva of the sandfly *Lutzomyia longipalpis* may propel parasite proliferation and dissemination by impairing antigen presentation of macrophages and Langerhans cells.[37-39] The proliferating parasites may cause circumscribed lesions, or disseminate to distal skin, mucocutaneous membranes or visceral lymphoid organs.

Leishmania parasites first attach to macrophages or dermal Langerhans cells via the C3bi (CR3) complement receptor (see chapter 2).[40] This dermal disturbance causes first a swift decrease in Langerhans cell density (unpublished observations), and after a few days a hyperplasia of epidermal Langerhans cells.[41,42] Concomitantly, IL-1 and GM-CSF is produced by epidermal cells, thus promoting Langerhans cells migration to the lymph nodes (Fig. 8.1).[12,13] It is not clear whether infected Langerhans cells return to the epidermis and then migrate or they get infected in their way to the lymph node. In the paracortical area of the lymph node the infected Langerhans cells prime naive T cells to become *Leishmania*-specific T cells.[43,44] Some of these memory T cells specifically migrate to the skin to initiate the inflammatory response against the parasite. In addition, Langerhans cells in the lymph node may also act as a reservoir of the parasites involved in sustained stimulation of T cell and immunological memory.[44]

The memory T cells travel to the skin and extravasate through the dermal perivascular unit, where its endothelial cells express E-selectin, VCAM-1 and ICAM-1 (unpublished observations). Furthermore, the serum detection of the soluble forms of these molecules revealed low levels for sE-selectin, normal levels for sVCAM-1, and increased levels of sICAM-1 in patients with localized cutaneous leishmaniasis (LCL).[45] After extravasation, T cells migrate to the epidermis and interact with the keratinocytes and a few activated Langerhans cells to receive proper

Fig. 8.1 (on opposite page) Events in the immune response of cutaneous leishmaniasis. LC = Langerhans cell, KC = keratinocyte, AM = amastigotes, P = sandfly proboscis, NK = Natural killer cell, MΦ = macrophage, L = lymphocyte


IL-12
mast cell
dermal perivascular unit
chemokines
rolled cell
Tm
Tm
epidermis
dermis
cortex
paracortex
lymph node

and essential accessory signals. T cells then return to the dermis to mold a granuloma infiltrate against the parasites, where infected macrophages process and present *Leishmania* antigens to T cells fostering clonal proliferation. Albeit most infiltrating cells are memory T cells, only a few are cutaneous-specific CLA+ T cells, suggesting that other lymphocytes probably primed to cross-reacting antigens may be sequestered to the inflammatory area (unpublished data).

THE EPIDERMAL INVOLVEMENT IN CUTANEOUS LEISHMANIASIS

Defects in the signaling properties of the epidermis can result in the generation of immunopathology in the three clinical forms of American cutaneous leishmaniasis (ACL).

The epidermis of patients with LCL contains many components associated with active inflammation.[46] These involve appropriate numbers of CD1a+ and CD83+ Langerhans cells, a marked expression of MHC class II by keratinocytes, and ICAM-1+ keratinocytes distributed in patches (Table 8.1). Furthermore, mRNA transcripts for IFN-γ, IL-1β, TNF-α, TNF-β and IL-8 (but not IL-6) can be detected in LCL epidermal sheets using the reverse transcriptase-polymerase chain reaction (RT-PCR).[47] IL-6, however, can be detected in the entire LCL lesion using the same procedure.[48] In contrast, diffuse cutaneous leishmaniasis (DCL) epidermis has few CD1a+ and CD83+ Langerhans cells,[31,49] and the keratinocytes fail to express MHC class II or ICAM-1.[49] DCL epidermis also lacks IL-6, and only a few patients express mRNAs for IL-1β and TNF-α,[48] indicating a possible failure at the level of monokine production by the APC. Indeed, the lack of IL-6 may be the result of the poor expression of IL-1β and TNF-α, as these cytokines are necessary to induce IL-6 production.[50] Similarly, the presence of IFN-γ in the DCL lesions may contribute to the lack of message for IL-1β in these patients, since IFN-γ is known to downregulate IL-1 production.[51] The most outstanding feature of mucocutaneous leishmaniasis (MCL) lesions is the absence of CD1a+ and CD83+ Langerhans cells in the mucosal epithelium.[49,52] This may reflect the selective migration of antigen-primed Langerhans cells from the epithelium to regional lymph nodes, or may be the result of a direct cytolytic effect of the parasite on these cells during the prolonged course of MCL. In MCL epidermis, both MHC class II and ICAM-1 are uniformly expressed, confirming the hypersensitivity state associated with this clinical form.

THE DERMAL IMMUNE RESPONSE IN CUTANEOUS LEISHMANIASIS

The LCL granuloma shows a predominance of Th1 cytokines,[49,53] with a CD4/CD8 ratio within the normal range, many CD25+ and CD83+ activated T cells, abundant memory T cells, and many γδ T cells.

There is high expression also of leukocyte function-associated antigen 1 (LFA-1, CD11a/CD18) and LFA-2 (CD2) also expressed by memory T cells (Table 8.1).[49] This cellular organization reflects the effector mechanisms associated with LCL, which include macrophage activation and lysis of the infected cells. Additionally, LCL lesions show deposits of IgG2 and IgG3, which are induced by Th1 cytokines, in the upper dermis near the basement membrane (Table 8.1).[54]

The presence of parasites in the skin induces the local production of IFN-γ within LCL lesions. This, and other cytokines such as TNFβ, could lead to the induction of keratinocyte expression of ICAM-1 and MHC class II, promoting the migration of epidermotropic T cells. Langerhans cells are potent stimulators of *Leishmania*-specific T cells[43] and can thus promote an effective Th1 response against the parasite. Once the parasite is eliminated, or masked from recognition by the host immune system, the epidermal accessory signals are downregulated. This process causes infiltrating cells to return to circulation or die by apoptosis, inducing healing of the cutaneous immunopathology that

Table 8.1. Levels of epidermal cells, infiltrating cells and immunoglobulin isotype deposits in lesions of different forms of cutaneous leishmaniasis*

Disease	LCL	DCL	MCL
Epidermal cells			
HLA DR+ keratinocytes	H	I	H
ICAM-1+ keratinocytes	N	L	H
CD1a+ Langerhans cells	H	L	A
CD83+ Langerhans cells	N	L	L
Infiltrating cells			
CD4/CD8	N	I	H
CD25	H	L	L
CD2	H	I	ND
CD83	H	L	L
γδ	H	L	L
CD30	L	H	ND
memory/naive	H	L	H
Immunoglobulin isotype deposits in upper dermis			
IgG1	L	H	L
IgG2	H	L	H
IgG3	H	L	H
IgG4	L	H	L
IgE	L	H	L

Abbreviations: LCL, localized cutaneous leishmaniasis; DCL, diffuse cutaneous leishmaniasis; MCL, mucocutaneous leishmaniasis; N, normal; A, absent; H, high; L, low; ND, not determined.
* Data summarized from Refs 31, 47, 49, 52, 54.

has caused lesion. In fact, treatment of *Leishmania*-infected mice with ultraviolet light, spectrum B (UVB) alleviates the dermatological manifestation, without reducing the parasite load.[55] Furthermore, the role of lymphocytes in immunopathology has recently been shown in T cell lacking SCID mice, which manifested the characteristic ulcer of leishmaniasis solely after transferring CD4+ T cells.[56]

The DCL granuloma shows a predominant Th2-mediated response, with many CD30+ T cells, deposits of IgG1, IgG4 and IgE toward the basement membrane, unusually high numbers of naive T cells and no particular microanatomic arrangement (Table 8.1). CD30, a member of the TNF/nerve growth factor (NGF) receptor superfamily, is preferentially expressed by T cells that produce Th2 cytokines.[57]

The disparity in the levels of CD1a and CD83 between LCL and DCL lesions may be related to cell triggering, since CD83 expression has been associated with antigen presentation and the cellular interactions that follow lymphocyte activation.[58] Indeed, the CD83 molecule may distinguish a subset of primed CD1a+ Langerhans cells that are responsible for presenting antigen to memory T cells. Thus, LCL lesions possess many accessory signals necessary to promote an effective immune response against the *Leishmania* parasite, whereas DCL lesions manifest an impaired epidermal function.

Certain risk factors may make individuals susceptible to DCL, such as the genetic background of the host,[59] species of the parasite[60] and sensitivity to UVB.[61] In this situation, the parasite evades the immune response of the host, and the cytokines produced cannot activate the keratinocyte to express ICAM-1 and MHC class II. In addition, Langerhans cells are diminished either by a direct effect of the parasites,[44] or by a lack of epidermal priming. These events lead to the genesis of a Th2 response, creating a state of progressive parasite-specific unresponsiveness, with a cutaneous paralysis of T cells.

The MCL granuloma shows a mixture of Th1 and Th2 cytokine patterns, characterized by an abundance of IFN-γ, IL-2, IL-4, IL-5 and IL-10.[48,53] In addition, the MCL granuloma shows a high CD4/CD8 ratio, which is distinguished by the marked accumulation of CD4+ T cells in the lesions and low numbers in peripheral blood[52,62] (levels of other T cell subsets are shown in Table 8.1).

Mucosal involvement arises in individuals many years after the primary cutaneous lesions have been cured. In fact, it is possible that individuals with no apparent skin lesions remain cryptically infected with *L. (Viannia) braziliensis*, the only recognized causative agent of MCL. The parasite and/or immunological memory may be activated by reinfection, immunosuppression or trauma (the so-called Köebner phenomenon in which traumatized uninvolved skin develops disease), causing a chronic immune response with associated mucosal damage. Another factor that may be involved is the vasodilator peptide maxadilan,[37,39] which may inhibit IFN-γ activity and antigen presenta-

tion by macrophages.[38] Thus, in a given endemic area, recurring sandfly bites may contribute to the hyperactive state of MCL lesions.

In MCL lesions, activation of the disease may be associated with the hyperproduction of keratinocyte-derived monokines, such as IL-1 and TNFα. These cytokines can upregulate the expression of ICAM-1 and MHC class II on endothelial cells and keratinocytes, and initiate T-cell traffic without concomitant antigen presentation. The lack of epithelial Langerhans cells may cause insufficient or inadequate signal transduction to accomplish the effector phase of the immune response. Further release of monokines by the keratinocytes could promote a persistent proinflammatory state, with associated tissue damage.

CONCLUSIONS AND OUTLOOK

The outcome of cutaneous leishmaniasis is decided by early immunological events occurring in the skin and draining lymph nodes. We propose the following model of events in cutaneous leishmaniasis, depicted in Figure 8.1.

Upon the sandfly inoculation of *Leishmania* parasites into the dermis, macrophages may take up the parasites and pass them to some Langerhans cells (LC) that then migrate in great numbers to the epidermis. Alternatively, the mechanical disturbance caused by the insect proboscis may induce the production of IL-1 and GM-CSF by keratinocytes, attracting dermally-infected Langerhans cells to the epidermis. These monokines may alter the regulatory role of extracellular matrix components in controlling Langerhans cell migration. Most of these activated Langerhans cells will rapidly travel to the paracortical area of the draining lymph nodes to start the immunostimulatory phase of immune response by sensitizing naive T cells into memory T cells. Concomitantly, after the entry of the parasites the perivascular unit is activated allowing the infusion of leukocytes into the skin. The extravasation of cells through the unit compels the sequential interactions of leukocytes with endothelial cells under the direction of an adhesion cascade that also involves chemokine secreted by keratinocytes (KC). Moreover, the epidermis guides the specific homing and inflammatory confine of the T cells that are specific for *Leishmania*. Accessory signals, such as the expression of ICAM-1 and MHC class II, and the secretion of cyrokines, direct the effector phase of the immune response that will take the form of a granuloma infiltrate. Failures at this level will cause an impaired immune response that is not only unable to eliminate the parasite, but which can also result in tissue damage. Indeed, a defect at the level of accessory signals in DCL lesions may account for the selective anergy that is observed in these patients. The macrophage dense DCL granuloma, which contains many naive and CD30+ T cells, may result from defective signaling by the epidermis and the selective migration of naive and Th2 cells. Similarly, defective signaling at the epithelial level may account for the

chronic proinflammatory state that causes tissue damage in MCL. It is important to note that although most lymphocytes conforming the LCL granuloma are memory T cells, only a small percentage are cutaneous-specific CLA+ T cells. These CLA+ T cells may be the first lymphocytes to migrate into the dermis and initiate the granuloma formation, which will be later accomplished by cross reacting memory T cells and other cell groups. The cellular components of innate immunity (natural killer cells, NK; macrophages, Mϕ; mast cells) may condition the cytokine milieu, and therefore the type of immune response.

It would be of interest to direct future studies toward the analysis of specific factors that may affect the cutaneous immune response in leishmaniasis, such as cytokine microenviroment, expression of cytokine receptors and role of the extracellular matrix.

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