Epidermal Langerhans cells are critical for immunoregulation of cutaneous leishmaniasis

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In leishmaniasis, macrophages are known to play a central role as modulators of the specific immune activity. In this article, Heidrun Moll presents evidence for the critical involvement of another component of the skin immune system, the epidermal Langerhans cell. She proposes that Langerhans cells take up parasites in the skin and transport them to the draining lymph node for presentation to T cells and initiation of the specific immune response.

Human leishmaniasis comprises a diverse group of diseases caused by protozoa of the genus *Leishmania*. The parasites belong to the family Trypanosomatidae; they alternate between the promastigote form in the sandfly vector and the obligatory intracellular amastigote form in the mammalian host. The diseases vary in severity from the naturally healing cutaneous leishmaniasis (oriental sore), which is characterized by a localized skin lesion at the site of the sandfly's bite, to the potentially fatal visceral leishmaniasis (kala-azar), in which the parasites disseminate from the site of infection and invade lymph nodes, spleen, liver and bone marrow. The clinical manifestation depends primarily on the species of parasite, but it also involves the genetic basis of the host's ability to develop an effective cell-mediated immune response, thus resembling the situation in leprosy.

The spectrum of disease patterns seen in humans can be reproduced by experimental infection of mice with *Leishmania major*, the cause of cutaneous leishmaniasis in the Old World. This mouse model has provided a wealth of information on the immune mechanisms underlying host resistance or susceptibility to leishmaniasis, with implications for a number of infectious diseases. The outcome of murine infection with *L. major* is not influenced by humoral immunity but appears to be determined by CD4$^+$ helper T cells (T_H cells) with different patterns of lymphokine activity. Thus, protective immunity can be attributed to T_H1-like cells producing interferon-gamma (IFN-γ), whereas T_H2-like cells releasing interleukin 4 (IL-4) and IL-10 facilitate survival of the parasites[1]. On the other hand, the findings that *L. major*-specific T_H1 cells can also exacerbate disease[2] and that local injection of IL-4 has a therapeutic effect[3] have emphasized the complexity of the interplay between the parasite and the host immune system.

Early events in *Leishmania* infection

Resistance to *L. major* infection can be induced in genetically susceptible mice by various treatments, for example, sublethal irradiation, immunization with parasite antigen or treatment with anti-IL-4 antibodies. In most cases, however, such manipulations are only effective if performed before infection or during the first week, probably because the differential stimulation of T_H-cell subsets is initiated within three days after inoculation of parasites[4]. These findings emphasize the importance of the early phase of infection.

The natural site of entry of *Leishmania* parasites into the mammalian host is the skin. It is here that the organisms invade host cells for intracellular multiplication and are first encountered by the immune system. In view of the above observations, it is very likely that the cutaneous immune response at this initial stage of infection is crucial to the course of disease. Within a few hours after parasite inoculation, a massive cellular infiltration occurs consisting predominantly of macrophages which rapidly take up parasites[5]. The signals mediating the influx of macrophages may include chemotactic cytokines such as the monocyte chemoattractant protein, MCP-1 (R. Gillitzer, I. Becker and H. Moll, unpublished). On the basis of *in vitro* and *in vivo* studies, it is widely accepted that macrophages play a central role in leishmaniasis[6]. They serve not only as host cells for the parasites, but also as antigen-presenting cells mediating the stimulation of specific T cells. In return, lymphokines released by activated T cells regulate the antimicrobial potential of macrophages which are the final effector cells for limitation of the deleterious spread of parasites.

In the very early phase of infection with *Leishmania*, there are few T cells in the dermal infiltrate[5]. Thus, infected macrophages expressing parasite antigen have a low probability of encountering T cells with the corresponding specificity required for triggering the cell-mediated immune response. It is necessary, therefore, to provide a principal sensitizing signal that activates antigen-specific quiescent T cells in the lymphoid organs and induces their emigration via the bloodstream to the lesional skin. Furthermore, to allow for effective antigen presentation by macrophages, the

surface expression of major histocompatibility complex (MHC) class II antigens has to be increased by stimulatory signals, such as IFN-γ, that are predominantly derived from activated T cells[7]. These considerations indicate that macrophages may not suffice for induction and modulation of the cell-mediated immune reactivity to *Leishmania* parasites and other intracellular microorganisms invading the skin. In fact, the skin harbours cells with potent accessory capacity, epidermal Langerhans cells, which may represent the 'missing link'.

Langerhans cells – a component of the dendritic cell system

Langerhans cells are derived from the bone marrow. They have a pronounced dendritic shape and can be identified, at the ultrastructural level, by organelles in their cytoplasm, termed Birbeck granules (reviewed in Ref. 8). Phenotypically, Langerhans cells share some features with macrophages since they express surface markers such as ATPase, nonspecific esterase and receptors for Fc and for complement. In contrast to macrophages, however, Langerhans cells (in the mouse) react with a monoclonal antibody directed against nonlymphoid dendritic cells (NLDC-145) (Ref. 9), and they constitutively express sizeable amounts of MHC class II molecules.

Langerhans cells are potent stimulators of antigen-specific and MHC-restricted T-cell responses[10]. Interestingly, their functional properties *in vitro* are dependent on the state of differentiation[11,12]. Freshly isolated Langerhans cells can process native antigen for presentation to previously sensitized T cells, but are weak activators of resting T cells. During short-term culture, however, Langerhans cells lose their processing capacity and acquire the unique potential to induce primary immune responses. Thus, they become remarkably similar to the dendritic cells found in lymphoid organs[13]. These observations showed that cultured Langerhans cells are *in vitro* equivalents of Langerhans cells that have ingested and processed antigen in the skin and have then migrated to the draining lymph node while developing into lymphoid dendritic cells[12,14]. Indeed, this translocation of Langerhans cells has been documented *in vivo* after epicutaneous application of contact allergens[15,16]. It would be an efficient mechanism for transport of antigen from the site of first encounter in the skin to the draining lymph node where a large variety of T cells can be found for initiation of the specific immune response.

Evidence for a role of Langerhans cells in leishmaniasis

The function of Langerhans cells *in vivo* has been analysed using contact hypersensitivity and skin transplantation as convenient models. However, the involvement of Langerhans cells during infectious diseases is only established for viral infections, such as human immunodeficiency virus and herpes simplex virus. With regard to skin-borne diseases caused by intracellular bacteria or parasites, there are reports on the distribution and turnover of Langerhans cells[17–19], but their function has not been defined. Attempts to address this issue by performing manipulations that result in an alteration of Langerhans cell density in the skin (for example, ultraviolet B irradiation, tape stripping or steroid treatment) have produced conflicting results depending on the type of infectious agent and the mode of treatment[19–21]. Furthermore, the contribution of Langerhans cells to the observed effects is difficult to evaluate because those treatments also affect other components of the skin immune system.

Parasite ingestion by Langerhans cells

We have assessed the function of Langerhans cells in experimental cutaneous leishmaniasis with *L. major*. Although Langerhans cells are considered to be minimally phagocytic, they express receptors for the complement component C3bi (CR3), which opsonizes *Leishmania* parasites for macrophages[22,23]. This would favor the idea that they can interact with these organisms. Indeed, Langerhans cells were able to phagocytose intact parasites *in vitro* and *in vivo*[24]. Ingestion of *L. major* could be detected only after incubation of amastigotes with freshly isolated Langerhans cells, but not with those cultured for more than 12 h (suggesting that only intracutaneous Langerhans cells display this activity) and was mediated by the CR3. Thus, it is conceivable that phagocytic activity is not a general feature of a given cell type, but a property dependent on the state of differentiation and on expression of the receptors that are required for interaction with the respective particle. In this context, it is of interest that Reis e Sousa and Austyn[25] have recently provided evidence for the ability of Langerhans cells to phagocytose zymosan, the uptake of which correlated with the activity of the mannose receptor. Together, these findings unambiguously show that Langerhans cells are able to take up particles of considerable size.

Presentation and transport of L. major *by Langerhans cells*

As compared with macrophages, the rate of infection and the parasite load of *L. major*-containing Langerhans cells was consistently low. This supports the notion that phagocytosis of *L. major* by Langerhans cells is not aimed at clearance of parasites but at acquisition of antigen for presentation to T cells. Such a concept is in concordance with our finding that epidermal Langerhans cells are potent stimulators of *L. major*-specific T-cell proliferation and lymphokine production *in vitro*[26]. In this respect, their activity was found to be much greater than that of macrophages.

In the course of infection with *L. major*, a dramatic change in the distribution of Langerhans cells was observed[24,27]. A considerable loss of NLDC-145$^+$ Langerhans cells in the segment of the epidermis overlying the parasite-containing infiltrate was concomitant with the appearance of NLDC-145$^+$ cells in the dermal layer of the lesion, some of which contained *L. major*. Langerhans cells of the epidermis were found not to be parasitized. These observations strongly suggest that Langerhans cells migrate from the epidermis to the site of infection in the dermis for uptake of *L. major* parasites. Furthermore, it was possible to demonstrate directly by *in vivo* tracking that epidermal

Langerhans cells infected with *L. major* have the ability to migrate from the skin to the draining lymph node[2]. Such a translocation was not seen with infected macrophages under similar conditions. The migratory lymph node dendritic cells presented the transported antigen to *L. major*-primed T cells *in vitro* and, most notably, activated resting T cells capable of mediating a parasite-specific delayed-type hypersensitivity response *in vivo*.

The model

On these grounds, I propose a central role for Langerhans cells in the early phase of leishmaniasis (Fig. 1). Upon inoculation of *Leishmania* into the skin, Langerhans cells migrate from the epidermis to the dermis and take up parasites. The signals inducing this translocation may be mediated by host-derived cytokines, such as IL-1β (Refs 28, 29) or tumor necrosis factor-α (Ref. 30) and/or by parasite-derived molecules. Whereas macrophages serve as scavengers and are the predominant site of parasite replication, the primary function of infected Langerhans cells appears to be the transport of organisms to the draining lymph nodes where they encounter a large variety of T cells with different specificities. During migration, the Langerhans cells develop into potent antigen-presenting cells with the ability to stimulate selected, but immunologically naive, T cells for initiation of the immune response. As a result, activated T cells with specificity for *Leishmania* antigens would emigrate via the blood into the lesion. At this site, infected macrophages as well as parasite-containing Langerhans cells that remained in the dermis are likely to present antigen to infiltrating T cells and regulate their effector activity, a process that may be enhanced by cascades of locally produced cytokines.

At this point, the question arises whether Langerhans cells should be considered merely as a functional alternative to macrophages in leishmaniasis. Several considerations argue against this supposition: (1) the migratory property of Langerhans cells is a distinctive feature that equips them to act as an outpost of the immune system; (2) Langerhans cells have the capacity to present antigen to T cells with extraordinary efficiency; and (3) Langerhans cells have the unique ability to induce the primary activation of antigen-specific T cells.

Thus, the involvement of Langerhans cells in initial events after infection may be a prerequisite for development of the specific cellular immune response. At later stages of infection, the remarkable ability of Langerhans cells to cluster high numbers of memory/effector T cells within the skin[31] may be important for maintenance of an effective response. On the other hand, macrophage functions are complementary to those of Langerhans cells in leishmaniasis. Their scavenger activity and ability, after appropriate activation, to produce high amounts of reactive oxygen and nitrogen metabolites emphasizes their role as the ultimate effector cells during clearance of infection[32]. It has yet to be determined whether Langerhans cells use similar mechanisms to control intracellular replication of parasites.

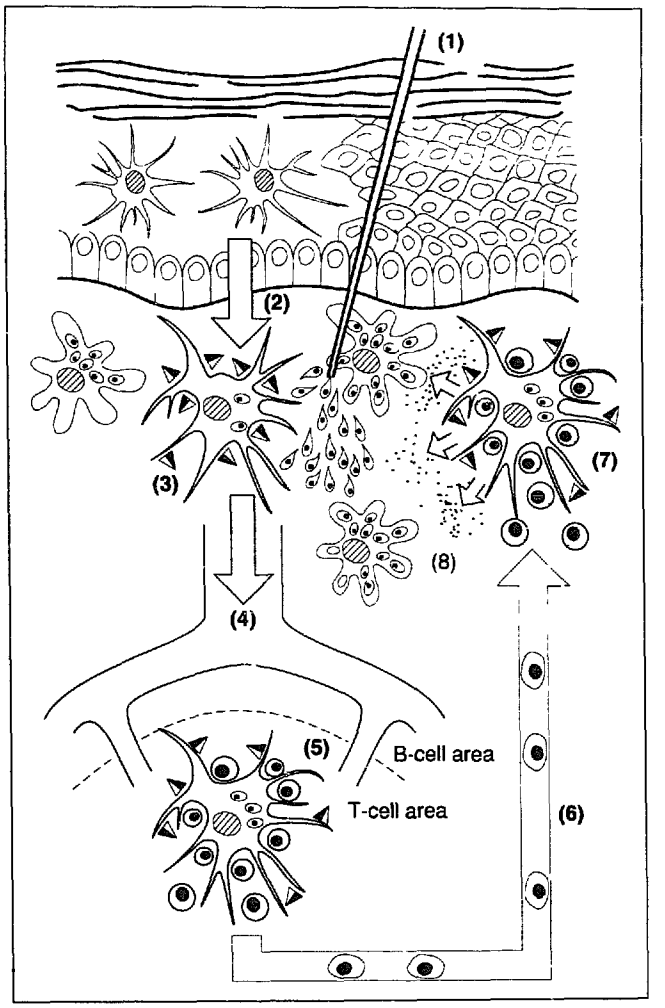

Fig. 1. *Hypothetical model of the functional activities of Langerhans cells in cutaneous leishmaniasis. After deposition of* Leishmania *promastigotes in the skin by a sandfly or by experimental infection (1), Langerhans cells migrate from the epidermis to the dermis (2) and, like macrophages, take up parasites (3). Appropriate processing results in expression of parasite antigen associated with MHC class II molecules on the cell surface (▲). A proportion of Langerhans cells transports parasites from the infected skin to the draining lymph node (4) for presentation to antigen-specific resting T cells in the paracortex (5). As a result, activated T cells emigrate via the blood into the lesion (6), where infected macrophages and Langerhans cells that remain in the dermis (7) regulate their effector activity by several mechanisms including cytokine secretion (8).*

Implications for the course of disease

On the basis of the findings discussed above, there are several important implications for our understanding of the pathogenesis of cutaneous leishmaniasis. First, with reference to the development of T_H-cell subsets producing different arrays of lymphokines. It has been suggested that the type of antigen-presenting cell determines the phenotype of responding T cells[33–35]. The described activities of Langerhans cells, however, can be detected in resistant and in susceptible mice, although we observed differences in the rate of infection (H. Moll, unpublished). In fact, the initial predominance of *L. major*-bearing dendritic cells in lymph nodes draining the lesions of both types of mice[27] may explain our previous observation that, at the early

stage of infection, those organs contain a comparable frequency of IL-4-secreting cells[36].

It is possible that the relative frequencies of different types of antigen-presenting cells, resulting in different densities of ligands (MHC class II-peptide) for the T-cell receptor, may influence the phenotype of responding T cells[37]. In addition, the development of T-cell subsets *in vivo* may not be restricted exclusively by the antigen-presenting cell, but may be influenced by the availability of co-stimulatory signals (for example, cytokines) from other cell types in the microenvironment[38,39].

A second implication relates to the role of parasite antigens. The various types of accessory cells may differ in their processing machinery and may thus favor the presentation of distinct antigens or particular epitopes of a given antigen. For example, freshly isolated Langerhans cells reflecting the intra-epidermal stage were found to display numerous acidic organelles that are probably involved in antigen processing[40]. These organelles virtually disappear in cultured Langerhans cells – those that resemble lymph node dendritic cells and have lost the ability to process native proteins, but retain the ability to present peptides[11,40]. Furthermore, MHC class II molecules are more stable and synthesis of invariant chain is increased in Langerhans cells as compared with macrophages[41]. The slow turnover of MHC class II molecules is of particular interest because it enables antigen-laden dendritic cells to retain immunogenic peptides during migration[14]. Thus, it may be possible to charge Langerhans cells with purified parasite antigen *ex vivo* for immunization and induction of a protective T-cell response *in situ*. Certainly, a more detailed knowledge of these issues will be important for choosing the route and the mode of administration of protective antigens and, thus, the development of new vaccination strategies.

Finally, it will be of interest to analyse the mechanisms underlying the disparate permissiveness of resident macrophages and Langerhans cells to infection with *L. major* and possibly other *Leishmania* species. This may help to understand the varying disease patterns caused by different species of parasites and to approach the well-documented phenomenon of parasite persistence in immune hosts.

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