

**EXPERIMENTAL CUTANEOUS LEISHMANIASIS:  
LANGERHANS CELLS INTERNALIZE *LEISHMANIA MAJOR*  
AND INDUCE AN ANTIGEN-SPECIFIC T-CELL RESPONSE**

Heidrun Moll

Institute of Clinical Microbiology  
University of Erlangen-Nürnberg  
Wasserturmstrasse 3  
W-8520 Erlangen, Germany

**INTRODUCTION**

Human leishmaniasis constitutes a diverse group of diseases caused by protozoa of the genus *Leishmania*. The parasites are transmitted by sandflies and the infection is initiated by introduction of promastigotes into the skin at the site of the vector's bite. In the mammalian host, *Leishmania* parasites exist as obligatory intracellular amastigotes residing in mononuclear phagocytes. The diseases vary in severity from the self-healing cutaneous leishmaniasis, characterized by a localized cutaneous lesion at the site of primary infection, to the progressive visceral leishmaniasis, where the parasites disseminate from the original skin lesion to local lymph nodes and then to spleen, liver and bone marrow. The clinical manifestation depends primarily on the species of parasite, but also on the genetic make-up of the host. The T cell-mediated immunity is crucial for the outcome of infection because activated T cells release various lymphokines that promote either the spreading or the elimination of parasites<sup>1,2</sup>.

The entry of *Leishmania* into host cells occurs via attachment to cell surface receptors and subsequent internalization by phagocytosis. Receptor-ligand binding involves the receptor for complement component C3bi, CR3, and the mannose-fucose receptor on macrophages<sup>3</sup>. Infected macrophages express parasite antigen on their surface and thus serve as antigen-presenting cells for induction of a specific T-cell response.

In the mammalian skin, there are two appropriate cell types that would be in contact with inoculated *Leishmania* parasites - dermal macrophages and epidermal Langerhans cells (LC). LC express CR3 (Refs. 4, 5) and they are potent antigen-presenting cells bearing high levels of major histocompatibility complex (MHC) class II antigens. In contrast to macrophages, however, which are known to play a central role in the course of infection<sup>6</sup>, the function of LC in leishmaniasis has so far not obtained adequate attention.

We used *Leishmania major*, the cause of human cutaneous leishmaniasis in the Old World, for analysis of the following issues in the mouse model: (1) the interaction of LC

with *L. major*, (2) the efficacy of LC to present *L. major* antigen to T cells, and (3) the ability of LC to transport *L. major* antigen from the skin into lymphoid tissues for stimulation of T cells.

## LANGERHANS CELLS CAN BE INFECTED BY *L. MAJOR*

In order to investigate the interaction of LC with *L. major* in vitro, single-cell suspensions of epidermal cells were prepared from ear skin of BALB/c mice by trypsinization procedures as previously described<sup>7</sup>. Freshly isolated epidermal cells were incubated with *L. major* amastigotes for 24 h. Subsequently, the cultures were harvested for removal of extracellular parasites and identification of infected epidermal cells by cytochemistry.

Double labeling combining immunoenzymatic labeling (ABC method<sup>8</sup>) to detect *L. major* antigens and immunofluorescence staining for expression of MHC class II (Ia) molecules revealed that only Ia<sup>+</sup> epidermal cells but not Ia<sup>-</sup> cells contained *L. major* antigen. Because LC are the only Ia<sup>+</sup> cells in the normal epidermis, this finding demonstrated that epidermal LC can be parasitized by *L. major*. Furthermore, ultrastructural studies showed that LC accommodated intact amastigotes which were secluded within phagosomes (Figure 1). The ingestion of *L. major* by LC was blocked by anti-CR3 antibodies but could not be reduced by a soluble inhibitor of the mannose-fucose receptor. This indicated that in contrast to the uptake of *Leishmania* by macrophages, which requires the combined action of CR3 and the mannose-fucose receptor<sup>3</sup>, the internalization by LC is mediated primarily by CR3.

Only freshly isolated LC were able to ingest *L. major*. When parasites were added to cultured epidermal cells, the rate of infection was inversely correlated with the time of preincubation. No uptake could be detected after overnight culture of epidermal cells prior to addition of parasites (Table 1).

*L. major*-containing LC could not only be identified after culture in vitro but were also detectable in situ in the lesions of mice that had been intradermally infected with *L. major* promastigotes. This was demonstrated in skin sections by mixed labeling<sup>8</sup> of LC with an antibody to nonlymphoid dendritic cells (NLDC-145, Ref. 10) and an antiserum to *L. major*. NLDC-145<sup>+</sup> LC expressing *L. major* antigen could only be traced in the dermal infiltrate, but not in the epidermis, and constituted less than 1% of nonlymphoid mononuclear cells in this area.

**Table 1.** The ability of LC to ingest *L. major* decreases with the time of culture.<sup>1</sup>

Time of culture before addition of <i>L. major</i> (hours)	infected LC (% of control ; $\bar{x} \pm SD$ )
0 (control)	100
2	59 $\pm$ 12
5	32 $\pm$ 11
9	8 $\pm$ 5
16	0 $\pm$ 0
24	3 $\pm$ 1

<sup>1</sup> *L. major* amastigotes were added to epidermal cell cultures at various time points. After additional 24 h, the cultures were harvested, extracellular parasites were removed, and the rate of infection of LC was determined by fluorescence microscopic analysis after staining with acridine orange and ethidium bromide<sup>9</sup>.



**Figure 1.** Electron microscopic documentation of a *L. major*-infected LC containing two amastigotes within one phagosome and a single organism within another phagosome. Note the unit membrane of the phagosome (arrowheads) and the flagellum (F). Magnification =  $\times 15,000$ . (Courtesy of Klemens Rappersberger, M. D.)

The maximum level of infection in culture was 25% of total LC and the majority of infected LC had only ingested one parasite (mean: 1.4 amastigotes). Most interestingly, time course analysis revealed that the average number of organisms per infected cell did not increase after 24 h of culture, and by one week, *L. major*-containing LC were no longer detectable. Thus, LC are able to restrain parasite replication. This is in contrast to macrophages which are permissive to parasite infection unless they are activated by cytokines such as as interferon- $\gamma^{11}$ .

The above findings showed that the parasite load of LC in vitro and in vivo is much lower than that of macrophages. This suggests that endocytosis of *L. major* by LC is not primarily aimed at antigen scavenging, the characteristic of macrophages. In addition, LC did not support intracellular parasite multiplication and are therefore unlikely to account for the massive expansion of amastigotes usually observed in the infected skin. It is conceivable, however, that the major function of LC, which are known to be amongst the most active antigen-presenting cells<sup>12</sup>, is the presentation of *L. major* antigen to T cells. To test this possibility, we investigated the ability of LC to stimulate *L. major*-primed T cells in vitro.

## **LANGERHANS CELLS ARE POTENT STIMULATORS OF AN ANTIGEN-SPECIFIC T-CELL RESPONSE TO *L. MAJOR***

To assess the efficiency of epidermal antigen-presenting cells, we used unselected epidermal cell populations which contain 2% to 4% LC. The results demonstrated that epidermal cells are able to present *L. major* antigen in vitro to T cells from primed mice<sup>13</sup>. The heterogeneous epidermal cells were remarkably efficient because the doses required for optimal responses were equivalent to merely 250 to 2000 LC per culture. The T cell-stimulatory effect of epidermal cells could indeed be attributed to LC because removal of those cells completely abrogated the response. LC stimulated both T-cell proliferation and lymphokine production; the reaction was antigen-specific and could be induced by antigen in *L. major* lysate as well as by antigen derived from viable parasites<sup>13</sup>. The finding that only freshly isolated LC, but not cultured LC, were able to present *L. major* antigen was in line with our observation that only fresh LC could internalize parasites. This is reminiscent of earlier reports showing that freshly explanted LC can process native antigen for presentation to primed T cells, whereas cultured LC have lost this ability but are highly active mediators of stimuli that do not require antigen processing<sup>14-16</sup>. Freshly isolated LC would reflect the properties of intracutaneous LC, and LC cultured for several days would be the in vitro equivalents of lymphoid dendritic cells derived from LC that have migrated to lymph nodes.

## ***L. MAJOR*-BEARING LANGERHANS CELLS MIGRATE FROM THE INFECTED SKIN TO THE DRAINING LYMPH NODE**

Evidence is accumulating that LC are capable of transporting antigens from peripheral non-lymphoid tissues via the lymphatics into lymphoid tissues for presentation to T cells<sup>17,18</sup>. Therefore, it was of interest to investigate whether dendritic cells carrying parasite antigen can be localized in lymph nodes draining the site of cutaneous infection with *L. major*. For this purpose, frozen sections of skin and lymph nodes from infected mice were analyzed by immunocytochemistry. In infected skin, the number of LC residing in the epidermis decreased rapidly within a few days after infection, whereas a gradually increasing number of LC appeared in the dermal compartment. Using mixed-labeling techniques<sup>8</sup>, dendritic cells displaying *L. major* antigen were found not only in the dermis but also in the regional lymph node. In the early phase of infection, expression of parasite antigen in the lymph nodes was confined to dendritic cells and could not be detected on macrophages. Those lymph node cells had the potency of presenting parasite antigen to T cells because they stimulated antigen-specific T-cell proliferation in vitro and, after transfer to naive recipients, they induced DTH reactivity to *L. major* antigen. These findings indicate that LC capture *L. major* antigen in the skin and transport it to the regional lymph node for initiation of the T-cell immune response.

## CONCLUDING REMARKS

In summary, our results suggest an important role of LC during cutaneous leishmaniasis. They support the concept that, upon inoculation of *L. major* into the skin, viable parasites are ingested not only by macrophages but also by LC that have migrated into the dermis. After appropriate processing of the organisms, LC would interact with antigen-specific T cells in two different ways. On the one hand, they are capable of carrying parasite antigen from the skin to the draining lymph node and presenting it to quiescent T cells of the circulating pool. On the other hand, LC may present parasite antigen to effector T cells infiltrating the cutaneous lesion and regulate the local immune response.

Several implications arise from these findings which are important for our understanding of the pathogenesis of cutaneous leishmaniasis. The first aspect relates to the well-documented phenomenon that resistance and susceptibility to infection can be attributed to CD4<sup>+</sup> T-cell populations with different patterns of lymphokine activity<sup>1,2</sup>. Release of interferon- $\gamma$  appears to promote protection, whereas interleukin 4 has been suggested to facilitate survival of parasites. The possibility exists that different types of antigen-presenting cells (i.e. LC, lymphoid dendritic cells, macrophages and B cells) favor the expansion of disparate T-cell subsets on the basis of providing different costimulatory signals. The second point relates to the role of different parasite antigens. The processing machinery of LC may differ from that of other antigen-presenting cells and favor the presentation of distinct antigens or particular epitopes of a given antigen. A more detailed knowledge of this issue will be important for choosing the route of administration of potentially protective antigens. Finally, it will be of interest to analyze the mechanisms underlying the disparate permissiveness of resident macrophages and LC to infection with *L. major* or other *Leishmania* species. This may help to understand the varying disease patterns caused by different species of parasites. Current work in our laboratory addresses these issues. The above considerations emphasize that experimental cutaneous leishmaniasis provides an excellent model to study the role of LC in vivo during infectious diseases.

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