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Epidermal inoculation of *Leishmania*-antigen by gold bombardment results in a chronic form of leishmaniasis

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Abstract

Experimental leishmaniasis represents a suitable model to analyze Th1-type associated immunity. In C57BL/6 mice healing of leishmaniasis correlates with activation of Th1 cells. Recently, it could be demonstrated that dermal dendritic cells rather than epidermal Langerhans cells are responsible for the activation of Th1 cells after infection, indicating a necessary reconsideration of the role of Langerhans cells. In our current work, epidermal application of *Leishmania*-antigen prior to infection resulted in an atypical course of disease that is characterized by an impaired *Leishmania*-specific Th1 response. Consequently, these mice cannot manage an efficient elimination of the parasites at the site of infection. These data point to the activation of immunomodulatory effects by epidermal incorporation of antigen.
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Keywords: Gene gun; Gold particles; T cell response; Epidermal antigen inoculation

1. Introduction

Leishmania parasites comprise obligate intracellular protozoan pathogens that are adapted to infect a variety of mammalian hosts including humans and mice. Depending on the transmitted parasite subspecies, *Leishmania* infections cause different clinical symptoms that range from cutaneous to visceral manifestations [1,2]. In the experimental model of leishmaniasis, mice are infected subcutaneously or intradermally with stationary phase promastigote *Leishmania* (*L.*) *major* parasites [3,4]. The course of disease predominantly depends on the genetically determined ability of the infected mouse strain to mount a protective T cell mediated immune response. This protective T helper (Th) 1 response is characterized by an early interferon-γ (IFN-γ) production and the expression of inducible nitric oxide synthase by activated macrophages [5,6]. Resistant mouse strains, such as C57BL/6 (B6.WT), are able to heal the pathogen-induced skin lesion and acquire immunity. In contrast, susceptible BALB/c mice develop an IL-4 dominated Th2-type response and finally succumb to a progressive generalized infection [5]. Since 1993, epidermal Langerhans cells (LCs) have been discussed to be crucial for the induction of a protective immune response against *L. major* [7]. However, sufficient tools to adequately dissect dermal dendritic cells (DCs) from epidermal LCs were limited or not established in the early 1990s. The identification of CD207 (Langerin), a LC-specific marker, now allows for discrimination between lymph node (LN) homing LCs and dermal DCs [8,9]. Recently it was demonstrated that inflammatory dermal $CD207^{+}/CD8\alpha^{-}$ DCs migrate to the draining LN to activate *Leishmania*-specific T cells [3,10]. It is important to mention that during an infection

Abbreviations: B6.WT, C57BL/6; DC, dendritic cell; LC, Langerhans cell; Ig, immunoglobulin; Th cells, T helper cells; CTL, cytotoxic T lymphocyte; LN, lymph node; EAI, epidermal antigen incorporation

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with *L. major* parasites, LCs also migrate to the paracortical area of skin-draining LNs. But, in contrast to dermal DCs, LCs do not induce *Leishmania*-specific T cell proliferation [3].

LCs migrate under steady-state conditions to the LN. This indicates that a permanent low-level influx of LCs to the paracortical area of the LN takes place [3,11,12]. Therefore, it is tempting to speculate that epidermal LCs present self-antigen to T cells. This hypothesis was indeed supported by Mayerova et al. who demonstrated that LCs are able to present keratinocyte-expressed self-antigen to naïve T cells [13], indicating that the cutaneous system might be involved in peripheral tolerance [14]. Because presentation of self-antigen is known to be crucial for induction of tolerance in general, LCs could therefore represent a subpopulation of DCs with immune regulatory capacities. A current model suggests, that DCs indeed are tolerogenic when they present antigen under steady-state non-inflammatory conditions. However, when stimulated by inflammatory mediators, they mature and induce effector T cell responses [15,16].

As described above, LCs can be detected (i) under steady-state and (ii) under inflammatory conditions in the skin-draining LN. Nevertheless, the biological function of LCs in the experimental model of leishmaniasis is still unresolved. Therefore, we established a method that allows us to incorporate *Leishmania*-antigen (L-Ag) into the epidermal compartment of the skin. With a conventional "gene gun" it is possible to expose epidermal cells to antigen, which is normally localized in the dermal or subcutaneous compartment. In 1992 Tang et al. for the first time used a biolistic device for *in vivo* transfection by propelling gold microprojectiles coated with plasmid DNA into the skin of mice, thereby eliciting an immune response against the encoded antigen [17]. The rationale to deliver vaccine antigens to the skin is to target antigen to epidermal LCs to facilitate antigen uptake and presentation and increase the magnitude of an induced immune response. Indeed, gene gun immunization with plasmid DNA has been demonstrated to be highly immunogenic and often superior to saline needle injection of plasmid DNA although much lower amounts of DNA are applied [18–20]. But, the type of immune response elicited *via* gene gun immunization markedly differs from immune responses induced by needle application. Although gene gun immunization can induce strong cytotoxic T lymphocyte (CTL) responses [21], which has been attributed to the intracellular delivery of antigen, the serological immune response is dominated by high levels of IgG1 and only low levels of IgG2a, indicating a Th2-biased reaction [20,22,23]. The differences in the observed immune reaction have been explained by the lower amounts of Th1 promoting immunostimulatory CpG motifs applied by gene gun immunization [20,24]. However, we have shown previously that gene gun application can bias a Th1 immune reaction towards a serological Th2-type reaction in an antigen-independent manner [25]. We speculated that activation of specific subsets of DCs *via* gold bombardment itself might account for the observed immune modulation.

Targeting epidermal LCs has not only been performed with DNA coated gold particles but also with sugar excipient based powder formulations of recombinant proteins, polysaccharides or inactivated pathogens (reviewed in [26]). However, these powder formulations are not delivered into the cytoplasm of epidermal cells, but diffuse into surrounding cells and are picked up by LCs from extracellular spaces. This results in a different processing and presentation of antigen and reduced induction of CTLs [27]. Nevertheless, epidermal powder immunization induced protective immune responses against influenza virus at a 10-fold lower dose compared to intramuscular immunization [27].

In the present study, we utilized a similar method as described by Maddelein et al. [28] to specifically deliver L-Ag to the epidermal compartment. Antigen was applied in a small volume of water to the skin of mice and after evaporation of liquid the skin was bombarded with 1.6 μm gold particles using a gene gun. With this technique of epidermal antigen incorporation (EAI) we are able to induce an antigen-specific T cell response in the skin-draining LN.

2. Materials and methods

2.1. Mice

Female B6.WT mice (aged 8–10 weeks) were purchased from Charles River (Sulzfeld, Germany). Ovalbumin (OVA) transgenic OT.II mice were maintained at the animal facility of the Bernhard-Nocht-Institute for Tropical Medicine or the University Hospital Hamburg, according to the guidelines for the care and use of experimental animals.

2.2. L. major parasites and the preparation of parasite antigen

The cloned virulent *L. major* isolate (MHOM/IL/81/FEBNI) was propagated *in vitro* in blood agar cultures as previously described [29]. The virulence of the parasites was maintained by monthly passage in BALB/c mice. For the preparation of *L. major* parasites, stationary phase promastigotes from the third to seventh *in vitro* passage were harvested, washed four times, and re-suspended in PBS. Alternatively, the parasites were subjected to four cycles of rapid freezing and thawing to prepare L-Ag [3].

2.3. L. major infection and evaluation of the systemic course of disease

Mice were infected *via* subcutaneous injection of 3×10^6 stationary phase promastigotes into one of the hind footpads in a final volume of 50 μl. The course of disease was monitored daily as described [3]. The parasite load in infected tissues was estimated using a real-time PCR assay as previously described in detail [30].

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