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Mini-review

Challenges and perspectives in vaccination against leishmaniasis

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ABSTRACT

The leishmaniases are a group of diseases caused by protozoa of the genus *Leishmania* and affect millions of people worldwide. The leishmaniases are transmitted to vertebrate hosts by phlebotomine sand flies. In this review, we focus on several issues that have been poorly addressed in ongoing efforts to develop a vaccine against *Leishmania*, namely: vaccination with antigens present in sand fly saliva, vaccines based on intracellular *Leishmania* antigens, and use of recombinant BCG as a vehicle for vaccination. Additionally, we address the differences between *L. major* and *L. braziliensis* and the impact that these differences may have on strategies for immunoprophylaxis.

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Leishmaniasis is a serious and increasing public health problem. Approximately 300 million people live in or travel to tropical and subtropical risk areas. Moreover, human leishmaniasis is endemic in more than 80 countries, and its prevalence exceeds 12 million cases worldwide; 1.5–2.0 million new cases occur annually, causing a burden estimated at 2,357,000 disability-adjusted life years (DALYs) (http://www.who.int/tdr/diseases/leish/diseaseinfo.htm). *Leishmania* infection can be classified into three main classical syndromes: cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL) and visceral leishmaniasis (VL). Clinical manifestations of the disease depend on several factors, including the species involved, and symptoms range from self-limiting cutaneous lesions through the severe mucocutaneous form to the often fatal visceral form. In VL, which encompasses a broad range of clinical signs, infection remains asymptomatic or subclinical in many cases, while in others it follows an acute or chronic course. Importantly, the disease does not resolve spontaneously, and the ensuing systemic infection may be fatal if left untreated [1]. In CL, lesions tend to heal spontaneously, and immunity that ensues following natural healing is lifelong. Therefore, prevention of leishmaniasis through prophylactic immunization seems feasible.

Ongoing approaches to vaccine development are largely based on identification of appropriate surface antigens of *L. major*. It is expected that a vaccine against leishmaniasis will likely combine more than one antigen and that antigens will preferentially be conserved among *Leishmania* species and present in both the amastigote and promastigote stages of the parasite. Although several current candidates fulfill such criteria, demonstration of protection by these antigens in more than one animal model is lacking. Additionally, some protective antigens are conserved among their mammalian orthologues, raising concerns over possible autoimmune reactions. This review will focus on areas poorly addressed in the ongoing efforts to develop a vaccine against *Leishmania*, namely, vaccination with antigens present in sand fly saliva, vaccines based on intracellular *Leishmania* antigens, and use of recombinant BCG as a vehicle for vaccination. An additional challenge that needs proper

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attention is the impact that recognized differences between *L. major* and *L. braziliensis* may have on strategies for immunoprophylaxis.

1. Vaccination with sand fly salivary antigens

Leishmania protozoans are transmitted to their vertebrate host by infected sand flies. While attempting to feed, these flies inject both saliva and *Leishmania* promastigotes. Sand fly saliva contains a vast repertoire of pharmacologically active molecules able to interfere with the host's hemostatic, inflammatory and immune responses. The effects of sand fly salivary products on leishmaniasis have recently been reviewed [2]. The actions of these salivary components during early interactions between *Leishmania* and the host's immune system are closely linked to disease evolution as well as to protection against the protozoan. Hence, characterization of salivary components is regarded as essential for understanding the pathogenesis of the disease as well as for providing a basis for development of novel strategies to hamper pathogen transmission. Indeed, maxadilan, a vasodilatory peptide isolated from *Lutzomyia longipalpis* saliva, decreased the secretion of IFN-γ and increased the production of IL-6 in mononuclear cells [3]. *Lu. longipalpis* saliva inhibited IL-10 and TNF-α production and enhanced IL-6, IL-8 and IL-12p40 secretion by LPS-stimulated human monocytes and dendritic cells (DCs) [4]. In parallel, reduced CD80 and increased HLA-DR expression were also observed.

These results show that sand fly salivary gland components have an immunomodulatory effect on human cells. This finding has important implications for vaccine development. Moreover, modulation of *Leishmania* infection by sand fly saliva has also been reported during experimental infection with *L. major* [5], *L. braziliensis* [6,7] and *L. amazonensis* [8]. Importantly, in a CL experimental model employing *L. major*, it was shown that prior exposure of mice to bites of uninfected sand flies conferred powerful protection against *L. major* [9] and that a DNA vaccine encoding SP15, a salivary antigen present in *Phlebotomus papatasi* saliva, provided similar protection [10]. However, when a similar approach was used in an attempt to prevent infection with *L. braziliensis*, immunization with saliva of *Lu. intermedia*, the main vector of *L. braziliensis*, was found to enhance infection [11]. Such results point to differences in the composition and immunomodulatory capacity of salivary antigens between distinct sand fly species.

With respect to the use of salivary components in the development of vaccines against VL, it has been demonstrated that immunization with a DNA plasmid coding for an 11 kDa protein from *Lu. longipalpis* saliva induced protection against intradermal co-inoculation of *L. chagasi* and salivary gland homogenate [12]. This protection was associated with the development of anti-sand fly saliva cellular immunity in the form of a DTH response and the presence of IFN-γ at the site of sand fly bites. Hence, immunity to a single salivary protein can confer protection against VL. Indeed, immunization of dogs with *Lu. longipalpis* salivary antigens led to the development of a recall response characterized by lymphocytic infiltration and expression of IFN-γ and IL-12 [13]. The recent observation that neutrophils persist at the site of sand fly bites and that this effect inhibits *L. major* elimination in mice vaccinated with *Leishmania* antigen + CpG [14] reinforces the case for development of combination vaccines, including parasite- and sand fly salivary antigens.

Sand fly salivary antigens may also serve as epidemiological tools to track vector exposure. For example, children residing in areas endemic for VL exhibit anti-*Lu. longipalpis* saliva IgG antibodies [15]. This humoral response appears simultaneously with an anti-*L. chagasi* cell-mediated immunity [16], supporting the hypothesis that induction of an immune response against saliva can facilitate induction of a protective response against leishmaniasis. Moreover, anti-sand fly saliva antibodies may be used as an important epidemiological marker of vector exposure and may even prove useful as a marker of protection. In another study, healthy volunteers exposed to laboratory-reared *Lu. longipalpis* developed anti-saliva antibodies (IgG1, IgG4 and IgE) [17]. Two major patterns of responses were observed in these volunteers: intense skin reactions with indurated nodules accompanied by delayed-type hypersensitivity (DTH)-like response with higher IgG/IgE ratio, and mild erythematous reactions with lower IgG/IgE ratio [17]. The most immunogenic proteins in individuals exposed to *Lu. longipalpis* sand flies had molecular weights of 45, 44, 43, 35, 27 and 16 kDa [16,17].

2. *Leishmania* vaccines based on intracellular antigens

Recent advances in the design of vaccines against leishmaniasis using various strains of inbred mice have shown that immunization with defined parasite antigens provides protection against challenge with several *Leishmania* species. Interestingly, many of these protective molecules have an intracellular location [18]. Some, such as A2 protein (an amastigote-specific molecule) [19] or the *Leishmania* sterol 24-c-methyltransferase (SMT) [20], are parasite-specific and are not found in mammalian cells. Other intracellular protective antigens are members of conserved protein families such as histones, the acidic ribosomal protein P0, the stress-inducible LmSTI1 protein, and LACK, the leishmanial homolog of the mammalian receptor for activated C kinase [21]. In natural infections, immune responses against these intracellular proteins are thought to result in immunopathology, since they predominantly stimulate non-protective specific humoral responses in patients with different forms of the disease [20,22–25] and in dogs with VL [26–28].

Interestingly, some of these antigenic proteins have also been implicated in the generation of protective responses. Thus, *Leishmania* histone H2B was able to stimulate the production of IFN-γ in a T cell clone established from an immune donor [29], and parasite histones H2B, H2A and H3 induced proliferation and IFN-γ production by peripheral blood mononuclear cells (PBMCs) from CL patients [30]. Similar results were observed with cells from resistant mice stimulated with A2 [31]. Generally, a high specificity was observed in the humoral and cellular responses elicited against highly conserved intracellular antigens, since T- and B-cell epitopes were restricted to the small blocks of non-conservative amino acid substitutions. Likely for that reason, neither reactive T cells [29] nor anti-intracellular protein antibodies recognized their mammalian orthologues [21]. Thus, it can be hypothesized that, depending on the immune response elicited against them, these antigens may play an important role in disease development.

The fact that the immune response to parasite antigens fails to provoke recognition of homologous regions within conserved proteins argues against nonspecific polyclonal activation as the basis of the immune response to these antigens. It therefore is expected that these antigens are exposed to the host immune system after infection. Extracellular promastigote cytolysis mediated by serum lytic factors [32] or neutrophil extracellular traps [33] may prime the immune response of the vertebrate host against intracellular antigens in the inoculation site. Later, when amastigotes replicate inside the macrophages, these antigens may be derived by spontaneous cytolysis within infected cells and become exogenously exposed after disintegration of the cells [34].

Intracellular antigens have been used for immunization in combination with Th1-inducing adjuvants or as DNA vaccines, since the development of vaccines based on these antigens is mainly focused on the generation of specific IFN-γ producing $CD4^+$ Th1 and $CD8^+$ T cells. Thus, immunization with *L. donovani* A2 DNA vaccines [35] or with A2 protein combined with *Propionibacterium acnes* [36] induced significant protection against VL caused by *L. donovani* in BALB/c mice. Protection was correlated with the generation of a Th1/Th2 mixed response and with A2-specific IFN-γ production. Similarly, immunization of BALB/c mice with SMT antigen plus monophosphoryl lipid A (MPL®) induced partial protection against *L. donovani* challenge and resulted in IFN-γ production upon *in vitro* stimulation with the antigen [20].

Although the generation of a vaccine-induced Th1 response is necessary for protection, it may not be sufficient. Thus, Th1 responses