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Failure of a multi-subunit recombinant leishmanial vaccine (MML) to protect dogs from *Leishmania infantum* infection and to prevent disease progression in infected animals

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Abstract

We report results of a Phase III trial of the multi-subunit recombinant *Leishmania* polyprotein MML for the protection of dogs against infection by *Leishmania infantum*. The antigen, also known as Leish-111f, is the first antileishmanial human vaccine entered Phase I clinical testing. The study was performed in a leishmaniasis endemic area of southern Italy. Three groups of 15 *Leishmania*-free beagle dogs each, received 3 monthly injections with vaccines A (MML + MPL®-SE adjuvant), B (sterile saline = control) and C (MML + Adjuprime adjuvant), respectively, before transmission season 2002. The surviving dogs received a second three-dose vaccine course 1 year later. The dogs were naturally exposed to sandfly bites for 2.5 months in 2002, and for 5 months in 2003. Every 2 months post vaccination, dogs were examined by clinical and immunological evaluation, and by specific serology, microscopy, culture and PCR. A weak lymphoproliferative response to MML was seen in A and C groups throughout the study period. One year after the first vaccine course, the cumulative incidence of leishmanial infections was 40% in group A, 43% in group B and 36% in group C. Two-year post-vaccination (1 year after the second vaccine course) the cumulative incidence was 87% in group A (with three symptomatic cases), 100% in group B (with no symptomatic cases) and 100% in group C (with two symptomatic cases). The efficacy of the MML vaccine as an immunotherapeutic agent for the prevention of disease progression (subpatent infection → asymptomatic patent infection → symptomatic patent infection) was evaluated through follow-up of dogs found infected prior to the second vaccination. Among 15 infected animals, progression to a subsequent stage of infection was found in 5/6 dogs of group A, 3/6 of group B and 2/3 of group C. We conclude that vaccination with MML is not effective to prevent leishmaniasis infection and disease progression in dogs under field conditions.

Keywords: Recombinant polyprotein; *Leishmania*; Canine leishmaniasis; Vaccine

1. Introduction

Zoonotic visceral leishmaniasis (ZVL) is a severe sandfly borne disease caused by the protozoan parasite *Leishmania infantum* and widely distributed in temperate and subtropical countries of both the Old and New World [1]. The domestic reservoir of ZVL are dogs, which may suffer from a severe disease characterized by chronic evolution of viscero-cutaneous signs occurring in less than 50% of infected animals [2]. On the other hand, both asymptomatic and symptomatic dogs with detectable antibodies can be infectious to phlebotomine vectors [3,4].

Mass detection of seropositive dogs followed by culling and/or drug treatment, or the mass application of deltamethrin-impregnated collars, were shown to have an impact in reducing human and canine ZVL prevalence in endemic areas of the Old World [5–7], although the efficacy of eliminating seropositive canines has been debated in Brazil [8,9]. The above control measures are either not

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acceptable, expensive or not very effective. Mathematical models used to compare the effectiveness of various tools for controlling ZVL, suggest that a dog vaccine may be the most practical and effective method [10]. Therefore, the development of vaccines able to protect dogs from leishmanial infections and/or to prevent disease progression in infected animals, is highly desirable for the implementation of ZVL control programs as well as for the veterinary community.

A few Phase I/II vaccine trials have been performed in dogs, using killed *Leishmania* promastigotes, purified leishmanial fractions or recombinant DNA [11,12]. Recently, a fucose-mannose-ligand (FML) enriched fraction of *Leishmania donovani* entered a Phase III vaccine trial against symptomatic canine leishmaniasis, with about 80% clinical efficacy [13,14]. The same antigen conferred 90% protection from disease progression when used for the immunotherapy of asymptomatic animals [15].

In this paper, we report results of a Phase III trial of the multi-subunit recombinant *Leishmania* polyprotein MML, also known as Leish-111f [16,17], for the protection of dogs against infection by *L. infantum*. This chimeric antigen was generated from three recombinant *Leishmania* antigens screened for their ability to elicit human and murine cellular immune responses. Recombinant TSA (=MAPS), obtained from an *Leishmania major* amastigote cDNA expression library, elicited strong T-cell immune responses in mice and conferred protective immunity against *L. major* when administered with IL-12. This antigen also stimulated proliferative responses in peripheral blood mononuclear cells (PBMC) from human leishmaniasis patients [18]. Recombinant LmSTI1 (=M15) was also selected from an *L. major* amastigote cDNA expression library. Both cellular and humoral responses against this antigen were shown in infected BALB/c mice and in human leishmaniasis patients. In particular, recombinant LmSTI1 was demonstrated to be capable of shifting these toward a Th1-type cellular response in mice with advanced *L. major* infection [19]. A mixture of TSA and LmSTI1 antigens, administered with IL-12 and alum, was found to protect from experimental cutaneous leishmaniasis in a non-human primate model [20]. Recombinant LeIF, originating from a *Leishmania braziliensis* expression library, was found to stimulate the production of IFN-γ and IL-2, but not IL-4 or IL-10, in PBMC from human leishmaniasis patients, and IL-12 in PBMC from both patients and uninfected individuals [21]. The ability of LeIF to influence an early Th1 cytokine profile by IL-12-dependent mechanisms was shown in a SCID mouse model [22]. Since LeIF confers only partial protection against *L. major* in BALB/c mice when used alone, it may have a potential role as a Th1-type adjuvant when used in combination with other leishmanial antigens. A candidate vaccine consisting of Leish-111f formulated in monophosphoryl lipid A stable emulsion (MPL® - SE) entered Phase I clinical testing in healthy volunteers in January 2003 [23].

2. Materials and methods

2.1. Study area and dogs

The study was performed in a rural setting of the Naples province, southern Italy. This area has long been under investigation due to a high incidence of human and canine ZVL. An average of about 40 human cases is reported annually from a cluster of villages and towns surrounding Vesuvius [24], whereas canine leishmaniasis seroprevalence averages 23% [6]. Adult females of the local phlebotomine vector, *Phlebotomus perniciosus*, are usually active from the end of May through to October. In the study area, this species was found to be naturally infected at high rates with *L. infantum* zymodemes known to cause disease in man and dog [25].

Forty-five beagle dogs (23 males) born in January 2002, purchased by a local dog breeder from a laboratory animal company located in a non-endemic area of northern Italy (Green Hill 2001, Montichiari, Brescia), were enrolled in the vaccine study. The dogs had received routine vaccinations against leptospirosis, distemper, adenovirosis-2, hepatitis, parainfluenza and parvovirus (CEPPiL, Merial, France), and were negative for anti-*Leishmania* antibodies by immunofluorescent antibody test (IFAT).

The first two doses of the vaccines under study were administered at the facilities of the laboratory animal company, while the third dose was given after the animals were moved to the study area in July 2002. Here, the dogs were placed in three contiguous open kennels and kept under constant veterinary care during the study period. The use of topical or environmental insecticides was avoided to allow natural exposure of dogs to sandfly bites. Tick control was affected by mechanical measures. The collection of biological samples from the dogs was performed in accordance with the national guidelines for animal welfare, under the supervision of the veterinary services of the Local Health Unit.

2.2. Vaccine and vaccination

Two vaccine preparations, differing in adjuvant composition, consisted of 45 μg/dose MML plus 50 μg/dose MPL®-SE (vaccine A), or 45 μg/dose MML plus 1 mg/dose Adjuprime (Pierce Chemical, IL, USA) (vaccine C), respectively, to give a final volume of 1 ml/dose. A third preparation consisted of 1 ml/dose sterile saline (vaccine control, B). The study was blinded, as the vaccine doses were prepared by Novartis Animal Vaccines Ltd. (Braintree, UK) following procedures reported by Skeiky et al. [17], and neither the veterinarian in charge nor the scientific staff were informed of the identity of the vaccine batches and compositions.

Dogs were randomized by sex and assigned to three groups of 15 animals each, to receive three subcutaneous injections with A, B and C vaccines, respectively, at 28-day intervals starting from 3rd June 2002. The surviving dogs (15 of group A, 14 of group B and 13 of group C) received a second three-dose vaccine course 1 year later, starting from 1st July 2003.