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Inhibition *in vivo* of both infective *Leishmania major* and *L. mexicana amazonensis* mediated by a single monoclonal antibody

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Abstract

Monoclonal antibodies raised against a strain of *Leishmania infantum* isolated in Greece were produced and tested for their protective effect in an *in vivo* system (in BALB/C mice). A single monoclonal antibody, IgG_2b isotype, can prevent the development of two *Leishmania* strains *in vivo*: one of *L. major* and one of *L. mexicana amazonensis*. This antibody-mediated protection may be dependent on complement.

Introduction

Monoclonal antibodies produced by hybrid cell lines have been raised, against the New World species of *Leishmania* (see MCMAHON-PRATT & DAVID, 1981). They have been used, in particular, to identify the subspecies of *L. braziliensis* (see MCMAHON-PRATT *et al.*, 1982). Recent studies have shown that monoclonal antibodies could kill parasites cultured within macrophages *in vitro*. Such induced mortality has been recorded in one series of experiments with *L. major*, a pathogenic agent of Old World leishmaniasis (HANDMAN & HOCKING, 1982).

Complete protection against *L. mexicana amazonensis* promastigotes inoculated with monoclonal antibodies has been similarly obtained *in vivo*, using a Winn assay system (ANDERSON *et al.*, 1983).

The data presented here show that a single monoclonal antibody is able to protect the BALB/c mouse from infection with either *L. major* or *L. mexicana amazonensis*.

Materials and Methods

Production of monoclonal antibodies

Monoclonal antibodies were produced as described elsewhere (ROSETO *et al.*, 1982; MONJOUR *et al.*, 1984b). We used a *Leishmania* strain isolated in Greece, *L. infantum* (LEM 497, Montpellier Collection), from the bone marrow of a sick dog and injected into a hamster. The amastigotes from the hamster spleen were deposited in a liquid medium (RPMI 1640 medium supplemented with 20% foetal calf serum) and after transformation into promastigotes at 24°C, the parasites were passaged not more than twice in the nutrient broth.

Promastigote preparations were used to immunize three BALB/c female mice two months old and serologically negative for *Leishmania*. Each animal was injected three times, every 15 days subcutaneously with 10^6 parasites + 10 µg of Quil A purified Saponin (Laboratories Superfos, Denmark). One month later the mouse with the highest anti-leishmanial titre was given a booster injection of 2×10^7 promastigotes administered intraperitoneally four days before cell fusion. Spleen cells were mixed with immunoglobulin-non-secreting, 8-azaguanine-resistant Sp2/0 myeloma cells (SHULMAN *et al.*, 1978) and fused with 50% polyethylene glycol, following the technique described by GALFRE *et al.* (1977). Fused cells were selected in a hypoxanthine/azaserine (HA) selective medium.

The screening of cell culture supernatants was performed by indirect immunofluorescence (IIF) (MONJOUR *et al.*, 1978). *L. infantum* (LEM 497), *L. major* and *L. m. amazonensis* were used as antigens. Mouse and human anti-leishmanial sera, normal sera and RPMI medium were used as controls.

Positive hybridoma cultures were cloned using the limiting dilution technique and mink CCL64 cells (American Type Culture Collection) as feeder cells. The supernatants of growing clones were rescreened by IIF. 104 clones proved to be positive and 10 were selected which had high antibody titres (> 3200). Specificity of the monoclonal antibodies secreted by these ten clones was determined by an indirect immunofluorescence test (as described by DE IBARRA *et al.*, 1982) using live promastigotes of the following strains : *L. infantum* (LEM 497), *L. major* (LEM 129 Montpellier Collection, Ref. MHRO/SU/59/Neal P.), *L. mexicana amazonensis* LV 79 (Liverpool Collection), *L. donovani* ITMAR 263 (Antwerp Collection) and using amastigotes, obtained as described elsewhere (MONJOUR *et al.*, 1984a).

Large quantities of antibodies were obtained by intraperitoneal injection of 2×10^6 hybridoma cells into pristane-treated BALB/C mice. Two weeks later ascitic fluids were recovered.

IgG classes were determined by the immunodiffusion method.

Protective immunity assay

One ascitic fluid (64B16 : IgG_2b) which proved to be positive by IIF with *L. infantum* (LEM 497), *L. major* (LEM 129) and *L. m. amazonensis* (LV79) was used for assays of protection *in vivo*.

50 µl of monoclonal-antibody ascites were mixed with 10^6 promastigotes of either *L. major* or *L. m. amazonensis*. These mixtures were maintained at 37°C for 30 min and then injected subcutaneously near the root of the tail of BALB/c female mice. When the monoclonal antibody-promastigote mixture was observed by microscopy before the injections, viability of promastigotes, based on motility, was approximately 95%.

Many controls were used under the same conditions, namely decomplemented (30 min–56°C) monoclonal antibody containing ascites: mouse ascites devoid of monoclonal antibodies and non-reactive in IFI with *L. infantum* (LEM 497), *L. major* and *L. m. amazonensis*, anti-*Plasmodium falciparum*, anti-HBS monoclonal antibody containing ascites and ascites induced by injection of Sp2/0 myeloma cells. Simultaneously, experimental infections were induced in control mice using either *L. major* or *L. m. amazonensis* to confirm infectivity of the parasites. The

animals in each case were observed for three months. Control animals developed cutaneous leishmanial lesions with detectable amastigotes. Each experimental group included five BALB/c mice.

Results

Before the protective immunity assay, we examined the monoclonal antibodies with respect to their isotype and their target antigens. Most belonged to the IgG isotype IgG_1 and the others to IgG_2b. Based on observation of strong fluorescent labelling of the *L. major* and *L. m. amazonensis* live promastigote surfaces, we selected one monoclonal antibody (64B16 : isotype Ig2b) as potentially protective. Fig. 1 indicates that pre-treatment of the two *Leishmania* species, with 64B16 abolished their capacity to induce a cutaneous infection. Ascitic fluid containing monoclonal antibodies against *P. falciparum* and HBS, whether decomplemented or not, did not prevent the experimental infection. Complete protection was observed for over three months even though as many as 10^6 promastigotes were injected (Table I). Lesions appeared in controls two months after infection. Lesion size was between 0·25 and 0·5 cm at three months and over 0·5 cm when the animal died about six months after infection. Using Giemsa staining, it was confirmed that the lesions were due to leishmaniasis.

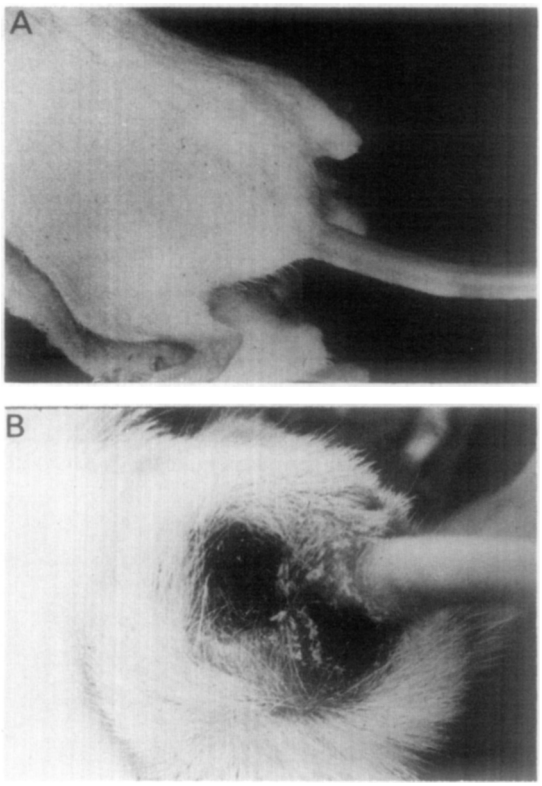

Fig. 1 : Cutaneous leishmaniasis.
The promastigote strains have been pretreated with a anti-*Leishmania* (A) monoclonal antibody, and a control monoclonal antibody (B).

Discussion

As seen in another *in vivo* system described elsewhere (MONJOUR *et al.*, 1984a) anti-leishmanial monoclonal antibodies raised against an Old World strain can afford protection against New World *Leishmania* including *L. donovani*. This cross protection has been confirmed by these data using *L. major* or *L. m. amazonensis* and BALB/c female mice for the protective immunity assay.

Recent studies proved that antibodies could be important in the development of immunity to leishmaniasis. They abolished the capacity of *L. major* to multiply within macrophages *in vitro* (HANDMAN & HOCKING, 1982), and provided protection against *L. mexicana* infections in mice (ANDERSON *et al.*, 1983).

In our experiments, we demonstrate that a single monoclonal antibody raised against *L. infantum* can prevent the development of one strain of *L. major* and one of *L. mexicana amazonensis*.

The mechanisms conferring possible resistance to *Leishmania* infections have frequently been discussed. This disease was considered to be controlled by cell-mediated immunity (MAUEL & BEHIN, 1981). Recent studies suggested that additional co-operation with humoral antibody could play an important part in effective protection (ARRENDONDO & PEREZ, 1979; ALEXANDER & PHILLIPS, 1980). Furthermore, HOWARD *et al.* (1982) in prophylactic immunization against experimental leishmaniasis noted that antibody response may be crucial in resistance to the infection. They considered that the development of cell-mediated immunity in immunization might not be necessary. At the present time, as did HANDMAN & HOCKING (1982) and ANDERSON *et al.* (1983), we report that antibodies, when used in an *in vitro* or an *in vivo* system, can abolish the development of parasites. The role of the complement (C') in this effect is controversial. It has been proved that antibody + C' caused the lysis of promastigotes (ADLER, 1964). In our own experiments, decomplemented monoclonal antibody ascites were not able

Table I—Protective action of different monoclonal antibodies against infection by *L. major* and *L. mexicana amazonensis*

Antigen	Monoclonal antibody 64B16	Ascitic fluid Sp2/0	Monoclonal anti-HBS	Monoclonal anti-*P. falciparum*
Leishmania major	5/5	0/5	0/5	0/5
Leishmania mexicana amazonensis	5/5	0/5	0/5	0/5

Symbols represent number of protected mice over total number of treated mice.

to prevent *Leishmania* infections. However, ANDERSON *et al.* (1983) using one antibody of isotype IgG_1 suggests that the mechanism of this antibody-mediated protection may be C' independent. Finally, according to HANDMAN & HOCKING (1982), isotype IgG_2b promotes *L. major* killing *in vitro* and isotypes IgG_2a or IgG_3 are cytotoxic to promastigotes in the presence of complement.

These last observations have to be confirmed in several *in vivo* systems to distinguish the mechanism by which these antibodies are protective. Their use may allow the identification of the *Leishmania* antigens inducing effective immunity.

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