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, IgG2b isotype, can prevent the development of two *Leishmania* strains in *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 McHahon-Pratt & David, 1981). They have been used, in particular, to identify the subspecies of *L. brasilienensis* (see McHahon-Pratt et al., 1982). Recent studies have shown that monoclonal antibodies could kill parasites cultured within macrophages in *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 Whim 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 10⁴ 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 × 10⁷ promastigotes administered intraperitoneally four days before cell fusion. Spleen cells were mixed with immunoglobulin-non-secretting, 8-azaguanine-resistant, Sp2/0 myeloma cells (Shulman et al., 1978) and fused with 50% polyethylene glycol, following the technique described by Galfré et al. (1977). Fused cells were selected in a hypoxanthine/azaserine (HA) selective medium.

**Protective immunity assay**

One ascitic fluid (64B16 : IgG2b) which proved to be positive by IIF with *L. infantum* (LEM 497), *L. major* (LEM 129 Montpellier Collection, Ref. MHRO/SU/59/Neal P.), *L. mexicana amazonensis* LV79 (Liverpool Collection), *L. donovani ITMAR 263* (Antwerp Collection) and using amastigotes, isolated in Greece were used as antigens. Mice 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 resealed 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 D'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* LV79 (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 × 10⁸ hybridoma cells into pristane-treated BALB/c mice. Two weeks later ascitic fluids were recovered.

IgG classes were determined by the immunodiffusion method.

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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 IgG1 and the others to IgG2b. Based on observation of strong fluorescent labelling of the \textit{L. major} and \textit{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 \textit{Leishmania} species, with 64B16 abolished their capacity to induce a cutaneous infection. Ascitic fluid containing monoclonal antibodies against \textit{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.

Discussion

As seen in another \textit{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 \textit{Leishmania} including \textit{L. donovani}. This cross protection has been confirmed by these data using \textit{L. major} or \textit{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 \textit{L. major} to multiply within macrophages \textit{in vitro} (HANDMAN & HOCKING, 1982), and provided protection against \textit{L. mexicana} infections in mice (ANDERSON et al., 1983).

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

The mechanisms conferring possible resistance to \textit{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 \textit{in vitro} or an \textit{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

| Antigen                  | Monoclonal antibody 64B16 | Ascitic fluid Sp2.0 | Monoclonal anti-HBS | Monoclonal anti-
|--------------------------|---------------------------|---------------------|----------------------|---------------------|
| \textit{Leishmania major}| 5/5                       | 0/5                 | 0/5                  | 0/5                 |
| \textit{Leishmania mexicana amazonensis} | 5/5                       | 0/5                 | 0/5                  | 0/5                 |

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

Fig. 1: Cutaneous leishmaniasis.

The promastigote strains have been pretreated with an anti-\textit{Leishmania} (A) monoclonal antibody, and a control monoclonal antibody (B).
to prevent Leishmania infections. However, ANDERSON et al. (1983) using one antibody of isotype \( \text{IgG}_1 \) suggests that the mechanism of this antibody-mediated protection may be \( C' \) independent. Finally, according to HANDMAN & HOCKING (1982), isotype \( \text{IgG}_b \) promotes \( L. \) major killing \( \text{in vitro} \) and isotypes \( \text{IgG}_a \) or \( \text{IgG}_3 \) are cytotoxic to promastigotes in the presence of complement.

These last observations have to be confirmed in several \( \text{in vivo} \) 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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