In vitro assessment of anti-*Leishmania* immunity of man acquired with a vaccine

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Summary. Monocytes, obtained from a human volunteer immunized with a *Leishmania infantum*-derived vaccine, when cultured in vitro displayed a strong parasiticidal activity against *L. major* promastigotes. In addition, immune serum conferred leishmanicidal activities to monocytes of normal, unexposed donors, and to murine macrophages.
Key words. Leishmania infantum; Leishmania major; vaccine; immunoprophylaxis.

Protective immunity against leishmaniasis has been achieved with a vaccine made from a 94-67 kD antigenic fraction (LIF 2), isolated from lysates of sonicated *Leishmania infantum* (MCAN/GR/82/LEM 497) promastigotes. This vaccine, when injected i.v. into C57BL/6 mice, induced protection against a challenge with *L. major*[1]. Associated with a water-soluble adjuvant, muramyl dipeptide (MDP), 3 s.c. injections of 15 µg (protein equivalent) of vaccine into BALB/c mice led to long-lasting resistance to Old and New World *Leishmania* species[2]. Dogs, natural hosts of *L. infantum*, were similarly immunized; their immune sera displayed protective activities in a Winn-type assay system[3].

A human volunteer was immunized by three s.c. injections at monthly intervals with 20 µg of LIF 2, associated with 100 µg of murabutide (Laboratoires CHOAY, Paris), a non-pyrogenic MDP derivative[4]. The activities of this immune serum, obtained 2 months after the last injection, were assessed by 3 different assays:

- the *Leishmania* replication inhibition test (LRI),
- the Winn-type assay system,
- the ability to induce monocytes to abolish the infectivity and/or destroy Leishmania parasites.

The first 2 assays were performed as described previously[5, 6]. For the third, peripheral monocytes obtained from subjects without previous exposure to *Leishmania* were isolated from heparinized blood on a Ficoll-Hypaque gradient[7]. Following 2 washes in 25 mM Hepes buffered RPMI 1640, 2×10^5 cells were seeded in the absence of serum into 35-mm diameter plastic Petri dishes (Falcon). After 2 h of incubation (37 °C in 5% CO_2-95% air), nonadherent cells were removed by vigorous rinsing with cold medium. The adherent cells were further incubated for 48 h in Hepes-RPMI medium supplemented with 20% of heat-inactivated fetal calf serum (FCS). Phenotyping of the adherent cells revealed the following distribution: monocytes = 81% ± 4.3 (My 4 antibody, Coultronics France); lymphocytes CD4 = 10.5% ± 1.6 (IOT4, Immunotech, Lumigny, France) and lymphocytes CD8 = 1.4% ± 0.2 (OKT8, Immunotech, Lumigny). After 2 washes, the cell monolayers were incubated for 30 min in the presence of 6% human serum, obtained from unexposed donors, the immunized volunteer, and of 3 patients with an active cutaneous *L. major* infection. After removal of the incubation mixture, 1.5 ml of RPMI-FCS 20% containing 1×10^5 *L. major* (zymodeme LON 1) promastigotes was added to the culture dishes.

Twenty-four, and 36 h after infection, the cells were detached with the help of a rubber policeman, washed in buffered RPMI, examined on Giemsa-stained smears, or transferred into 45-ml culture flasks containing 7 ml RPMI-FCS and placed at 24 °C so that the transformation of amastigotes into promastigotes could be observed.

The results of the experiments are shown in the table.

Human serum, obtained from unexposed donors, and the volunteer's serum before immunization, used to treat promastigotes in the LRI and Winn-type assays, did not diminish the parasite infectivity in these 2 tests relying on BALB/c mice as hosts. These sera prevented neither the uptake of promastigotes by human monocytes and their further intracellular growth into amastigotes (fig. 1), nor the ability of the latter to transform into promastigotes when placed in RPMI + FCS at 24 °C. By contrast, the post-immunization serum inhibited the growth and viability of *L. major* parasites in the LRI and Winn-type assays; monocytes treated with the immune serum phagocytosed promastigotes normally (fig. 2), but the resulting amastigotes replicated inefficiently and after 48 h of culture, only a limited number of them could be seen (fig. 3); furthermore, when transferred at 24 °C, these amastigotes were unable to complete their cycle, and to differentiate into promastigotes. A further serum sample, drawn 8 months after immunization, displayed similar properties. A complete neutralization was observed with

Source of serum	LRI test		Winn-type assay[c]	Monocyte parasiticidal activities	
	% of parasitized murine cells[a]	No. of parasites /ml (24 °C culture)[b]		% of parasitized monocytes[d]	No. of parasites /ml (24 °C culture)[e]
Normal human serum	55–60	$> 1 \cdot 10^6$	Positive	77 ± 7	$> 1 \cdot 10^6$
Pre-vaccination serum	56	$> 1 \cdot 10^6$	Positive	73	$> 1 \cdot 10^6$
Immune post-vaccination serum, + 2 months	3	0	Negative	2.2 ± 1.7	0
+ 8 months	ND[f]	ND	ND	3.1 ± 2.3	0
L. major-infected patient	63	$> 1 \cdot 10^6$	Positive	68 ± 10	$> 1 \cdot 10^6$.

[a] Percentage of parasitized (amastigote) peritoneal cells (PEC) observed 12–15 days after i.p. injection of $1 \cdot 10^4$ *L. major* promastigotes, pre-treated with one type of serum, + $2 \cdot 10^4$ murine sarcoma TG 180 cells.
[b] Number of promastigotes detected per ml of culture medium inoculated with the murine PEC, and cultured at 24 °C for a period of 12 days.
[c] One thousand *L. major* promastigotes, incubated for 30 min at 37 °C with one type of serum, were injected into the base of the tail of 8-week-old female BALB/c mice.
[d] Percentage of parasitized human monocytes observed after 48 h of culture at 37 °C, 5% CO_2/95% air. The results are the mean ± SEM of 8 normal human sera, of 2 post-vaccination sera tested 4 and 2 times respectively, and of 3 patients. All tests undertaken in duplicate.
[e] Number of promastigotes found per ml of medium after a 12-day culture at 24 °C following inoculation of 1.000 ± 200 monocytes.
[f] ND: not done.

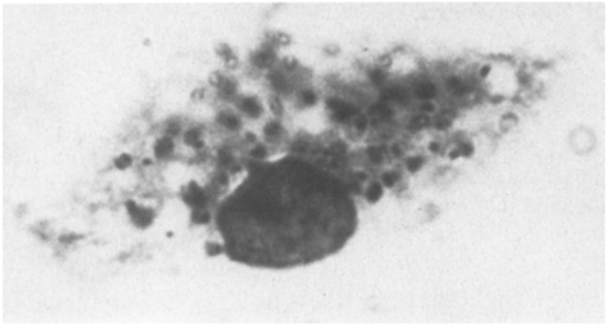

Figure 1. Monocyte from a non-exposed donor, treated with normal human serum, examined 36 h after infection with *L. major* promastigotes (Giemsa stained × 800).

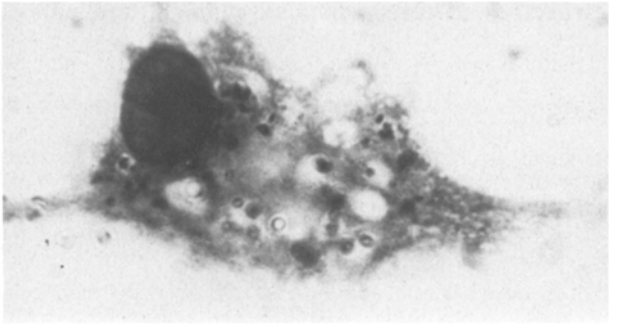

Figure. 2. Monocyte from a non-exposed donor, treated prior to *L. major* promastigotes infection with a human immune serum, examined after 36 h of co-culture (monocytes + parasites), (Giemsa stained × 800).

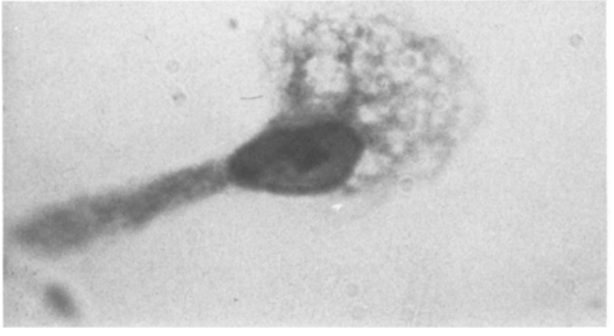

Figure. 3. As figure 2, but examined 24 h after the onset of infection (Giemsa stained × 800).

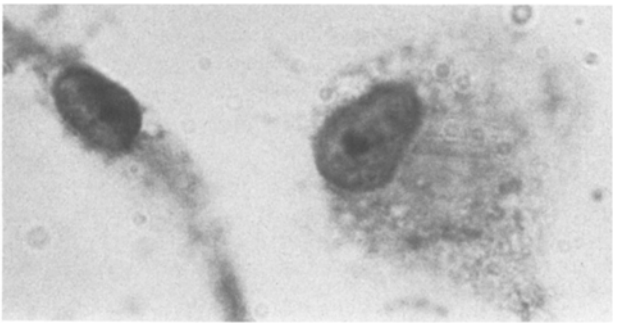

Figure. 4. Monocytes from an immune donor, pre-treated with autologous serum, photographed 36 h after infection with *L. major* promastigotes (Giemsa stained × 800).

the vaccinated person's monocytes treated with autologous immune serum: promastigotes did temporarily attach to the phagocyte surface, but failed to enter the cell and were unable to survive (fig. 4).

Thus, serum and/or monocytes of a human immunized with an anti-*Leishmania* vaccine, express a consistent and pronounced parasiticidal effect in 3 different assay systems. Similar results were observed in the experimental BALB/c mouse model, in which these leishmanicidal activities coincide with resistance to an in vivo challenge; besides that, an increased level of gamma interferon was found, 24 h after infection, in the supernatants of cultures of peritoneal macrophages obtained from mice immunized with LIF 2 (unpublished results). The assays carried out with monocytes are fairly easy and inexpensive to perform, and possess, in our view, a real potential in the evaluation of immunoprophylaxis programs.

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