DEVELOPMENT OF EOSINOPHILIA IN DOGS INTRADERMICALLY INOCULATED WITH SAND FLY SALIVA AND *LEISHMANIA (LEISHMANIA) CHAGASI* STATIONARY-PHASE PROMASTIGOTES

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Salivary gland lysates of the sand fly Lutzomia longipalpis *have been shown to enhance the infectivity of* Leishmania *in mice. As shown herein, the simultaneous inoculation of* Leishmania chagasi *stationary-phase promastigotes and* L. longipalpis *salivary gland lysate by the intradermal route in a group of mongrel dogs induced a statistically significant eosinophilia, in relation to dogs inoculated with* Leishmania *or with salivary gland lysate only. These dogs had no evidence of infection, in spite of the high infectivity of the promastigotes when inoculated by the intravenous route.*

Key words: *Leishmania chagasi* – *Lutzomia longipalpis* – sand fly saliva – eosinophilia – infectivity – experimental infection – dogs

The infection of syngeneic mice by low numbers of *Leishmania major* or *L. amazonensis* was shown to be enhanced by sand fly (*Lutzomia longipalpis* or *Phlebotomus papatasi*) saliva (Titus & Ribeiro, 1988; Theodos et al., 1991), a phenomenon which was attributed to local haemodinamic effects of the saliva (reviewed by Ribeiro, 1989). This enhancing effect of sand fly saliva on *Leishmania* infectivity could play a role in the development of human leishmaniases in endemic areas, provided it also happened in human beings and/or in animal reservoirs of the disease.

The dog is the main reservoir of *Leishmania chagasi*, a protozoan that causes visceral leishmaniasis in the New World (Deane & Deane, 1955). When inoculated experimentally with *L. chagasi*, however, dogs usually develop mild infections, with little or no development of pathological alterations (Cunha, 1938; Oliveira, 1988). The high frequency of heavily infected, sick dogs in endemic areas suggests that other factor(s), in addition to parasite entry in the skin, could play a role in the development of the infection. A study

designed to identify possible effects of the simultaneous inoculation of sand fly salivary gland extracts and *L. chagasi* promastigotes in dogs is described in this paper.

MATERIALS AND METHODS

Animal – Three-month-old mongrel dogs, weighing 6.5 to 9 kg, with no clinical signs of disease and normal haemograms, were used. The animals were treated for gastro-intestinal parasites (mebendazol, 200 mg/day for three days) two months prior to this study.

Parasites – *L. chagasi* (BHTO 02) stationary-phase promastigotes were obtained from LIT (liver infusion-tryptose, Difco Laboratories, Detroit, USA) cultures at 26 °C (Sadigurski & Brodskin, 1986). These promastigotes were shown to be highly infective when inoculated intravenously in dogs and hamsters. The *Leishmania* species was confirmed by the pattern of reactivity of the promastigotes against a panel of species-specific monoclonal antibodies (Grimaldi Jr. et al., 1987).

Sand fly salivary gland extract – This was prepared in accordance with Titus & Ribeiro (1988). Briefly, *L. longipalpis* salivary glands were dissected, placed into distilled water containing 0.1% bovine serum albumin (Sigma Chemical Co., St. Louis, USA) and frozen.

After storage at −20°, the extract was brought to isotonicity with 1.5 M phosphate-buffered saline pH 7.2.

Leishmania-antibody serum levels − These were determined in an ELISA utilizing microtitre plate wells sensitized with *L. chagasi* promastigote extract, as described elsewhere (Voller et al., 1976).

Bone-marrow cultures − Bone marrow aspirates were seeded in biphasic medium, consisting of brain-heart infusion-agar containing 20% normal rabbit blood as solid-phase and LIT with 20% fetal calf serum (Sigma Chemical Co., St. Louis, USA) as liquid phase (Marin, 1982). These were incubated at 26 °C for two months and examined microscopically for *Leishmania* promastigotes every week. These culture conditions could readily reveal the presence of *Leishmania* in the bone-marrow of dogs intravenously inoculated with the parasites used in the present studies.

Infection and follow-up of dogs − Five dogs were inoculated with a total of 2×10^5 viable stationary-phase promastigotes each, divided in three 20 μl intradermal injections in the ear, mixed with extract of *L. longipalpis* salivary gland. The amount of extract inoculated per animal corresponded to a whole gland (two acini). Control dogs received identical numbers of *L. chagasi* without salivary gland extract (four animals) or were inoculated with saline only (four animals). In addition, a third control group consisted of three dogs that received the same amount of salivary gland extract without parasites.

The animals were followed up for five and a half months with (1) weekly physical examinations, (2) monthly determinations of *Leishmania*-antibody levels and hemograms, and (3) bone marrow examinations for the presence of *Leishmania,* by light microscopy and *in vitro* cultures, one month and five months after infection. After this period of study, all dogs were inoculated intravenously with 10^8 *L. chagasi* promastigotes, obtained as described above.

RESULTS

No clinical, parasitological or sorological signs of infection were observed in any of the dogs inoculated intradermically with *L. chagasi,* with the exception of a dog that re-ceived *Leishmania* in saline, which had a positive ELISA result (i.e., above the mean of the results obtained with the sera of 17 normal dogs plus three standard deviations) five months after infection.

All the dogs studied were readly infected by the intravenous inoculation of 10^8 *L. chagasi* promastigotes, five and a half months after the intradermal inoculation of the parasites, having all positive bone-marrow cultures and positive sorology.

A statistically higher blood eosinophilia could be observed in the group which received sand fly saliva, when compared with the other two groups, 30 days (p ≤ 0.01, Kruskal-Wallis' test) and 60 (p ≤ 0.05, Kruskal-Wallis' test) days after the inoculation of parasites (Fig.). Injection of the same amount of saliva, without parasites, in three normal animals had no effect on eosinophil counts (data not shown).
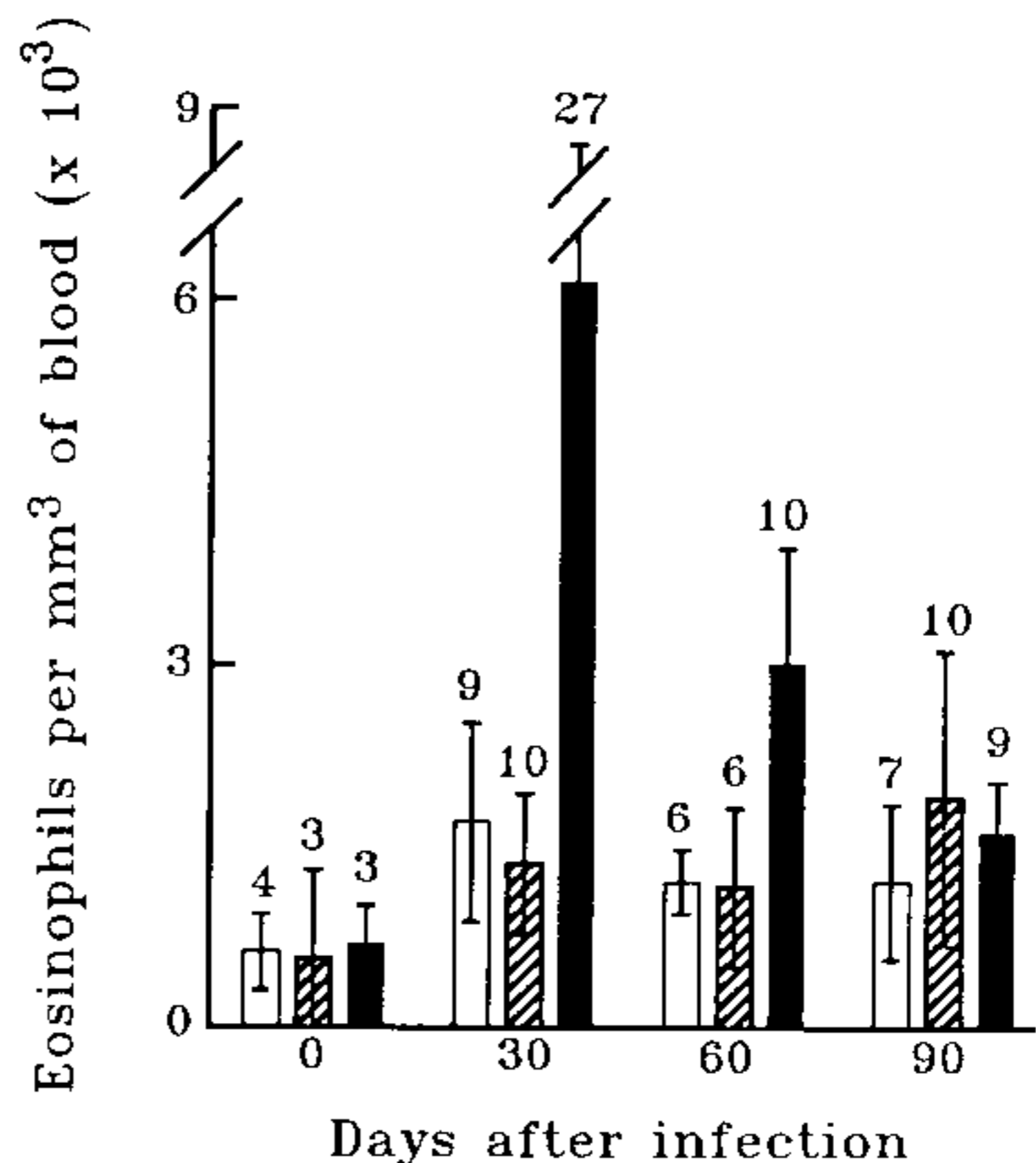
Eosinophilia in dogs inoculated with a mixture of *Leishmania chagasi* stationary-phase promastigotes and *Lutzomia longipalpis* salivary gland lysate. Columns represent the mean number of eosinophils per mm^3 of blood in groups of dogs inoculated with saline (▢; n = 4), with *Leishmania* promastigotes and saline (▨; n = 5) and with promastigotes and salivary gland lysate (■; n = 4). Vertical bars represent the standard deviations of means. Numbers above each column refer to the percentages of eosinophils in relation to the total numbers of leukocytes in the blood.

DISCUSSION

The intradermal inoculation of potentially infective culture-derived *L. chagasi* stationary-phase promastigotes failed to infect well-fed mongrel dogs, whether mixed or not with sand fly salivary gland extract, as described herein. Since the intradermal route is the natural via of transmission of leishmaniases to mamals, this finding suggests that others factors, absent in the present study, may be involved in the development of the infection. Possible differences in the infectivity of insect-derived promastigotes in relation to culture-derived stationary-phase promastigotes, as used in this study, could explain the present results.

Salivary gland extracts, prepared exactly as in the present experiment, were shown to enhance *L. amazonensis* and *L. major* infection in syngeneic mice (Titus & Ribeiro, 1988; Theodos et al., 1991). This apparent discrepancy with the present results in dogs can be obviously explained by differences in the animal and/or *Leishmania* species utilized. In fact, syngeneic strains of mice differ in their susceptibility to the enhancing effect of sand fly saliva on *Leishmania* infection (Theodos et al., 1991).

One unexpected finding in the work described herein was the high eosinophilia observed in the group of dogs that received *Leishmania* associated with salivary gland extract, in relation to the other groups of dogs. Whether this phenomenon could be an indication that sand fly saliva (possibly associated with live *Leishmania* or with *Leishmania* products) would affect the canine immune system in a way that, in the contingency of other factors, could facilitate the infection, is open to speculation. In fact, eosinophils are well represented in the local cellular response to *Leishmania* in susceptible mice (Pompeu et al., 1990) and T-cell lines producing IL-5, a potent stimulator of eosinophil growth and differentiation (Dent et al., 1990), were shown to exacerbate murine experimental leishmaniasis (Scott et al., 1988).

ACKNOWLEDGEMENTS

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