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INTERLEUKIN-2 PRODUCTION DURING MURINE INFECTION BY *LEISHMANIA MEXICANA AMAZONENSIS*

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Highly susceptible BALB/c mice, resistant C57B1/6 and their F_1 progeny (BDF_1) were infected subcutaneously in the foot pad with Leishmania mexicana amazonensis. *At various times after infection, spleen or draining popliteal lymph node cells were assayed for their capacity to generate Interleukin-2 (I1-2) by Concanavalin A (ConA) stimulation. In both BALB/c and C57B1/6 strains there was a transient increase in their capacity to produce I1-2, from the 3rd to the 10th week post-infection. Return to pre-infection levels ocurred between 13th to 16th week post-infection in all three strains. BALB/c mice always produced higher titers of I1-2 than C57B1/6, but such differences were statistically significant only at 3 and 10 weeks post-infection. BDF_1 mice had titers similar to those observed in BALB/c mice. I1-2 production by ConA-stimulated lymph node cells was lower as compared to the spleen, but with a similar pattern among the three mice strains. Our data show that susceptibility to infection by* L. mexicana amazonensis *is not associated with deficient ConA-stimulated I1-2 production.*

Key words: cutaneous leishmaniasis – Interleukin-2 – *Leishmania* (immunology) – murine leishmaniasis – lymphokines

Several animal models have been developed for producing the different aspects of human cutaneous leishmaniasis in the Americas (Coelho & Coutinho-Abath, 1965, Mata et al., 1968; Perez, Labrador & Torrealba, 1979; Grimaldi, Moriearty & Hoff, 1980; Barral et al., 1983; Andrade et al., 1984). We have shown (Barral et al., 1983) that C57B1/6 mice infected with *L. mexicana amazonensis* develop a late metastatic ulcerative disease resembling mucocutaneous leishmaniasis, in sharp contrast to the progressive and diffuse nodular pattern observed in BALB/c mice. The immune status of the host is thought to be contributory to disease manifestation caused by *L. mexicana* (Arredondo & Perez, 1979; Barral et al., 1983) as well as *L. tropica* (Howard, Hale & Liew, 1980; Scott & Farrell, 1981). In all cases a severe T cell-mediated immunoderegulation was associated with nonhealing leishmanial infections in BALB/c mice.

Interleukin-2 (I1-2) appears to be fundamental in the regulation of T-cell-dependent immune responses (Smith, 1980; Watson & Mochizuki, 1980; Ruscetti, 1984). It is a soluble factor produced by T cells in the presence of Interleukin-1 and mitogen or antigen, and is an amplifier of the effector phase of the immune response by sustaining proliferation of helper and effector T lymphocytes. The central role of I1-2 in regulating the immune response prompted us to investigate its production by resistant or susceptible mice during the course of infection with *L. mexicana amazonensis*.

MATERIAL AND METHODS

Mice – BALB/c, C57B1/6 and (C57B1/6 x BALB/c)F_1 (BDF_1), from the FIOCRUZ colony, were used at 6-8 weeks of age. The animals were maintained with balanced mouse ration and water *ad libitum*.

Parasite – The Josefa strain of *L. mexicana amazonensis* (MHOM/BR/76/Josefa), provided by Dr. Philip Marsden (University of Brasília), was typed by isoenzymic pattern and monoclonal antibodies panel (courtesy of Dr. Gabriel Grimaldi, FIOCRUZ, Rio de Janeiro), as well as kDNA analysis (courtesy of Dr. Peter Jackson; WRAIR, Washington, D.C.). The parasite is maintained in our laboratory by serial subcutaneous passage in BALB/c mice.

Infection – Promastigotes for infection were cultivated in liver infusion tryptose medium supplemented with 10% fetal calf serum for 7-10 days at 25°C. Mice were infected subcutaneously into the left hind foot-pad, with $5x10^6$ viable promastigotes, at a volume of 0.025ml. Infection was followed by serial measurements of foot-pad thickness.

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In vitro **stimulation of I1-2 production** – Spleen cells from three normal or infected mice, obtained at 3, 10, 13 and 16 weeks post-infection, were washed three times with RPMI 1640 tissue culture medium (GIBCO, Grand Island, N.Y.) supplemented with 10^{-5} M 2-mercaptoethanol, 2mM L-Glutamine, 50U/ml Penicillin, 50μg/ml Streptomycin (complete medium) plus 5% fetal calf serum (FCS). One ml aliquots of cell suspension, at $5x10^6$ cells/ml, were incubated with or without ConA (Sigma, St. Louis, MO), at a final concentration of 5μg/ml, at 37°C in a humid atmosphere with 5% CO_2. After 24 hr of incubation cell-free supernatants were collected and stored at -20°C until tested.

Assay of I1-2 activity – Performed as described by Lelchuk, Rose & Playfair (1984), using ConA blasts as target cells.

a) **T-cell blast preparation**. Spleen cells from normal mice, at a concentration of $1x10^7$ cells/ml, were incubated in complete medium plus 5% FCS, with 3μg ConA per ml. After 48hr at 37°C in an atmosphere of 5% CO_2, the cells were washed twice in RPMI containing 20mg/ml of alpha-methyl-D-mannopiranoside (Calbiochem-Bhering Corp., La Jolla, CA), and twice in plain RPMI. Blast cells were counted and adjusted to a concentration of $2x10^5$ cells/ml complete medium with 20% FCS.

b) **Measurement of I1-2 activity**. Blasts ($2x10^4$ cells/well) were recultivated for 72hr, in flat bottomed microtiter plates, in triplicate serial dilutions (1/2 to 1/16) of supernatants to be tested for their I1-2 activities. Four hours before harvesting, cultures were pulsed with 1μCi ^{3}H-thymidine per well. In each experiment the proliferation induced by a supernatant from normal mice spleen cells stimulated with ConA, was used as a reference for I1-2 activity. The same batch was stored in aliquots at -20°C and used for all experiments.

Units refer to the reciprocal of the dilution causing stimulation above the level represented by mean plus three standard deviations of counts obtained in 24 wells with unstimulated blasts.

Assay for delayed-type hypersensitivity – Performed as described by Barral et al. (1983). Briefly, animals were injected with 50μg protein of leishmanial antigen solution into the ventral face of the right hind foot-pad. Results are presented as thickness value (in millimeters $x10^{-2}$) measured 24hr after antigen injection minus the measurement of foot-pad thickness before injection.

Statistical analysis – Comparisons among the three groups were performed by one-way analysis of variance. Tests of significance of differences between the means of each two groups were made by independent samples Student's *t*-test.

RESULTS

I1-2 production – Spleen cells from normal BALB/c or BDF_1 mice produced higher (but not statistically significant) titers of I1-2, upon stimulation by ConA, than normal C57B1/6 (Table I). In both BALB/c and C57B1/6 mice infection by *L. mexicana amazonensis* determined an early rise in I1-2 production, from 3 to 10 weeks post-infection. In both time points, differences were statistically significant ($p < 0.05$, with F ratios of 7.80 and 7.00 respectively). From 13th to 16th week there was a fall in I1-2 titers, with a tendency to reach pre-infection levels for each strain. During the whole period of observation I1-2 production by the susceptible strain (BALB/c) was always similar (not statistically significant) to BDF_1 mice, and higher than in C57B1/6, the resistant mouse strain. I1-2 production by ConA-stimulated lymphnode cells had a similar pattern to that observed with spleen cells, but with lower titers in all three strains.

DTH responses – Table II shows that all three mouse strains developed a transient low level DTH response at three weeks post-infection. Such reactivity is suppressed later on in BALB/c mice (10 and 16 weeks post-infection), at a time when I1-2 production by these animals are at normal or increased levels. On the other hand, C57B1/6 animals, at this period, showed a stronger and sustained DTH responsiveness with lower levels of I1-2 production. BDF_1 animals remained with low grade positive responses during the entire period of observation post-infection.

TABLE I

Production of interleukin-2 by ConA-stimulated spleen cells from normal mice or after infection by *Leishmania mexicana amazonensis*

Mouse strains / Weeks Post-Inf.	BALB/c*	C57B1/6*	BDF_1*
0	10.7 ± 2.7	3.3 ± 0.7	12.0 ± 4
3	16.0 ± 0	6.7 ± 1.3	12.7 ± 0.7
10	16.0 ± 0	8.0 ± 0	13.3 ± 2.7
13	11.0 ± 3	6.7 ± 1.3	16 ± 0
16	12.0 ± 4	2.0 ± 0	8 ± 0

* Mean ± S.E.M. of units of I1–2 per ml.

TABLE II

Delayed type hypersensitivity reactions to *Leishmania* antigen in normal mice or after infection by *L. mexicana amazonensis.*

Mouse strains / Weeks Post-Inf.	BALB/c*	C57B1/6*	BDF_1*
0	2.5 ± 4.2	2.3 ± 0.7	6.1 ± 2.5
3	18.7 ± 1.3	17.5 ± 1.2	17.9 ± 1.5
10	8.3 ± 2.5	34.2 ± 2.3	14.1 ± 1.2
16	9.4 ± 3.7	43.6 ± 2.6	16.4 ± 2.6

* Mean ± S.E.M. of measurements at 24hr minus pre-injection measurements; in mm x 10^{-2}.

DISCUSSION

This report shows that ConA-stimulated I1-2 production is not impaired during the course of *L. mexicana amazonensis* infection in highly susceptible BALB/c mice. Indeed the I1-2 production is higher than in resistant (C57B1/6 mice or in animals of intermediate susceptibility as C57B1/6 x BALB/c) F_1 mice.

Interleukin-2 has been reported to induce thymocyte proliferation after mitogenic stimulation (Shaw et al., 1978), and has also been shown to support antigen-specific activated helper T cells in long-term culture (Watson et al., 1979). Since T-cell mediated mechanisms are important for recovery and resistance in leishmaniasis a defect in I1-2 production has been suspected in cutaneous leishmaniasis (Mitchell, 1984). I1-2 has also been implicated in the induction of gamma-interferon (Weigent, Stanton & Johnson, 1983), and this lymphokine seems to be important in the destruction of *Leishmania* by macrophages (Murray, Rubin & Rothermel, 1983). Despite all these suggestions there was no deffective I1-2 production in susceptible mice. Antigen-driven I1-2 production in mice infected by *Leishmania* was not detected by us with the methods used in this study, what is similar to the observations of other investigators (J. Louis, personal communication). We cannot exclude that a selective deffect, only evident with antigen stimulation, could be demonstrated in these animals. This is unlikely however since ConA stimulation was capable of detecting deficiency of I1-2 production in other parasitic diseases, as in the infection by *Mycobacterium lepraemunium* (Hoffenbach, Lagrange & Bach, 1983), by *Trypanosoma cruzi* (Hasel-Bellan et al., 1983), by *Plasmodia* (Lelchuk, Rose & Playfair, 1984) and even by *L. donovani* (Reiner & Finke, 1983).

The progressive course of cutaneous leishmaniasis in BALB/c mice with normal I1-2 production suggest a normal function of T-cell immune mechanism. Recently Titus et al. (1984) demonstrated exacerbation of cutaneous leishmaniasis in BALB/c mice by adoptive transfer of parasite-specific helper T cell populations. They suggested that such cells were attracting permissive monocytes to the lesions, providing an increased number of host cells for the multiplication of parasites. Therefore it is possible that there is no impairment of T cell function in

highly susceptible BALB/c mice. Along this line, the same group has demonstrated a curative effect on cutaneous leishmaniasis in BALB/c mice by administering anti-L3T4 monoclonal antibodies (Titus et al., 1985). Since anti-L3T4 antibodies inhibit T cell activation by antigen, it seems that L3T4 antigen-specific T cells may participate in the development of lesions by inducing an influx of monocytes into the lesion. Our results also suggest that disease in genetically-susceptible mice during cutaneous leishmaniasis, is a result of an excess of T-cell function.

RESUMO

Camundongos BALB/c (susceptíveis), C57B1/6 (resistentes) ou sua geração F_1 (BDF_1) foram infectados subcutaneamente na pata traseira com *Leishmania mexicana amazonensis.* Avaliamos, em diferentes períodos de infecção, a capacidade de células do baço ou de linfonodo poplíteo, de produzir Interleucina-2 (I1-2) em resposta à estimulação por Conconavalina A (ConA). Nos camundongos BALB/c e C57B1/6 observamos, da 3ª à 10ª semana pós-infecção, uma elevação transitória da capacidade de produzir I1-2. Da 13ª à 16ª semana pós-infecção houve um retorno dos níveis de produção pré-infecção. Camundongos BALB/c produziram títulos mais elevados de I1-2 que os C57B1/6, mas tais diferenças só foram estatisticamente significantes na 3ª e 10ª semanas pós-infecção. Camundongos BDF_1 apresentaram títulos semelhantes aos dos BALB/c. Os níveis de I1-2 (estimulada por ConA) produzidos por células do linfonodo foram mais baixos que os do baço, porém com padrão semelhante. Nossos dados mostram que a susceptibilidade à infecção por *L. mexicana amazonensis* não está associada a um defeito de produção de I1-2, estimulada por ConA.

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