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The role of natural killer cells in the early period of infection in murine cutaneous leishmaniasis

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Abstract

In order to study the role of natural killer (NK) cells during the early period of *Leishmania* infection, BALB/c mice were selectively and permanently depleted of NK cells by injection with ^{90}Sr and subsequently infected with *Leishmania (Leishmania) amazonensis* (HSJD-1 strain). ^{90}Sr is known to selectively deplete NK cells, leaving an intact T- and B-cell compartment and preserving the ability to produce both interferon alpha and IL-2. This method of depletion has advantages when compared with depletion using anti-NK cell monoclonal antibodies because the effect is permanent and neither activates complement nor provokes massive cell death. In the present study, after one month of treatment with ^{90}Sr, the depletion of NK cells was shown by a more than ten-fold reduction in the cytotoxic activity of these cells: 2 x 10^6 spleen cells from NK-depleted animals were required to reach the same specific lysis of target cells effected by 0.15 x 10^6 spleen cells from normal control animals. The histopathology of the skin lesion at 7 days after *Leishmania* infection showed more parasites in the NK cell-depleted group. This observation further strengthens a direct role of NK cells during the early period of *Leishmania* infection.

Key words
- Cutaneous leishmaniasis
- NK cells
- Strontium 90
- *Leishmania (Leishmania) amazonensis*

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Both innate and specific elements of the immune system contribute to the control or the progression of leishmaniasis. At the beginning of the infection, innate elements have been shown to have an important role in influencing the outcome of the disease. Among them, complement has been shown to contribute to the evasion of the parasite and for visceral dissemination in hamsters infected with *Leishmania (Leishmania) chagasi* (1). It has also been shown that nonimmune natural killer (NK) cells are important in *Leishmania* infection as a source of IFNγ with the potential to trigger the Th-1 type of immune response in cutaneous leishmaniasis (2,3). In addition, using the mutant beige mice with low NK activity, the direct importance of NK cells in the development of visceral leishmaniasis has been shown (4). Recently, in mice with an intermittent suppression or depletion of NK cells by anti-asialo GM1 or anti-NK1.1 monoclonal antibodies resulted in an increased susceptibility of mice to *Leishmania major* (5).

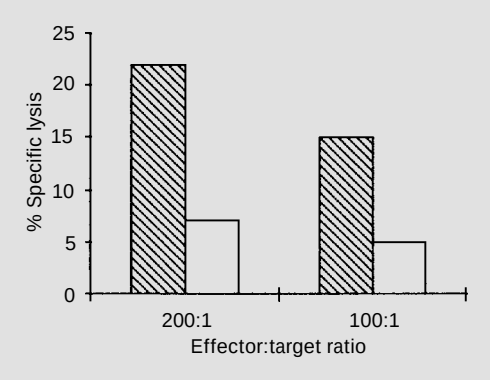

Figure 1 - *In vitro* spleen cell NK activity from normal (striped column) or NK-depleted (open column) mice. Spleen cells from control or ^{90}Sr-treated mice (0.6 µCi/g body weight intraperitoneal injection) were tested in a 4 H [^{51}Cr]-release microcytotoxicity assay at the indicated effector to target ratio using YAC-1 as described in Ref. 6. Specific lysis = ((release (cpm) with effector cells - release in medium alone)/(release in distilled water - release in medium alone)) x 100. Data are from two separate experiments yielding similar results. Data represent the mean of 5 animals in each group and the SD was less than 5% of the mean.

In the present study we have used ^{90}Sr to deplete NK cells. This treatment is well established and provides an intense local irradiation of the bone marrow leading to severe bone marrow aplasia with concomitant extramedullar myelopoiesis in the spleen (6,7). The treatment has been shown to lead to a severe and permanent depletion of NK cell activity in the spleen, in the lymph nodes and in the periphery without any noticeable alteration in the T- or B-cell compartment or in the capacity to rapidly produce IL-2 or interferon alpha upon stimulation (6). Here we studied the effect of NK cell depletion by ^{90}Sr on the course of *Leishmania (Leishmania) amazonensis* infection.

Ten newly weaned BALB/c mice were depleted of NK cells by intraperitoneal injection of ^{90}Sr (0.6 µCi/g body weight) as previously described (6). After 30 days (i.e., after elimination of free ^{90}Sr) they were infected subcutaneously in the hind footpad with 5 x 10^7 stationary phase promastigotes of *Leishmania (L.) amazonensis* (HSJD-1 strain) characterized by Prof. J.J. Shaw (Instituto Evandro Chagas, Brazil) according to the reactivity to monoclonal antibodies specific for *L. (L.) amazonensis*, *L. (V.) panamensis* and for the subgenus *Viannia,* and also by Dr. S.R. Uliana (Department of Parasitology, ICB, University of São Paulo, Brazil) according to the reactivity to subunit ribosomal DNA probes for *L. amazonensis* and the subgenus *Viannia* (8). Samples were taken to evaluate NK activity of spleen cells at the time of inoculation by a ^{51}Cr release cytotoxic assay of YAC-1 target cells. The level of parasite growth was verified by histopathological analysis of the skin lesion at seven days of infection.

The severe depletion of NK activity was confirmed by the lytic activity against YAC-1 cells in ^{90}Sr-treated animals (Figure 1). The calculated number of cells required in control animals to obtain the same level of lysis of target cells as found in NK-depleted animals (i.e., 7% specific lysis) was shown to be 0.15 x 10^6 cells versus 2 x 10^6 spleen cells in NK-depleted animals. This demonstrated that the NK cell activity of ^{90}Sr-

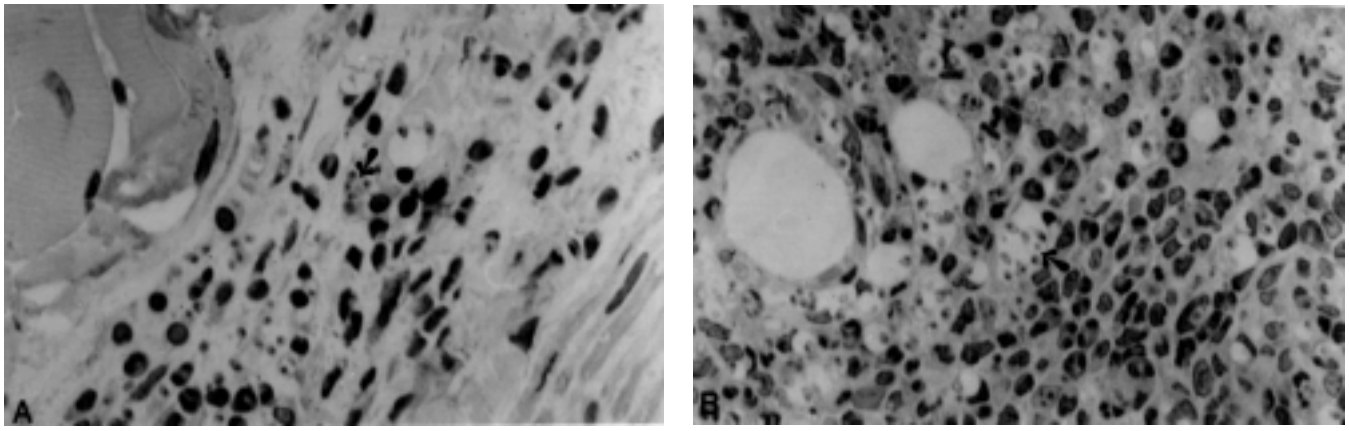

Figure 2 - Histopathology of skin lesions from control or NK-depleted mice infected with *Leishmania* (*L.*) *amazonensis.* Hematoxylin and eosin. Magnification, 40X. *A*, Mixed inflammatory infiltrate with few parasites is shown in control BALB/c mice. *B*, Mixed inflammatory infiltrate with more parasites is shown in NK-depleted BALB/c mice.

treated mice was reduced more than ten times. As shown in Figure 2A and B, seven days after infection more parasites were observed in the skin lesion in ^{90}Sr-treated mice. The inflammatory infiltrate characterized mainly by mononuclear cells with few polymorphonuclear neutrophils was similar in both groups.

Previous studies have similarly indicated a role for NK cells in leishmaniasis; however, our study clarified several important points. Several color mutants, including the beige mutant, have been shown to have reduced NK cells compared with their wild type counterpart (9). These mice also have severe alterations in the lysosomal compartment which affect macrophage and neutrophil functions. Therefore, there is uncertainty about the data obtained in beige mice and how the defects in phagocytes could interfere with the susceptibility of these mutant mice to *Leishmania* infection. The use of anti-asialo GM1 or NK1.1 antibodies leads to an intermittent and short-lived depletion of NK cells (5,10) and furthermore, as is the case for any antibody used to deplete cell components *in vivo*, to a rapid activation of the complement that is known to be important in the initial phase of infection (1). Finally, it is highly likely that the rapid elimination of a sizable portion of the lymphocyte pool by the antibody can cause secondary effects due to complement activity and massive cell death. The ^{90}Sr-treated mice have an advantage since they do not present any apparent change in monocyte function, as shown by the ability to mount a normal T- and macrophage-dependent response to Con A detected by IL-2 production (6).

In a system with a selective depletion of NK cells along with an intact T- and B-cell compartment and with preserved ability to produce both interferon alpha and IL-2, we have shown increased *Leishmania* growth in the skin lesion. We conclude that the present data further support a direct role of NK cells in the early period of *Leishmania* infection.

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