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Cutaneous Leishmaniasis in Mast Cell-Deficient W/W^v Mice

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Genetically mast cell-deficient W/W^v mice infected with *Leishmania amazonensis* showed progressive development of ulcerative skin lesions. However, no significant differences between the W/W^v mice and the normal littermates with respect to size of the lesions, anti-*Leishmania* immunoglobulin E antibody, and the number of eosinophils accumulated in the lesions were observed.

The skin functions as a physiomechanical and immunological barrier to infection by various pathogens, including parasitic organisms (15). Protozoan parasites of the genus *Leishmania* are agents responsible for simple cutaneous, mucocutaneous, and visceral leishmaniasis, depending on parasite properties and the host's immunity (1, 10, 18, 20). A histopathological feature of cutaneous leishmaniasis is a granulomatous inflammation in the dermis and subcutaneous tissue (18). Although immunological interactions between parasitized macrophages and infiltrated T lymphocytes appear to be important for the control and development of lesions (1, 6, 10, 19, 20), resident skin cell populations such as natural killer cells, γδ T cells, and mast cells can be considered to contribute to the formation of the leishmanial granuloma. Interestingly, the outcome of cutaneous leishmaniasis is often associated with increased serum immunoglobulin E (IgE) antibody levels (6, 11) and eosinophil infiltration in the lesion (5, 14). Mast cells are present in quite high numbers in the skin. Mast cells mediate immediate hypersensitivity reactions through binding of antigens to IgE molecules on the cell surface, resulting in the release of a variety of chemical mediators, including the eosinophil chemotactic factor, from the cells (8). Furthermore, mast cells could produce interleukin-4 (IL-4) (3, 17), which appears to be required for establishment of *Leishmania major* infections in susceptible mice (4).

In the present study, we examined the possible role of skin mast cells in cutaneous leishmaniasis using W/W^v mice, which are genetically deficient in mast cells but have T cells, B cells, and natural killer cells with normal immunological functions (8). We compared immunopathological responses between W/W^v and normal +/+ mice after infection with *Leishmania amazonensis*.

Promastigotes of *L. amazonensis* (MPRO/BR/72/M1845) were transformed from amastigotes of the skin lesions of infected BALB/c mice. A tissue sample of the lesions was cultured in medium 199 containing 10% heat-inactivated fetal calf serum and 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid). The culture was expanded for a week, and promastigotes at the late log phase were harvested and washed with phosphate-buffered saline. The rumps of female 6-week-old mast cell-deficient (WB × C57BL/6)F_1 W/W^v mice and their normal +/+ littermates (Japan SLC Inc., Hamamatsu, Japan) were shaved and injected with 10^7 promastigotes in a volume of 50 μl. Lesion diameters were measured by a dial caliper at weekly intervals.

Total serum IgE levels in infected mice were determined by enzyme-linked immunosorbent assay with two anti-mouse IgE monoclonal antibodies as described previously (7). Anti-*Leishmania* IgE antibody in sera was detected by passive cutaneous anaphylaxis reactions in Wistar rats (21). Passive cutaneous anaphylaxis titers were expressed as the highest dilutions eliciting skin reactions. *Leishmania* antigen was prepared by the freezing and thawing of promastigotes, followed by centrifugation at 12,000 × *g* at 4°C for 20 min. The resultant supernatant was used for passive cutaneous anaphylaxis reactions.

For histopathological examinations, skin lesions were fixed with picric acid-formalin and were embedded in paraffin blocks. Paraffin sections were stained with hematoxylin-eosin. The sections were also stained with acidified toluidine blue for counting mast cells or with a Congo red solution for counting eosinophils (12). The numbers of mast cells and eosinophils surrounding the granuloma were expressed as the numbers of cells per five fields at magnifications of ×400 and ×1,000, respectively.

The animals used in this study were treated in accordance with the guidelines for animal experiments of the Jikei University School of Medicine.

Both W/W^v and +/+ mice exhibited progressive enlargement of ulcerative lesions on the rump, which had been inoculated with *L. amazonensis* promastigotes (Fig. 1A). Increased total serum IgE levels were detected in both mouse strains (Fig. 1B), and the kinetics of IgE levels was parallel to the development of the lesions (Fig. 1A and B). However, no significant differences in terms of the size of the lesions and total serum IgE levels between mast cell-deficient and control mice were found. The IgE concentration in sera collected from W/W^v mice 150 days after the infection (1,200 ng/ml; mean for four mice) was approximately 50 times as great as the concentration in sera obtained before the infection (25 ng/ml; mean for five mice). Parasite-specific IgE antibodies were also detected in the infected mice. Mean anti-*Leishmania* IgE passive cutaneous anaphylaxis titers in sera collected at 150 days of infection were 1:160 for four W/W^v mice and 1:160 for five +/+ mice. In a different experiment, we obtained a similar result, indicating no significant differences in the size of the leishmanial granuloma and in the concentration of serum IgE between the two mouse strains (data not shown).

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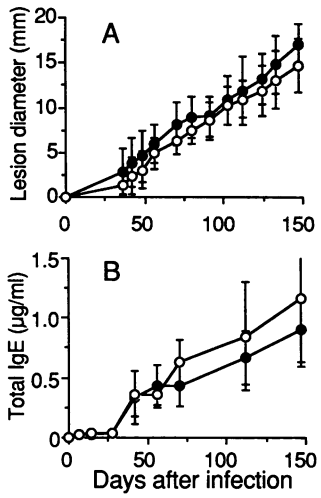

FIG. 1. Kinetics of lesion sizes (A) and total serum IgE levels (B) in mast cell-deficient *W/W^v* mice (○) and normal +/+ littermates (●) inoculated in the rump with 10^7 promastigotes of *L. amazonensis*. Each point represents the mean ± standard deviation for four or five mice.

Histological examination of chronic lesions caused by *L. amazonensis* revealed that a granulomatous response was associated with the massive accumulation of macrophages and mononuclear cells, including eosinophils, in the dermis and subcutaneous tissue. Macrophages were vacuolated and contained numerous amastigotes. In infected *W/W^v* mice (Fig. 2A), few or no mast cells were detected around the periphery of the granuloma as expected, but eosinophils (192 ± 103 per five fields at a magnification of ×1,000; mean ± standard deviation for four mice) accumulated within the granuloma. In infected +/+ mice (Fig. 2B), eosinophil infiltration was also observed in the lesion, but the number of eosinophils in the +/+ mice (226 ± 26 per five fields at a magnification of ×1,000; mean ± standard deviation for five mice) was comparable to that in the *W/W^v* mice. Mast cells (120 ± 29 per five fields at a magnification of ×400; mean ± standard deviation for five mice) was detected around the periphery of the lesions of the +/+ mice.

Little has been reported with respect to the role of skin mast cells in the immune response to parasites, although mucosal mast cells have been demonstrated to have a role in expulsion of parasitic nematodes from the intestine by using mast cell-deficient *W/W^v* mice (9). In the present study, we demonstrated that murine cutaneous leishmaniasis occurs in the absence of mast cells.

IgE antibodies produced in *L. amazonensis*-infected *W/W^v* mice could not induce mast cell-dependent type I allergy in the skin. Nevertheless, histopathological features of chronic lesions in *W/W^v* mice were essentially indistinguishable from those observed in normal +/+ mice. These results suggest that mast cell-dependent anaphylaxis may have a minimum role in the progression or control of cutaneous leishmaniasis. This finding is in sharp contrast to the immune response against larval *Haemaphysalis longicornis* ticks in *W/W^v* mice, in which acquired immunity appeared to result from the immediate hypersensitivity reaction mediated by skin mast cells and antigen-specific IgE antibodies (12, 13).

The present study also revealed that eosinophil infiltration in chronic leishmanial lesions occurred regardless of the presence or absence of mast cells. This may be due to critical

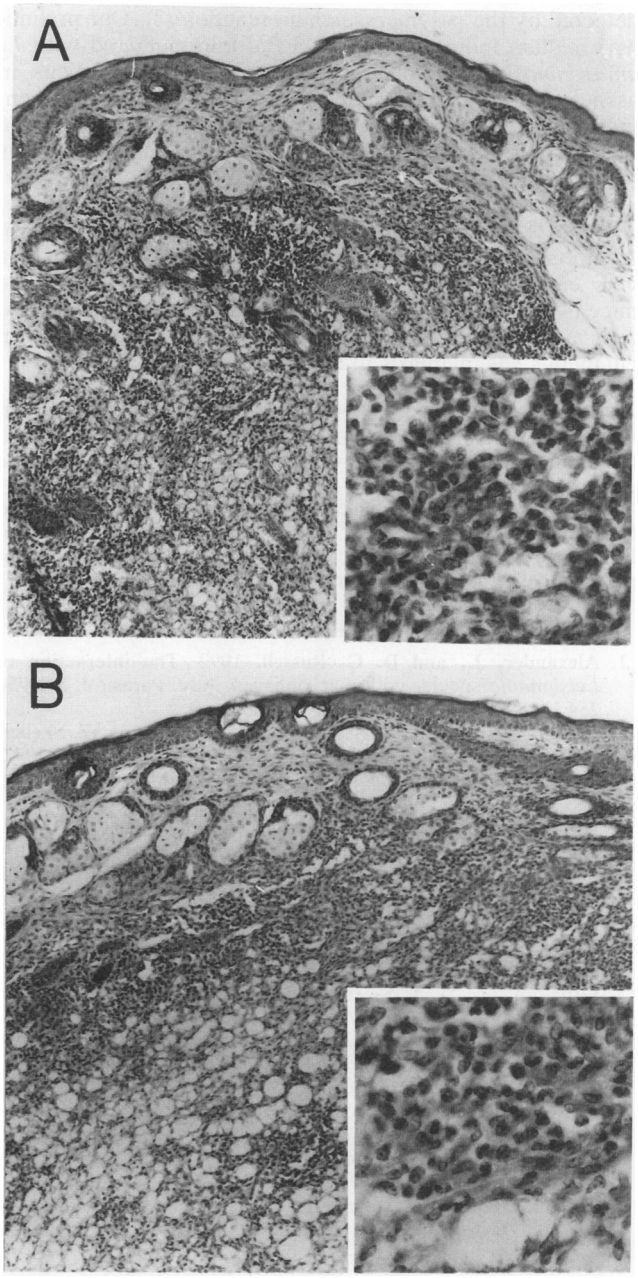

FIG. 2. Histological sections showing skin lesions 150 days after the *L. amazonensis* infection (hematoxylin and eosin staining). A granulomatous response was noted in the dermis and subcutaneous tissue (magnification, ×97) of *W/W^v* (A) and +/+ (B) mice. (Insets) infiltration of eosinophils and mononuclear cells around parasitized macrophages. Magnification, ×388.

levels of interleukin-5 (IL-5) production in *L. amazonensis*-infected *W/W^v* mice. Increased serum IgE levels in the mice was suggestive of an induction of Th2 CD4$^+$ T cells, which are capable of secreting not only IL-4 but also IL-5 (16). The former cytokine is known as regulatory cytokine for IL-4 production, and the latter is the major proliferating and differentiating factor for eosinophils.

In susceptible BALB/c mice infected with *L. amazonensis*, IL-4 mRNA, but not gamma interferon mRNA, was

detected by the polymerase chain reaction (2). Our preliminary results indicated that BALB/c mice infected with *L. amazonensis* developed progressive ulcerative lesions in association with increased serum IgE levels and antigen-induced IL-4 production by the lymph node cells in vitro and that C57BL/6 mice also developed nonhealing granulomas (unpublished data). Gamma interferon is the cytokine most associated with resistance to *L. major* infection in mice, whereas IL-4 has been linked to susceptibility (6, 10, 19, 20). Additionally, it has been reported that IL-4 is required at a very early phase of infection with *L. major* in susceptible mice (4). When these observations are taken together, mast cells may not be possible sources for critical levels of IL-4 in cutaneous leishmaniasis in mice.

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