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Rejection of the Intestinal Parasite *Nippostrongylus brasiliensis* by Mast Cell-Deficient W/W^v Anemic Mice

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The ability of W/W^v anemic mice to accumulate mucosal mast cells and to reject the intestinal parasite *Nippostrongylus brasiliensis* was examined. W/W^v mice did not accumulate mucosal mast cells in response to infections with *N. brasiliensis*. They eliminated a primary infection more slowly than did their normal littermate controls but were as refractory as controls to second and third infections. W/W^v mice had higher serum titers of worm-specific immunoglobulin E than did controls. These results indicate that mucosal mast cells are not an absolute requirement for *N. brasiliensis* rejection.

Mucosal mast cells (MMC) in the small intestine have been proposed to play a role in the rejection of many intestinal parasites (15, 23) because mast cells accumulate in large numbers in the intestinal mucosa during certain parasitic infections in sheep, cattle, guinea pigs, rats, and mice (2, 6, 22, 24, 28, 34, 38). Congenitally athymic (nude) mice, which do not accumulate MMC, are unable to reject many parasites, including *Hymenolepis diminuta* (1, 9), *Hymenolepis nana* (10), *Nippostrongylus brasiliensis* (12), and *Trichinella spiralis* (29, 30). Athymic mice infected with *N. brasiliensis* fail to accumulate MMC, fail to make worm-specific immunoglobulin E (IgE), and fail to reject the parasites. Thymus cells or thymus gland grafts repair these defects (28). IgE or another antibody is apparently not required for expulsion because mice panspecifically suppressed for antibody production reject worms normally (13). To determine whether mast cells are required for parasite rejection, we have evaluated the ability of mast cell-deficient W/W^v mice to accumulate MMC, produce worm-specific IgE antibody, and reject *N. brasiliensis*.

At least two distinct populations of mast cells in the mouse have been proposed (3, 5). One type of mast cell is found in the skin and connective tissues, is thymus independent, and is found in normal numbers in athymic mice. The other type of mast cell, MMC, is found in the intestinal mucosa, accumulates during some parasitic infections, is thymus dependent, and is absent in athymic mice. Others have reported (16, 29, 40) and we have also observed (28) that athymic mice possess normal or elevated numbers of mast cells in the skin but that mast cells are absent from the intestinal mucosa. Mast cells have been shown to be much decreased or absent altogether in the skin, stomach, cecum, and mesentary of uninfected W/W^v anemic mice (18). At birth, the number of mast cells in the skin of W/W^v mice is about 5 to 10% of normal. This number decreases as the mouse age increases and is about 1% of normal at 100 days of age. Mast cells are not found in the muscularis or adventitia of the stomach of W/W^v mice at any age (18). Unlike the MMC defect of nude mice, the connective tissue mast cell defect in W/W^v mice is not corrected by thymus gland grafts (19). It is, however, corrected by grafts of bone marrow or spleen (18-20). Little is known about the response of W/W^v anemic mice to intestinal parasitic infections or their ability to accumulate MMC.

We infected mast cell-deficient W/W^v mice with the intestinal parasite *N. brasiliensis* and found that these mice lack intestinal MMC at rest and do not accumulate them in response to infection. In a primary infection, W/W^v mice were slower than normal littermates to reject their worm burden. Both groups, however, were refractory to second and third infections. Thus, rejection and resistance to intestinal parasites are possible in the absence of MMC.

MATERIALS AND METHODS

Mice. W/W^v anemic mice have a hereditary macrocytic anemia. They lack melanocytes in the skin and have a substantial decrease in the number of erythrocytes (about 60% of normal); however, erythrocyte size is increased and the total amount of hemoglobin is, therefore, only slightly less than normal. These mice appear to have delayed maturation of erythroid stem cells (31). Other stem cells (e.g., intestinal epithelium) appear to divide at the normal rate (27). W/W^v mice are fully viable, living to maturity, but are

not fertile. They are bred as the F_1 generation (WBB6F_1-W/W^v) of WB/ReJ − +/W × C57BL/6J − +/W^v mice. These strain backgrounds have been found to be particularly favorable for vigor and survival. In this study, littermates of W/W^v mice included WBB6F_1 − +/+, WBB6F_1 − +/W, and WBB6F_1 − +/W^v. Groups were distinguished by their distinctive coloring. W/W^v mice are white, +/W and +/W^v mice are spotted, and +/+ mice are solid black. Mice used in these experiments were young adults 3 to 4 months old at the time of initial infection. Congenitally athymic (nude − *nu/nu*) mice and their normal thymus-bearing (euthymic − +/+ or +/*nu*) littermates were from our colony in which cross-intercross mating is in progress to derive a line of athymic mice congenic with BALB/c mice.

Parasites. The mouse-adapted strain of *N. brasiliensis* (36) was maintained in our laboratory as previously described (12). Mice were innoculated subcutaneously in the inguinal region with 600 infective larvae (L_3). The course of infection for each group of mice was monitored by determining the number of worm eggs per gram of feces (EPG) with a modified McMaster technique (37). To count total worms, the entire small intestine was removed from each mouse, washed with saline, slit open, and pressed between two glass plates. All worms remaining in the small intestine or in the dish of intestinal washing were counted, using a dissecting microscope.

Assay of homocytotropic antibodies. Worm-specific IgE responses of *N. brasiliensis*-infected mice were determined by passive cutaneous anaphylaxis tests in rats (26); we have published elsewhere the details of this test (13).

Enumeration of mast cells. Mice were killed by cervical dislocation, and the entire small intestine was removed. Starting 10 cm distal to the pyloric sphincter of the stomach, a 4-cm piece of intestine was removed, slit along the mesenteric attachment, flattened on a wet strip of paper, folded in half, and immersed in Carnoy fixative (7). Tissue was fixed 3 to 8 h before dehydration and infiltration with paraffin. Tissues were sectioned at 10 μm thickness, stained with Astra blue (4), washed in a hematoxylin and eosin bluing agent, and counterstained with eosin. A villus crypt unit was as defined by Miller and Jarrett (22). Counts of intraepithelial and lamina propria mast cells were combined and treated as a single population of cells.

RESULTS

In response to infection with 600 L_3 of *N. brasiliensis*, MMC in the intestine of BALB/c mice and littermates of W/W^v mice increased from a resting level of less than 5 to peaks of 312 and 190 per 10 villus crypt units, respectively (Table 1). In contrast, no MMC were seen in the intestine of W/W^v mice before infection, and they did not accumulate in response to infection. MMC counts were determined for each group of mice on days 12, 19, and 28 post-inoculation (PI). At each of these times, BALB/c mice and littermates of W/W^v mice were MMC positive.

TABLE 1. *MMC accumulation in response to infection with N. brasiliensis*[a]

Mice	No. of MMC per 10 villus crypt units at day after:					
	Infection 1			Infection 2		Infection 3
	12	19	28	12	28	12
W/W^v	0	3	0	ND	0	0
LM of W/W^v	60	190	90	49	27	36
BALB/c	312	ND	74	110	96	78

[a] ND, Not done; LM, littermates.

However, at none of these times did W/W^v mice show MMC accumulation. Representative mice from each group were reinfected on day 48, and some mice were reinfected again on day 98, after primary infection. Even two- and three-times infected W/W^v mice showed no MMC accumulation (Table 1).

Counts of EPG in W/W^v, littermates of W/W^v, and athymic mice were made daily after infection with 600 L_3 (Fig. 1). Athymic mice do not reject this parasite and functioned as a positive control for larval infectivity. Athymic mice attained very high levels of EPG and maintained those levels throughout the experiment. Littermates of W/W^v mice were egg positive from day 5 to day 9 PI, and W/W^v mice were egg positive from day 5 to day 23 PI. (In other experiments, some W/W^v mice remained egg positive for as long as 30 days PI.) However, even though W/W^v mice remained egg positive for a longer time than did their littermates, the EPG in W/W^v mice decreased dramatically on day 11 or 12 from a peak of 70,000 to between 5,000 and 10,000. On day 48 PI and again on day 98 PI, some W/W^v mice and their littermates were reinfected with 600 L_3. Both W/W^v mice and their littermates were refractory to second (Fig. 1) and third (data not shown) infections with this parasite.

To determine whether worms remained in the gut of W/W^v mice for an extended period, some mice from each group were killed at various times after infection with 600 L_3 (Table 2). Athymic mice were used as a positive control for larval viability. On day 7, when EPG were highest (Fig. 1), all groups of animals were worm positive. By day 11, however, littermates of W/W^v mice and BALB/c mice both were worm negative. In contrast, both W/W^v and athymic mice showed high worm burdens until day 16. This high worm burden in W/W^v mice occurred at a time when egg count, determined by fecal EPG, was falling, suggesting that egg production

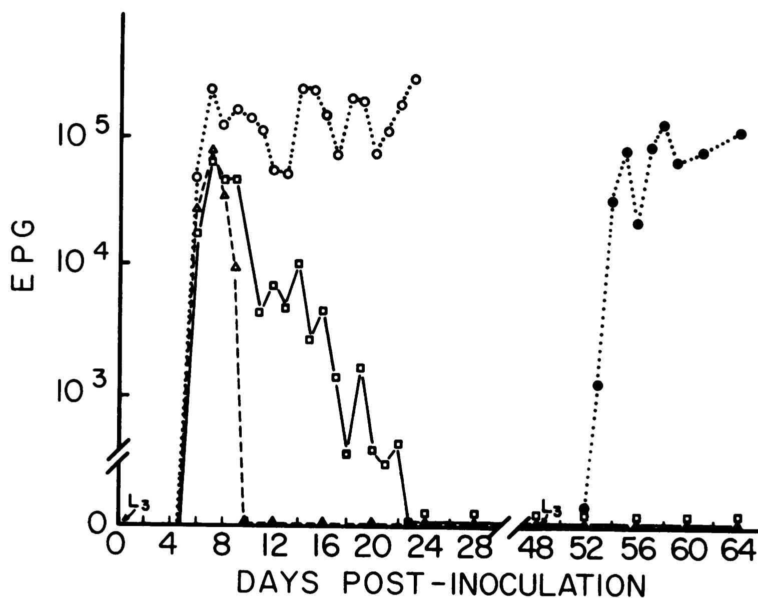

FIG. 1. *EPG in nude (○) and (●), W/W^v (□), and littermates of W/W^v mice (△) after infection with 600 L_3 of N. brasiliensis. W/W^v and littermates of W/W^v mice were infected on day 0 and again on day 48. Different groups of nude mice were infected on day 0 (○) and on day 48 (●).*

TABLE 2. *Number of worms harvested from entire small intestine of mice infected with 600 L_3 of N. brasiliensis*

Mice	No. of worms per mouse at day[a]				
	7	11	16	23	28
W/W^v	78 (22–162)[b]	173 (9–291)	173 (80–420)	11 (0–21)	11 (0–40)
LM[c] of W/W^v	90 (6–239)	0	0	0	0
BALB/c	129 (45–191)	0	0	0	0
BALB/c nude	188 (126–256)	273 (227–328)	285 (115–388)	280 (126–439)	ND[d]

[a] Day after infection of mice with 600 L_3.
[b] Mean number of worms for group of mice; range given within parentheses.
[c] LM, Littermate.
[d] ND, Not done.

per worm was decreased. This may have been due to damage of worms in the intestine prior to actual rejection (25). After day 16, the number of worms in the intestine of W/W^v mice fell sharply, and by day 28, the average number of worms per mouse was 11.

Serum titers of worm-specific IgE 28 days after infection with *N. brasiliensis* were determined for all groups of mice. W/W^v mice had substantially higher titers (>512) of worm-specific IgE than did their littermates (64), BALB/c mice (16), or athymic mice (0). Passive cutaneous anaphylaxis titers were determined in Lewis rats.

DISCUSSION

Unlike their normal littermates or BALB/c mice, W/W^v anemic mice did not accumulate gut MMC in response to infection with *N. brasiliensis* parasites. W/W^v mice rejected a primary infection of *N. brasiliensis* parasites more slowly than did their littermates or BALB/c mice, but all three groups of mice were refractory to second and third infections. After infection with *N. brasiliensis*, W/W^v mice had higher serum titers of worm-specific IgE than did their normal littermates or BALB/c mice.

The fact that W/W^v mice did not accumulate

MMC and yet successfully rejected their parasites indicates that MMC are not an absolute requirement for parasite rejection. On the other hand, because in a primary infection mast cell-deficient mice took two to three times longer than normal mice to reject their parasites, it is possible that MMC normally contribute functionally to the primary rejection mechanism. In contrast to our results, other investigators (35) have reported that W/W^v mice eliminate their parasite burden in a primary infection as rapidly as do normal mice.

Interestingly, W/W^v mice were as refractory as normal littermates or BALB/c mice to second and third infections. This suggests that the mechanisms involved in primary rejection may differ somewhat from those in second and third infections. In normal littermates and BALB/c mice, it appeared that MMC numbers were lower after second and third infections than after a primary infection (Table 1); thus, MMC may be more important in primary than in second or third infections.

Delayed parasite rejection after primary infection in W/W^v mice may have another explanation. It may be due to an immune defect. One group of investigators reported that they could not repair the hemopoietic defect in W/W^v mice with +/+ littermate bone marrow if the cells had been treated with anti-thy 1.2 antiserum (32, 39). They concluded that a thymus-derived cell was required for the regulation of hemopoiesis and that the W/W^v mouse had a defect of this cell as well as a bone marrow stem cell defect. W/W^v mice may lack a thymus-derived cell which contributes to normal parasite rejection. The precise immune status of W/W^v anemic mice is not known with certainty. Some investigators have reported that the spleens of homozygous W^v/W^v mice contain only 1/3 to 1/2 of the normal number of anti-sheep hemolysin-forming cells (G. M. Shearer and G. Cudkowicz, Fed. Proc. **26**:688, 1967). Others, however, found that heterozygous W/W^v mice had normal or elevated plaque-forming cell responses to sheep red cells and that tests of bone marrow-thymus synergism showed a higher-than-normal response when W/W^v tissues were used (21). It is essential that the immune status of W/W^v mice be clarified to accurately interpret the role of mast cells in parasite rejection mechanisms.

Worm-specific IgE has also been proposed to play a role in parasite rejection mechanisms (14, 23, 25). It is clearly not alone a sufficient stimulus for normal parasite rejection, however, because even the very high titers of IgE which we found in W/W^v mice did not produce normal rejection of *N. brasiliensis*. The absence of mast cells from the gut and elsewhere may account for this high IgE titer. The large number of MMC which typically accumulate in response to *N. brasiliensis* infection may function as an IgE "sink," keeping large quantities of this immunoglobulin out of the circulation and decreasing the serum titer in normal animals. Alternatively, the high titers of worm-specific IgE seen in W/W^v mice may suggest a thymus-dependent suppressor cell defect. We propose to do heterologous adoptive cutaneous anaphylaxis (17) to distinguish between these possibilities.

W/W^v mice will be valuable animals in characterizing the functions and control of MMC and connective tissue mast cells. They may also help determine whether two distinct populations of mast cells really exist. Kitamura et al. were able to repair the skin mast cell defect in W/W^v mice with bone marrow or spleen cell grafts and showed that the source of skin mast cell precursors was in the marrow (19). They were not, however, able to repair W/W^v skin mast cell defects with thymus or thymus cell grafts. Thus, skin mast cells derive from the marrow but not the thymus. The origin of MMC may be different. Because athymic mice have mast cells in the skin but none in the intestinal mucosa, the thymus has been suggested as a possible source of MMC. The observation that mast cells can be grown from thymus cell cultures (8, 11) supports this possibility. Do MMC derive from the thymus but not the marrow? Or do they derive from the marrow but remain impotent in the absence of a T-regulatory cell? We intend to repair W/W^v mice with various tissues (marrow, spleen, thymus) and purified cell populations to answer these questions.

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