Jpn. J. Vet. Res., 31, 133–140 (1983)

PRELIMINARY OBSERVATION ON THE ABSENCE OF GLOBULE LEUCOCYTES IN MAST CELL-DEFICIENT W/W^V ANEMIC MICE AFTER *TRICHINELLA SPIRALIS* INFECTION

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(Received for publication August 11, 1983)

No proliferation of globule leucocytes was observed in congenitally mast cell-deficient (WB × C57BL/6)F$_1$-W/W^V anemic mice during the time course between 5 and 35 days postinfection with *Trichinella spiralis*. On the contrary, in the intestine of C57BL/6-bg^J/bg^J and C3H/He mice, which had sufficient number of mast cells, an increasing number of globule leucocytes was observed intraepithelially on day 10 postinfection, after which it reached respective peaks of cell-proliferation on day 14 postinfection. The worms in W/W^V mice were sustained for a longer period than those in bg^J/bg^J and C3H/He mice. These results suggest a certain positive correlation between the infiltration of globule leucocytes and the rapidity of *T. spiralis* expulsion from the intestine.

Key words: *Trichinella spiralis*, self-cure, globule leucocyte, mast cell deficiency, W/W^V mouse

INTRODUCTION

It has been suggested that the number of subepithelial mast cells and intraepithelial globule leucocytes in the small intestine of various animal species including ruminants and laboratory rodents are responsible for the expulsion (self-cure) of many gastrointestinal helminthic parasites.[3,15,17]

The absence of subepithelial mast cell and globule leucocyte proliferation was observed in *T. spiralis* infected athymic (nude) B10LP mice in which the worms were not rejected.[18] But thymus transplantation induced globule leucocyte proliferation and the expulsion of adult worms from the intestine within the normal time frame as shown in normal mice. This indicated that the thymus itself, and/or factors originating from the thymus, play a crucial role in the appearance of these cell types. However, only a little information is available concerning the relationship between subepithelial mast

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cells and globule leucocytes, as well as their function in the expulsion of adult *T. spiralis* from the intestines.

Mutations at the dominant spotting locus (W, W^V) of the mouse genome resulted in deficiency of pluripotent stem-cell compartment, which is expressed as gross reduction in the number of tissue mast cells as the mouse age increases, i. e., less than 1% of the normal number at 100 days old,[9] as well as in the conditions of anemia,[12] sterility and lack of hair pigmentation.[19] Since the major function of T- and B-cells in these animals appears to be normal,[13] though with minor immunodeficiencies related to graft-versus-host diseases as suggested by Sharkis et al. (1978), the mast cell-deficient W/W^V mouse is a useful animal in examining the relative importance of subepithelial mast cells and intestinal globule leucocytes to the *T. spiralis* expulsion (self-cure), and the origin of these cell types.

The results of UBER et al. (1980) showed that the expulsion of *Nippostrongylus brasiliensis* from the intestines of W/W^V mice lacking mast cells occurred as rapidly as that from those possessing these cells, whereas KOJIMA et al. (1980), CROWLE & REED (1981) and MITCHELL et al. (1983) showed that rejection is delayed in W/W^V mice. Using mast cell-reconstituted W/W^V mice, CROWLE (1983) has shown that the presence of mucosal mast cells is not an absolute requirement for *N. brasiliensis* expulsion.

The following preliminary experiments were designed to determine the kinetics of globule leucocytes during *T. spiralis* expulsion from the intestine of mast cell deficient W/W^V mice.

MATERIALS AND METHODS

Mice

Male (WB × C57BL/6)F_1-W/W^V (abbreviated as W/W^V) anemic mice, aged 5 weeks, and male C57BL/6-bg^J/bg^J (abbreviated as bg^J/bg^J) beige mice, aged 5 weeks, were purchased from the Jackson Laboratory, Bar Harbor, Maine, USA. Male C3H/He mice, aged 5 weeks, purchased from Nippon CLEA, Tokyo, Japan, were used as control mice having sufficient number of mast cells in order to examine the normal time frame of worm expulsion. They were maintained on a mouse diet (CE-1 : Nippon CLEA, Tokyo, Japan) with sterilized distilled water in a barrier facility with filtrated air. The mice were 14 weeks old when used in the experiments.

Parasite

The strain of *T. spiralis* used was originally isolated in 1968 from a polar bear, *Thalarctos maritimus*, at Maruyama zoo, Sapporo, Japan, and had been maintained as stock infections in mice. Infective stage larvae were recovered by digestion of the stock mice, which had been infected for at least 8 weeks. The mice were killed under ether-anaesthesia, skinned, eviscerated and cut into pieces. The carcasses were then digested in artificial gastric juice (0.5% pepsin in 0.5% HCl) at 37°C for 3 hr

at a concentration of about 20 ml of the gastric juice to 1 gr of the material. Undigested sediment was filtered off on a coarse metal sieve and the larvae collected by repeated sedimentation and washing with physiological saline. The mice were orally inoculated with 400 infective stage larvae using a syringe under ether-anaesthesia.

To count the total number of adult worms, the entire small and large intestines, were removed and the former divided into 4 equal parts. A 1 cm pieces of the anterior portion of these parts was removed for histological examination. The rest of the intestines were slit longitudinally in a petri dish filled with physiological saline. Using a dissection microscope, all the worms remaining in the intestinal washing were counted.

Enumeration of globule leucocytes.

One mouse (W/W^V and bg^J/bg^J) or two mice (C3H/He) were killed at various intervals from 5 to 35 days after infection. The presence of globule leucocytes was determined by examining a 1 cm portion of the uppermost small intestine, which was fixed overnight in 10% formalin. Then, the tissues were dehydrated and embedded in paraffin wax according to conventional procedures and processed so that 4 μm thick sections were cut. Sections were stained with a modified Dominici stain,[11] which differentiated the globule leucocytes from other types of cells. After passage through water, the sections were stained for 30 min in a mixture of 0.5% eosin Y and 0.6% orange G. After a quick rinse in 60% ethanol, they were stained for 1 min in 0.3% toluidine blue O. By this staining, the globule leucocytes were characterized by the presence of a lymphocytic, pressed eccentric nucleus, large eosinophilic granules, which were larger than those of mast cells and eosinophils, unstained cytoplasm and localization in the epithelium mainly at the base of the villous. The number of globule leucocytes was counted on 4 random microscopic fields covering 1 mm^2.

Results

As shown in table 1, no accumulation of globule leucocytes was observed in W/W^V mice during the time course of infection between 5 and 35 days postinfection. While in the intestinal mucosa of the bg^J/bg^J and C3H/He mice, increasing numbers of globule leucocytes were observed on day 10 postinfection, i. e., 174 and 63 cells/mm^2, respectively, then they reached respective peaks of 465 and 1010 cells/mm^2 on day 14 postinfection. Thereafter, decreasing to 190 and 112 cells/mm^2, respectively, on day 21 postinfection were observed. The number of adult *T. spiralis* per mouse in the infected W/W^V and the other mouse strains possessing sufficient number of mast cells were presented in table 2. The mean numbers of globule leucocytes and adult worms in two C3H/He mice of each interval were also recorded in the respective tables. The W/W^V mice showed consistent adult worm-burden, i. e., from 124 worms on day 5 to 3 worms on day 35 postinfection. In contrast, no worm was found in the bg^J/bg^J

TABLE 1 *Number* [1] *of globule leucocytes in the small intestines of* W/W^V, bg^J/bg^J *and C3H/He mice infected with Trichinella spiralis* [2]

MOUSE STRAIN	NUMBER OF GLOBULE LEUCOCYTES Days postinfection					
	5	10	14	21	28	35
W/W^V	0	0	0	0	0	0
bg^J/bg^J	NE[3]	174	465	190	NE	NE
C3H/He	0	63	1010	112	99	NE

1) Figures represent the number of globule leucocytes by counting 4 microscopic fields covering 1mm^2 of one mouse (W/W^V and bg^J/bg^J) or two mice (C3H/He) on the average.
2) Infective stage larvae, 400 for each mouse, were inoculated orally.
3) NE : not examined

and C3H/He mice by day 14 and 21 postinfection, respectively. In the C3H/He mice especially, the worms were rejected rapidly, i. e., from 57.3% recovery rate on day 5 to 0.5% on day 10 postinfection, which was prior to the onset of globule leucocyte proliferation. The worms in the W/W^V and C3H/He mice were recovered mainly from the upper part of the small intestine, whereas in the bg^J/bg^J mice they were collected mainly from the caecum.

DISCUSSION

Following the clear elucidation by KITAMURA et al. (1978) of the mast cell-deficency in W/W^V mice from the histological studies of toluidine blue stained sections obtained from various tissues including the skin and the gastrointestinal tract, UBER et al. (1980) and CROWLE & REED (1981) confirmed the absence of intestinal mast cells in W/W^V mice infected with *N. brasiliensis*. Their use of the term "mucosal mast cell" or "intestinal mast cell" included both the lamina propria mast cells and the intraepithelially located globule leucocytes. Intestinal globule leucocytes have even been termed as intraepithelial mast cells (reviewed by Miller, 1980), and are thought to be equivalent to "degranulated mast cells".

WEILL (1919) originally described an intraepithelially located cell with a small, dark "lymphoid" nucleus and an unstained cytoplasm containing eosinophilic granules, and named it "Schollenleukocyten". The term was later translated as "globule leucocytes" by KEASBEY (1923) in his report on the subject in the sheep abomasum. Since then, various works have been done describing its distribution, morphology and cytochemical

TABLE 2 *Recovery*[1] *of adult Trichinella spiralis from infected W/W^V, bg^J/bg^J and C3H/He mice*[2]

MOUSE STRAIN	WORM-BURDEN Days postinfection					
	5	10	14	21	28	35
W/W^V	124(31.0)	87(21.8)	70(17.5)	17(4.3)	5(1.3)	3(0.8)
bg^J/bg^J	NE[3]	143(35.8)	80(20.0)	0(0)	NE	NE
C3H/He	229(57.3)	2(0.5)	0(0)	0(0)	0(0)	NE

1) Figures represent the number of adult *T. spiralis* collected from one (W/W^V and bg^J/bg^J) or two (C3H/He) mice on the average. Percentage recoveries indicated in parentheses.

2) Infective stage larvae, 400 for each mouse, were inoculated orally.

3) NE : not examined

properties in various hosts at the light microscopic level. They are known to become abundant during parasitic infections.[3,6,7,15,21,22,23,26,27] However, views on the function and origin of these cells are still controversial since it is not clear whether or not mast cells and globule leucocytes are related cell types (reviewed by GREGORY, 1979). Recent evidences based on an extensive study of parasitized rats and ruminants showed that globule leucocytes are partially degranulated end-stage of mucosal mast cells. Using chickens (White Leghorn, N strain), KITAGAWA et al. (1979) presented some evidences that globule leucocytes are not only thymus-dependent but originated from thymus lymphocytes. In this primary infection with *T. spiralis*, we have shown that a deficiency of globule leucocyte in W/W^V mice is confirmed, and that the adult worms remain for a longer period in W/W^V than in bg^J/bg^J and C3H/He mice whose globule leucocytes accumulate in the intestine. These findings show behavioral similarity between mast cells and globule leucocytes, and suggest some positive participation of globule leucocytes in the self-cure of *T. spiralis*, although this action is not essential.

Our results are not compatible with those of UBER et al. (1980), who reported that expulsion of *N. brasiliensis* from the intestine of W/W^V mice was achieved in the same duration as that of normal mice. It should be noted that there is a difference in parasitizing behaviors between *N. brasiliensis* and *T. spiralis*. In the former, an extensive somatic migration occurs before the worm finally arrives in the alimentary canal, whereas in the latter, no migration occurs and the worms burrow into the intestinal epithelial cells to release larvae through the intestinal mucosa to striated skeletal muscles via the lymphatic and blood stream.[28] It is difficult to determine which responses are elicited by the enteral and which by the parenteral forms of the migratory nematodes. The *in vitro* assay using parasitized sheep globule leucocytes and the mucosal mast cell isolation method[5] may give a clue to clarifying the disputed function of these cell types. Since little information is available concerning the ability of other parasite-sensitive cell populations in the W/W^V mice, further investigation is required to determine the implications of other cell populations such as eosinophils and goblet cells in the "self-cure".

ACKNOWLEDGMENTS

We wish to thank Prof. M. OHBAYASHI, Faculty of Veterinary Medicine, Hokkaido University, for his critical review of the manuscript. Thanks are also due to Mrs. S. KUDO for bibliographic assistances. This study was supported by Grant-in-Aid for scientific research (Grant No. 548340) from the Ministry of Education, Science and Culture, Japan.

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