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Genetic factors controlling the intestinal mast cell response in mice infected with *Trichinella spiralis*

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SUMMARY

Inbred strains of mice showed marked variation in their mast cell (MC) response to infection with *Trichinella spiralis*. Variation was under genetic control, the ability to respond to infection being inherited as a dominant trait. MHC-linked genes may influence the absolute level of response, but overall response kinetics appear to be controlled by genes which are not linked to the MHC. An enhanced MC response was transferred adoptively with immune mesenteric lymph node cells (IMLNC), but reciprocal adoptive transfers between H-2 compatible rapid (NIH) and slow (B10.G) responder strains showed that the degree of enhancement was determined by the response phenotype of the recipient, not that of the donor. Similarly, in bone marrow (BM) chimaeras, produced by reconstituting lethally irradiated F_1 (B10.G × NIH) mice with parental BM, the MC response to *T. spiralis* was determined by the response phenotype of the BM donor, whether or not rapid responder IMLNC were transferred. The data are discussed in terms of a T lymphocyte regulated, bone marrow stem cell origin of mucosal MC and interpreted as showing that genetic regulation of the MC response is expressed at the level of stem cell or precursor response to T cell derived mastopoietic factors.

INTRODUCTION

Intestinal mastocytosis is a common consequence of infection with parasitic nematodes, yet its functional significance remains obscure. In recent years a number of parameters of the mucosal mast cell (MC) response to infection have been defined. Thus mastocytosis is thymus-dependent (Ruitenberg & Elgersma, 1976; Mayrhofer & Fisher, 1979), requires continuing antigenic stimulation (Mayrhofer, 1979; Alizadeh, 1981) and can be accelerated by transfer of serum and lymphocytes from immune donors (Befus & Bienenstock, 1979; Nawa & Miller, 1979; Alizadeh & Wakelin, 1981a). However, fundamental questions concerning the origin and control of this cell population have yet to be answered. For example, although MC have been cultured *in vitro* from suspensions of thymic lymphocytes, mesenteric node lymphocytes and other cells, it is not known whether mucosal MC have similar origins *in vivo* (Askenase, 1979).

In mice infected with *Trichinella spiralis* there is a temporal correlation between the onset of protective immunity (leading to expulsion of the intestinal adult worms) and an increase in mucosal MC (Alizadeh & Wakelin, 1981b). Previous work has shown that there is a marked strain variation in ability to expel *T. spiralis* and some of the underlying mechanisms have been elucidated (Wakelin & Donachie, 1981). This paper describes the results of a study of strain variation in mucosal MC

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response to *T. spiralis*. Experiments were designed to define some of the genetic and immunological mechanisms involved and to throw light upon the regulation of intestinal mastocytosis.

MATERIALS AND METHODS

Animals. Male mice were used throughout and were infected when approximately 8 weeks old. Inbred NIH (H-2^q) were purchased from Hacking and Churchill Ltd; inbred C57BL/10(H-2^b), CBA(H-2^k) and B10 congenic mice (B10.G, H-2^q; B10.D2, H-2^d and B10.BR, H-2^k) were purchased from Olac, 1976 Ltd; inbred DBA_1(H-2^q) and hybrid B10.G × NIH were bred in the laboratory.

Parasite. The strain of *T. spiralis* used and the procedures for parasite maintenance, infection and recovery have been described previously (Wakelin & Lloyd, 1976). Mice were routinely infected with 300 larvae.

Cell transfers. Mesenteric lymph node cells (MLNC) were taken from mice infected 8 days previously. The methods used in preparing suspensions and transferring cells have been described (Wakelin & Wilson, 1977). Twenty million cells were transferred in each experiment.

Irradiation. Mice were irradiated using a ^{60}Co source (output 1050 rad/min) and reconstituted with 1×10^7 bone marrow cells within 3 hr. Antibiotic was given in the drinking water to both irradiated and control mice.

Histology. MC counts were taken from 5 cm portions of the small intestine, processed by the Swiss roll technique (Alizadeh & Wakelin, 1981b). Where worm counts were also required the remainder of the intestine was then incubated for worm recovery. Tissues were fixed in Carnoy's fixative for 3–6 hr and embedded in polywax (DIFCO Laboratories, Glasgow, Scotland). Sections were cut at 5 μm and stained with Alcian blue (1% w/v in 0·7 N HCl, pH = 0·3, for 30–45 min) followed by Safranin 'O' (0·5% w/v in 0·125 N HCl, pH = 1·0, for 5 min). After rinsing in distilled water the sections were processed rapidly through an alcohol series (70–100%), cleared in xylene and mounted in DPX. Compared with rat MC, mouse cells stain less intensely, nevertheless MC are clearly identifiable by their blue staining cytoplasmic granules. Cells were counted on a villus-crypt unit (vcu) basis and all cells showing the characteristic staining, whether situated in the lamina propria or the mucosal epithelium were recorded. A total of 20 vcu (approximately 4% of those present in any one section) was counted per mouse and the results are presented as mean number of MC (±s.d.) per 20 vcu per group of mice, usually three–six animals.

Statistics. Student's *t*-test (two-tailed) was used to evaluate the significance of differences between mean values. A probability of $P < 0{\cdot}05$ was considered significant.

RESULTS

Variation in MC response between strains

In two experiments responses were compared between three categories of mice, (a) strains sharing the H-2^q haplotype with a rapid (NIH, DBA_1) or slow (B10.G) worm expulsion phenotype, (b) strains sharing the C57BL/10(B10) genetic background and a slow responder phenotype but differing in H-2 haplotype (B10, H-2^b; B10.G, H-2^q; B10.D2, H-2^d and B10.BR, H-2^k) and (c) strains sharing the H-2^k haplotype but with a slow (B10.BR) or intermediate (CBA) expulsion phenotype. Mice were killed in groups of at least five on days 8, 12 and 16 after infection. The results are shown in Fig. 1. With the exception of B10.BR mice, all strains had large numbers of MC by day 12 after infection, but only in the rapid expulsion phenotype mice (NIH, DBA_1) were numbers high at day 8. B10.BR mice failed to show a substantial rise even by day 16, whereas the H-2^k identical CBA showed a response similar to that of the other B10 strains. Control, uninfected, mice of all strains had negligible numbers of MC (< 50 per 20 vcu). Worm recovery data confirmed the expulsion phenotypes of the strains used. NIH and DBA_1 were worm free by day 12 and CBA by day 16, B10 background mice all had substantial numbers of worms on day 16.

Inheritance of the MC response

Rapidity of worm expulsion is inherited as a dominant characteristic, thus the F_1 progeny of a cross

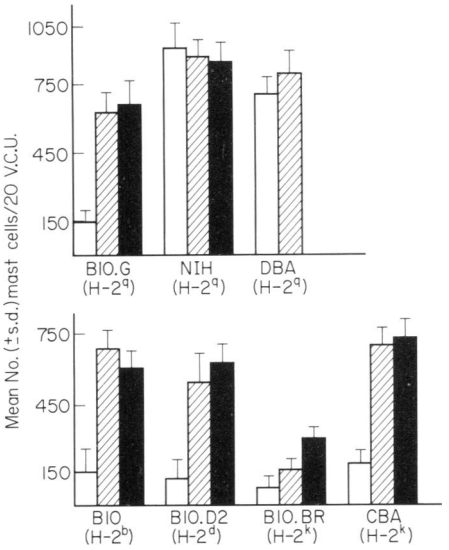

Fig. 1. Intestinal mast cell response of inbred mouse strains to infection with *Trichinella spiralis*. (□ = day 8; ▨ = day 12; ■ = day 16).

between rapid responder NIH and slow responder B10.G mice, both H-2^q, behave as NIH mice (Wakelin & Donachie, 1981). The MC responses of parental and F_1 mice are summarized in Fig. 2. Although the F_1 mice had significantly fewer MC than NIH on day 8, their response was clearly that of the rapid responder parental type.

Transfer of the MC response with MLNC from immune donors

Preliminary work has shown, in NIH mice, that immune (I) MLNC can transfer the capacity to make an accelerated MC response to infection (Alizadeh & Wakelin, 1981a); normal (N) MLNC have no effect. In the first experiment IMLNC were transferred reciprocally between H-2^q histocompatible NIH and B10.G mice (rapid and slow MC response phenotype respectively) to assess the role of donor and recipient phenotype in the adoptive response. The design and results of the experiment are shown in Table 1 (mice were killed on day 6 to allow an earlier assessment of the accelerated response). The data show clearly that the level of the response is determined primarily by the recipient phenotype. B10.G mice showed only a moderate MC response whether given B10.G or NIH cells. In contrast, NIH mice mounted a greater absolute response after transfer, indeed they showed the largest response when given B10.G cells.

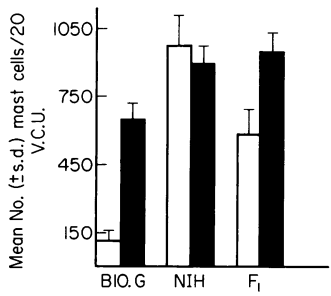

Fig. 2. Intestinal mast cell response of B10.G, NIH and (B10.G × NIH) F_1 mice to infection with *Trichinella spiralis*. (□ = day 8; ■ = day 12).

Table 1. Effect of reciprocal adoptive transfers (2×10^7 IMLNC) upon intestinal mast cell responses to *T. spiralis* infection in rapid (NIH) and slow (B10.G) responder strains of mice, both of which are H-2^q haplotype

Group	Recipient strain	Cell donor strain	Number of mast cells per 20 vcu 6 days after infection: Mean	s.d.
1	NIH	no cells	286	32
2	NIH	NIH	544*†	61
3	NIH	B10.G	1042*†‡	96
4	B10.G	no cells	105	48
5	B10.G	B10.G	242*	57
6	B10.G	NIH	260*	40

* Mean significantly greater than corresponding no cells control.
† Mean significantly greater than B10.G recipients.
‡ Mean significantly greater than NIH recipients.

To confirm and extend this observation two further experiments were performed. The first was based on the fact that in mice irradiated before infection, the MC response is ablated, but can be partially restored by transfer of IMLNC; NMLNC have no effect (Alizadeh & Wakelin, 1981a). The experimental design used above was repeated, with mice irradiated at 400 rad 1 day before cell transfer and infection. It was assumed that, under these conditions, any differences associated with donor phenotype ought to be more obvious, as the inherent capacity of the recipient to respond would be reduced. (400 rad irradiation is sub-lethal and delays, but does not prevent, expulsion of *T. spiralis* when IMLNC are transferred. This fact is interpreted as showing that myeloid populations are restored relatively rapidly from endogenous stem cells (Wakelin & Wilson, 1980).)

Mice were killed 8 days after infection and the results are shown in Table 2. The numbers of MC were much reduced, compared with those shown in Table 1, but the same overall pattern of response

Table 2. Effect of reciprocal adoptive transfers (2×10^7 IMLNC) upon intestinal mast cell responses to *T. spiralis* infection in sub-lethally irradiated (400 rad) rapid (NIH) and slow (B10.G) responder strains of mice, both of which are H-2^q haplotype

Group	Recipient strain	Cell donor strain	Number of mast cells per 20 vcu 8 days after infection: Mean	s.d.
1	NIH	no cells	5	2
2	NIH	NIH	218*†	30
3	NIH	B10.G	321*†‡	27
4	B10.G	no cells	6	3
5	B10.G	B10.G	72*	25
6	B10.G	NIH	66*	18

§ Mean significantly greater than corresponding no cells control.
† Mean significantly greater than B10.G recipients.
‡ Mean significantly greater than NIH recipients.

Table 3. Intestinal mast cell responses to *T. spiralis* infection in (B10.G × NIH) F_1 bone marrow chimaeric mice,* given 2×10^7 IMLNC from rapid responder NIH mice

Group	Donor of BM cells	2×10^7 NIH IMLNC	No. of mast cells per 20 vcu 7 days after infection. Mean	s.d.
1	NIH	−	425	85
2	NIH	+	980†	75
3	B10.G	−	338	92
4	B10.G	+	550	152

* Mice lethally irradiated and reconstituted for 12/15 weeks with 1×10^7 NIH or B10.G BM.

† Mean significantly greater than all other groups.

was evident, with NIH recipients again showing the greatest number of cells. In this experiment the activity of the IMLNC was also monitored in terms of the effect on worm expulsion in unirradiated NIH mice. Whereas control mice had a mean burden of $174{\cdot}8 \pm 24{\cdot}3$ worms on day 8, recipients of NIH or B10.G cells had respectively $73{\cdot}6 \pm 15{\cdot}4$ and $37{\cdot}5 \pm 21{\cdot}7$ worms, a significant reduction in each case.

Both the above experiments had involved reciprocal transfer of IMLNC between H-2 compatible, but otherwise genetically distinct mice. To overcome this limitation, and to examine the role of non lymphoid cells more directly, mast cell responses were examined in chimaeric mice prepared by irradiating F_1 (B10.G × NIH) mice at 850 rad and reconstituting with either NIH or B10.G bone marrow cells. The mice were rested for a minimum of 12 weeks to allow repopulation by cells of donor origin and, at the end of this time, were given IMLNC from NIH donors, infected with *T. spiralis* and killed after 7 days (Table 3).

Mice restored with B10.G bone marrow showed no significant increase in MC numbers after transfer of NIH IMLNC, whereas those given NIH bone marrow responded markedly to transfer.

DISCUSSION

Strain variation in intestinal MC response to parasite infection has been observed in both mice (Ruitenberg *et al.*, 1980) and rats (Nawa & Miller, 1979), but has not received detailed study. The present work shows that variation can affect both time of onset and level of the response and confirms that variation is under genetic control.

In the seven strains tested there was a temporal correlation between the rise in MC and the response that resulted in worm expulsion. Thus NIH and DBA_1 mice, both rapid responders in terms of worm expulsion, showed the earliest rise in MC, and B10 background mice, all slow responders, showed a delayed mastocytosis. CBA mice, intermediate in expulsion kinetics, were closer to B10 mice in their MC response. This correlation between the parameters of expulsion and MC response extended to the underlying genetic control. As with the former (Wakelin & Donachie, 1980) ability to mount a rapid MC response was inherited as a dominant characteristic. In addition, the genes which appear to control the overall response pattern were not linked to the MHC. MHC-linked genes may, however, influence the detailed pattern of response. Although all B10 background strains had a slow MC response, the absolute number of cells present in B10.BR mice remained much lower than in the other congenics.

Identification of the H-2 compatible NIH and B10.G strains as rapid and slow MC responders allowed analysis of the control of the response and of the cellular component through which genetic

control was expressed. The results of reciprocal adoptive transfers between these strains can be summarized as follows:

(1) transfer of IMLNC enhanced the MC response in normal recipients and partially restored the response in sub-lethally irradiated recipients. The magnitude of the response in each case was determined primarily by the phenotype of the recipient.
(2) in chimaeric (B10.G × NIH) F_1 mice, prepared by reconstituting lethally irradiated animals with parental bone marrow, the extent of the MC response was determined by the phenotype of the marrow donor.

The ability of IMLNC to accelerate mastocytosis could, as has been pointed out (Befus & Bienenstock, 1979), imply that this population provides a source of MC precursors, or of helper cells which influence proliferation and maturation of such precursors. If precursors are present, then their development must be regulated by factors present in the recipient, as similar inocula of IMLNC generated quite different levels of intestinal mastocytosis in NIH and B10.G mice (Table 1). By the same token, if helper activity is present in IMLNC, then translation of this activity into intestinal mastocytosis must again be recipient regulated. It might be possible to equate the reduced MC response seen in recipients of the B10.G strain with some form of active suppression, but this seems a less likely explanation of the strain differences that were still apparent between irradiated recipients (Table 2).

The results obtained with chimaeric mice show clearly that bone marrow cell phenotype plays a major role in determining the level of response to infection, whether or not IMLNC are transferred. Thus an explanation of the present data could be that suspensions of IMLNC contain helper cells which regulate the proliferation and maturation of mucosal MC from a bone marrow-derived stem cell and that genetic control is expressed primarily through the ability of the stem cell population to respond to the factors which the helper cells produce. The data presented in Tables 1 and 2 suggest that there may also be a significant difference in ability of IMLNC from different strains to produce such factors, in that NIH mice showed a much greater MC response when given B10.G cells than when given NIH cells. It is interesting that a similar differential effect has been seen in terms of worm expulsion after reciprocal adoptive transfer between these two strains (Wakelin & Donachie, 1980). However, in both instances, it is clear that the overriding genetic control is recipient-mediated and depends upon the response of recipient bone marrow-derived stem cells to donor lymphocytes. A number of experimental observations support this interpretation as far as the MC response is concerned. Thus it is possible to culture MC from bone marrow cells (Nagao, Yokoro & Aaronson, 1981) and bone marrow cells can restore tissue MC in congenitally deficient W/W^v mice (Kitamura, Go & Hatanaka, 1978). There is evidence from *in vitro* culture work with MC (Nabel, *et al.*, 1981) and other haemopoietic cell populations (Ruscetti, Cypess & Chervenick, 1976) that development is under T lymphocyte control and this has been extensively investigated in the case of P cells, bone marrow-derived cells with many characteristics of MC, which are generated in long term culture of spleen cells (Schrader, 1981). In this context it is significant that with *T. spiralis* (Alizadeh, 1981) as with other nematodes (Nawa & Miller, 1979) T cell enriched fractions of immune lymphocytes are the effective populations in adoptive transfer of intestinal mastocytosis.

It is clear from *in vitro* culture studies that both IMLNC and NMLNC contain cells capable of differentiating directly into MC (Denburg, Befus & Bienenstock, 1980), but the data reported here suggest that such cells play a relatively small part in the enhanced response seen after adoptive transfer. It seems likely that these immediate precursors are bone marrow-derived and migrate to the MLN before differentiation, but that during an active response to intestinal infection there is a rapid transit time, cells spending only a relatively short period in the MLN before migrating to the intestinal mucosa.

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