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Accelerated elimination of *N. brasiliensis* from the small intestine after auto-anti-IgE induction

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SUMMARY

Immunization of rats with a purified IgE myeloma (IR2) induced an auto-anti-IgE response. Such treatment inhibited total IgE levels in the serum of conventional IgE-producing rats (Marshall & Bell, 1985) and increased the number of mucosal mast cells (MMC) in the intestine. The present study has investigated the ability of auto-anti-IgE induction to influence the course of a *Nippostrongylus brasiliensis* infection, to modify IgE synthesis, or to affect the number of MMC in the intestine following infection. Auto-anti-IgE induction was found to have a surprising effect on worm elimination. IR2-immunized rats were able to rid themselves of this nematode with an accelerated tempo—a small but significant effect after primary infection, but a substantial enhancement of worm loss after reinfection. Auto-anti-IgE induction was not able to prevent the typical increase in IgE that accompanies an *N. brasiliensis* infection, nor did it alter the helminth-induced intestinal mastocytosis. When MMC degranulation was measured by assaying the serum levels of a specific rat mast protease (RMCP II) following secondary infection, the amount of RMCP II released was less in auto-anti-IgE-producing rats. These findings have implications for the importance of IgE, MMC and other cells of inflammation in an anti-parasitic response.

INTRODUCTION

The role of the IgE isotype has become firmly linked with the body's defence mechanism against a number of parasites, including the helminths, largely because of the dramatic increase in serum IgE following infection (Jarrett & Bazin, 1974). The mast cell has also been implicated: the number of mast cells in the gut mucosa increases enormously during the response to an intestinal nematode infection (Wells, 1962; Kelly & Ogilvie, 1972). Furthermore, high affinity binding of IgE to specialized Fc receptors on mast cells provides a sensitive trigger, which can release a miriad of pharmacologically active substances capable, in theory at least, of damaging intestinal nematodes or expelling them by peristalsis. These observations have led more than one group to propose a specific IgE-induced immediate hypersensitivity reaction as a mechanism of nematode expulsion (Askenase, 1980; Jarrett & Miller, 1982).

Attention to tissue fixation (Enerback, 1966) and improved methods of staining have led to the realization that connective tissue mast cells (CTMC) in sites such as skin or the peritoneal cavity and mast cells in the gut mucosa, so-called atypical or mucosal mast cells (MMC), have substantial differences (Jarrett & Haig, 1984). It is the MMC that increase in number during nematode infections and release a specific rat mast cell protease (RMCP II) from their granules after reinfection (Miller *et al.*, 1983) or following initiation of a worm antigen-induced systemic anaphylaxis (King & Miller, 1984). However, the mechanism operating to eliminate a nematode, e.g. the requirement for IgE or the precise role of MMC or eosinophils, is far from agreed. The issue is further complicated by the fact that T lymphocytes play a role either directly or indirectly by helping IgE synthesis (Tada, 1975) or stimulating mast cell development (Ruitenburg & Elgersma, 1976).

Recently we reported the successful induction of an auto-antibody response against the IgE isotype in the rat (Marshall & Bell, 1985). One of the consequences of initiating an auto-anti-IgE response was to reduce both total serum IgE levels (Marshall & Bell, 1985) and a specific IgE response (unpublished work). In addition, mast cell populations were affected: CTMC were reduced in a number of non-mucosal sites, whereas MMC were increased by 34% in the intestinal mucosa (unpublished work). We thought that such a model would be useful for investigating events in *N. brasiliensis* infection. We asked whether the observed changes in IgE and mast cell numbers were maintained under the strong stimulus evoked by the intestinal parasite, or whether auto-anti-IgE induction could alter the survival of *N. brasiliensis* in the rat.

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The powerful stimulation of *N. brasiliensis* infection was able to override the control of IgE synthesis provided by auto-anti-IgE. However, to our surprise, auto-anti-IgE induction had a small but significant effect on the tempo of worm loss during a primary *N. brasiliensis* infection, and a marked effect in eliminating this nematode following a secondary infection. The reasons for this accelerated disappearance of worms from the small intestine was considered in relation to the changes in IgE, MMC and eosinophils.

MATERIALS AND METHODS

Animals

The PVG.RT1^u congenic strain of rat was isolator bred in SPF conditions in the Animal Unit of the Manchester University Medical School. At weaning offspring were removed from the isolator and housed in a conventional environment.

Reagents

IR2 and IR162 IgE myeloma proteins were provided by the courtesy of Dr H. Bazin and purified by ion exchange chromatography on DEAE-cellulose and high performance liquid chromatography on a TSK G3000 SW column. Purity was assessed by sodium dodecyl sulphate–polyacrylamide gel electrophoresis and immunodiffusion techniques. Only those preparations found to be more than 95% pure were used. Human gamma globulin (HGG) was purchased from Sigma, Poole, Dorset.

Total IgE determinations

Paper radioimmunosorbent test (PRIST) assays were carried out according to the method of Karlsson *et al.* (1979).

Assay for auto-anti-IgE

A plate radioimmunoassay was used as previously described (Marshall & Bell, 1985) with slight modifications. Briefly, activated polyvinyl chloride plates (Flow Ltd, Irvine, Ayrshire) were coated with IR162 rat IgE myeloma at 100 μg/ml in borate-buffered saline (BBS), pH 8·3. The plates were washed, the remaining binding sites blocked with a 1% w/v solution of bovine serum albumin (BSA) in PBS, pH 8·3, and washed again. Sera were diluted 1/10 and 1/40 in phosphate-buffered saline +0·3% w/v BSA and 0·02% v/v Tween 20. Fifty microlitres of each dilution were tested in triplicate. The plates were incubated for 90 min at room temperature and washed. Rat IgG bound to the plate was detected by adding 50 μl of a ^{125}I-labelled sheep anti-rat IgG (SAR-IgG) that had been extensively absorbed against IgE as described elsewhere (Marshall & Bell, 1985).

Worm counting

Nippostrongylus brasiliensis larvae were cultured according to the method of Keeling (1960). The rats were infected by subcutaneous injection with an estimated mean inoculum of infective larvae. Rats were killed by ether anaesthesia. The entire small intestine was removed and divided into eight approximately equal segments numbered from 1 (pylorus) to 8 (ileocaecal junction). Each segment was cut open along its length and the number of adult worms or larvae present counted with the aid of a dissecting microscope.

Histology

A 2-mm transverse section of intestine was taken from the distal end of each segment of gut. A piece of tissue was removed from the terminal portions of the lungs. For examination of mast cell populations tissues were fixed in carnoys solution and for examination of eosinophil populations 2% glutaraldehyde buffered with sodium cacodylate. All tissues were parrafin-embedded and 3-μm sections stained with Alcian blue (pH 0·3) and counterstained with Safranin O (pH 1·0) according to the method of Strobel, Miller & Ferguson (1981) or with 2% vital new red according to the method of Smith & Crocker (1984).

MMC and eosinophils were counted microscopically using a $\times$10 eyepiece and $\times$100 oil emersion objective. Results were recorded as the number of cells per 100 high power fields (h.p.f.).

RMCP II assays

A 4–5-cm section of gut was taken from the distal end of segment 2, homogenized in three volumes of 0·2 M KCl and assayed by immunodiffusion for RMCP II according to the method of Woodbury & Miller (1982). Serum samples were frozen and assayed using an ELISA technique according to Miller *et al.* (1983).

Statistics

Values were recorded as means $\pm$ SE. Differences between groups were assessed by Student's *t*-test. A *P* value of 0·05 or less was considered to be significant.

RESULTS

PVG.RT1^u rats were injected i.p. on Days 0 and 14 with 100 μg of purified alum precipitated (ap) rat myeloma IgE (IR2) or with ap HGG as a control. On Day 42 all animals were injected subcutaneously (s.c.) with equal doses of approximately 1000 *N. brasiliensis* stage 3 larvae (L3). Prior to infection, all animals were bled and the tissues of six experimental and six control animals were taken for an assessment of mast cell and eosinophil populations. In agreement with our earlier studies (Marshall & Bell, 1985), serum IgE levels of the IR2-immunized group which had detectable auto-anti-IgE antibodies were significantly decreased compared with the control group of animals (Table 1). The number of MMC in both the small intestine and the lung

Table 1. The effect of auto-anti-IgE induction on serum IgE levels, MMC and eosinophils before primary infection with *N. brasiliensis*

	Control		Anti-IgE	
	n	Mean $\pm$ SE	*n*	Mean $\pm$ SE
[IgE] (ng/ml)	36	320 $\pm$ 20	36	76 $\pm$ 12***
[Anti-IgE] (ng SAR IgG/ml)	36	0†	36	420 $\pm$ 25***
Gut MMC/100 h.p.f.	6	260 $\pm$ 16	6	348 $\pm$ 28**
Lung MMC/100 h.p.f.	6	11 $\pm$ 2	6	22 $\pm$ 3*
Gut eosinophils/100 h.p.f.	6	54 $\pm$ 8	6	48 $\pm$ 4

*$P < 0·05$, **$P < 0·01$, ***$P < 0·001$.

† Radioactivity bound was not significantly above that of normal PVG.RT1^u serum.

Table 2. Total number of worms and number of MMC in the small intestine of control and IR2-immunized rats after primary (1°) and secondary (2°) infection with *N. brasiliensis*

Experiment	Infection	Dose	Days after infection	Total worms in small intestine: Control (mean ± SE)	Total worms in small intestine: Anti-IgE (mean ± SE)	MMC/100 h.p.f.: Control (mean ± SE)	MMC/100 h.p.f.: Anti-IgE (mean ± SE)
A	1°	1000	8	1103 ± 116	848 ± 108*	12 ± 6	21 ± 9
	1°	1000	14	1·3 ± 1·1	0·8 ± 0·5	666 ± 92	379 ± 84
B	2°	500	4	68 ± 9	36 ± 4**	915 ± 30	1016 ± 49
	2°	500	8	0·4 ± 0·3	0·5 ± 0·2	1566 ± 173	1285 ± 116
	2°	500	12	0·5 ± 0·3	0·8 ± 0·6	1790 ± 159	1643 ± 139
C	1°	1000	6	594 ± 27	570 ± 38	124 ± 22	94 ± 14
	1°	1000	8	523 ± 54	346 ± 55*	116 ± 23	148 ± 27
	1°	1000	42	0·8 ± 0·3	0·6 ± 0·4	1342 ± 84	1400 ± 102
	2°	1000	2	736 ± 82	726 ± 59	1245 ± 128	1072 ± 71
	2°	1000	4	951 ± 71	721 ± 58*	730 ± 49	795 ± 84
	2°	1000	6	467 ± 159	20 ± 13**	494 ± 74	487 ± 64

*$P < 0.05$, **$P < 0.01$.

tissue was significantly increased in the IgE-immunized groups, but there was no difference in the number of eosinophils present (Table 1). Six days after primary infection, the number and distribution of adult worms were examined in the small intestine. No significant difference was observed between IgE-immunized and control animals (Table 2), indicating that similar numbers of larvae successfully migrated through the lungs into the gut in both groups of rats. The bulk of the worms were resident in the first four segments of gut (Fig. 1). Examination of the small intestine at Day 8 showed that total numbers of worms were significantly reduced in the IgE-immunized animals compared with the control animals (Table 2). A small reduction in number was observed in each of the first four segments, so that the overall distribution pattern was unchanged in the IR2-immunized rats (Fig. 1).

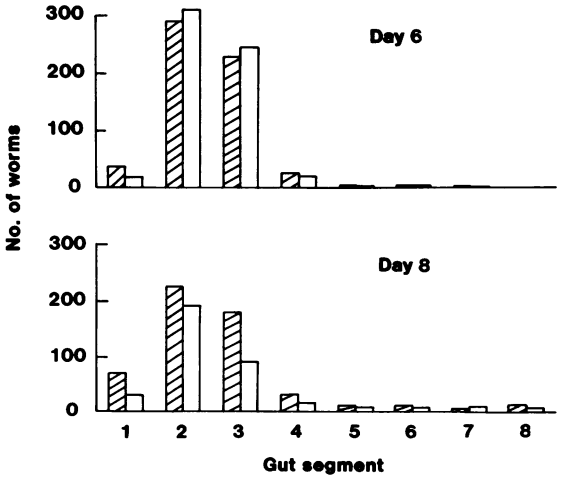

Figure 1. Distribution of worms in the small intestine of control (hatched) and IR2-immunized (open) rats 6 and 8 days after primary *N. brasiliensis* infection. All rats were injected s.c. with 1000 L3 *N. brasiliensis* larvae on Day 0. Histograms are means of six rats.

MMC numbers in the small intestine were also assessed at Days 6 and 8 following *N. brasiliensis* infection. From other studies it has been suggested that MMC declined in number within a week after *N. brasiliensis* infection, which correlated with MMC degranulation (Woodbury & Miller, 1982). This reduction in MMC numbers was observed here on Days 6 and 8 in both control and anti-IgE groups of rats (Tables 1 and 2). It is likely that the loss of distinguishing granules through degranulation rendered the MMC unrecognizable histologically.

Six weeks after the primary infection, animals were reinfected with 1000 L3 larvae s.c. At the time of secondary infection total serum IgE levels were similar in both IgE-immunized and control animals (Table 3), although now increased 50–70-fold compared with preinfection levels (Table 1). Although IgE levels were grossly suppressed in the anti-IgE-producing animals prior to infection (Table 1), auto-anti-IgE induction did not prevent the huge rise in serum IgE concentration that follows *N. brasiliensis* infection (Jarrett & Bazin, 1974). This rise in serum [IgE] may have been responsible for the lack of detectable auto-anti-IgE in the sera of infected animals (Table 3). In uninfected PVG.RT1^u rats the anti-IgE response was detectable for over a year following IR2 immunization, but in the presence of a high [IgE] it was likely that anti-IgE was complexed and thus undetectable by the plate radioimmunoassay. The effect of inducing an auto-anti-IgE response was examined in rats receiving a secondary *N. brasiliensis* infection. Groups of rats were killed on Days 2, 4 and 6 following a second s.c. injection with 1000 L3 larvae. An earlier experiment showed that over 98% of worms were expelled in both IgE-immunized and control animals by Day 8 (Table 2). At Day 2 (Fig. 2) similar numbers of L4 larvae were observed in both the control and the anti-IgE rats. As in the primary infection, anti-IgE induction did not prevent subcutaneously injected larvae from migrating to the gut. By Day 4 there was a significant reduction ($P < 0.05$) in

Table 3. A comparison between control and IR2-immunized rats 42 days after the primary at the time of secondary infection with *N. brasiliensis*

	Control		Anti-IgE	
	n	Mean ± SE	*n*	Mean ± SE
[IgE] ng/ml	25	6310 ± 960	2	5702 ± 890
[Anti-IgE] (ng SAR IgG/ml)	25	0†	24	0†
Gut MMC/100 h.p.f.	6	1398 ± 102	6	1342 ± 92
Lung MMC/100 h.p.f.	6	13 ± 3	6	20 ± 6
Gut eosinophils/100 h.p.f.	6	382 ± 46	6	562 ± 52*

*$P < 0.05$.

† Radioactivity bound was not significantly above that of normal PVG.RT1^u serum.

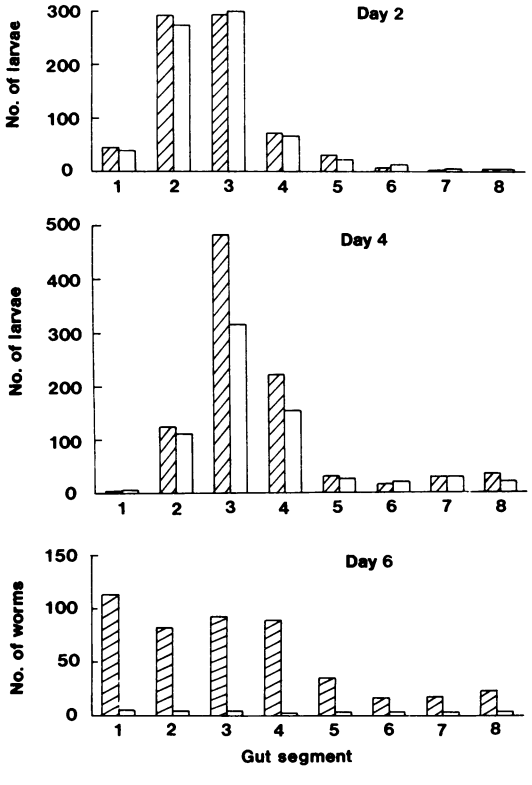

Figure 2. Distribution of larvae or worms in the small intestine of control (hatched) and IR2-immunized (open) rats 2, 4 and 6 days after a secondary infection of *N. brasiliensis*. Histograms are means of five to seven rats.

the number of larvae in the small intestine of IgE-immunized rats compared with control animals without a change in distribution pattern (Fig. 2). This enhanced loss of *N. brasiliensis* in the IgE-immunized group was even more striking at Day 6 (Fig. 2); more than 95% of the worms had disappeared from the small intestine of the anti-IgE animals, whereas 40% of the infecting dose remained in the control rats (Table 2). Two rats that had been injected with IR2 failed to produce auto-anti-IgE antibodies before primary infection and were excluded from the

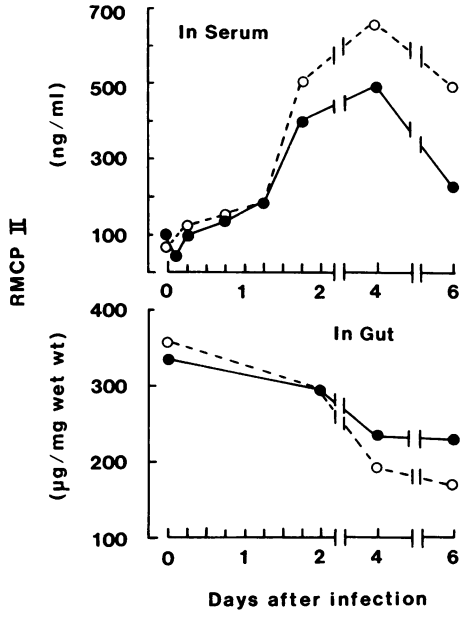

Figure 3. Release of RMCP II into the serum of extracted from gut tissue of control (○- - -○) or IR2-immunized (●——●) rats after secondary infection with *N. brasiliensis*. The animals are the same as those in Fig. 2. Each point is the mean of five to seven values.

analysis (one each on Days 4 and 6). Worm numbers in these rats resembled those of control animals. Inclusion of these rats in the analysis did not change the significance of the results.

In an attempt to elucidate the mechanism of this enhanced anti-parasitic response, we examined the MMC response to secondary infection by histological examination and by RMCP II assessment. At no time was there a significant difference in the number of MMC between the anti-IgE and control groups (Table 2). A more detailed picture of MMC degranulation was obtained by assaying serum levels of RMCP II (Miller *et al.*, 1983). Measurements of RMCP II extracted from gut tissue can be used to assess the remaining MMC.

Prior to reinfection, serum levels of RMCP II were similar in control and anti-IgE groups (Fig. 3). This suggests that any free anti-IgE or IgE–anti-IgE complexes in the IR2-immunized group were not causing a substantial ongoing degranulation of MMC. By 42 hr after reinfection there was the expected rise in serum RMCP II levels in both groups. However, the control animals had substantially more RMCP II than the IgE-immunized group ($P < 0.01$). This differential degranulation between groups was also observed on Days 4 and 6 (Fig. 3).

A reciprocal pattern of RMCP II was found by examining the gut tissue from these same groups of rats. Following reinfection there was a steady decline in RMCP II extracted from gut tissue in both groups, but once again control and experimental groups differed. The controls had significantly more RMCP II remaining in the gut than the anti-IgE rats by Days 4 ($P < 0.05$) and 6 ($P < 0.01$) (Fig. 3). The greater loss of RMCP II from the gut tissue of control rats corresponds with the increased levels of MMC protease in the serum. Thus, the augmented ability of anti-IgE rats to eliminate *N. brasiliensis* does not correspond with the degree of MMC degranulation taking place.

Possibly of more interest was the eosinophil population. This increased dramatically in both groups of animals following primary infection, and was 43% greater in the IgE-immunized group than in the control group at the time of reinfection (cf. Tables 1 and 3).

DISCUSSION

Having established an experimental model in which auto-anti-IgE induction resulted in a decrease in serum IgE (Marshall & Bell, 1985) and a change in mast cell numbers (to be published), the model was tested against a nematode infection. IgE-immunized rats were able to rid themselves of a *N. brasiliensis* infection in an accelerated fashion.

Part of the investigation was devoted to examining the effects of *N. brasiliensis* on the auto-anti-IgE response itself. Anti-IgE autoantibodies were unable to contain the massive rise in serum [IgE] following nematode infection. Since free autoantibodies were not detected following this rise, either the auto-anti-IgE response was in some way switched off, or autoantibodies were present but complexed with IgE and undetected by the assay. The fact that anti-IgE was not detected in sera containing high levels of native IgE confirms the specificity of the anti-ε antibodies.

Dessein *et al.* (1981) previously claimed that IgE synthesis was suppressed, even after a nematode infection, by giving neonatal rats repeated injections of a heterologous anti-IgE. However, the suppression of IgE was not substantiated by another group (Bazin & Pauwels, 1982). It was more likely that the suppressed IgE levels that persisted throughout a *Trichinella spiralis* infection in the Dessein experiments were directly attributable to the continued presence of the heterologous anti-IgE serum.

In a detailed study of mast cell changes after IR2 immunization (to be published), it was observed that MMC increased in number. This expansion of MMC in auto-anti-IgE rats was confirmed in the present experiments and took place before *N. brasiliensis* infection. However, the nematode infection overwhelmed any control there may have been of MMC; by the time of reinfection MMC were four to five times more numerous in both groups, and the differential between the auto-anti-IgE and control rats was lost. It was interesting to note that there was now a significantly increased number of eosinophils. In view of the fact that mast cells are a rich source of eosinophil chemotactic factors (Kay, 1985), it is interesting to speculate whether the enlarged population of eosinophils was orchestrated by the heightened levels of MMC stimulated by auto-anti-IgE induction before primary *N. brasiliensis* infection.

Despite the apparent loss of regulation over IgE synthesis and MMC expansion, the auto-anti-IgE induction still influenced the course of *N. brasiliensis* infection. The IR2 immunization lead to a reduction in the number of *N. brasiliensis* worms in the intestine 8 days after a primary infection—a small effect that was spread among the first four gut segments. Following secondary infection, the reduction of worms in the anti-IgE group of rats was first apparent by Day 4, although not by Day 2, suggesting that larvae were not held up in the lungs or otherwise prevented from reaching the gut. By Day 6 auto-anti-IgE had already completed the stage of 'rapid expulsion' while the control animals were just entering it. As a caveat, we realize that our timing may have been fortuitous since worm loss in primed rats can occur within a few hours (Jarrett, Jarrett & Urquart 1968). The accelerated elimination in the anti-IgE-producing rats could therefore represent a relatively modest enhancement, but nonetheless of great interest.

Since specific IgE, MMC and eosinophils have all been implicated in the control and elimination of nematodes (e.g. Capron *et al.*, 1986), theoretically the auto-anti-IgE effect could operate through one or more of these elements. For example, *N. brasiliensis*-bound IgE could become the target of the autoantibody. Complement activation by a IgG class anti-IgE could augment killing of the worm, or, at the mast cell level, auto-anti-IgE could facilitate degranulation. It is now known that eosinophils, platelets and macrophages also have Fc receptors for IgE (Capron *et al.*, 1986), extending the possible targets that could be activated by auto-anti-IgE. Studies using schistosomula larvae *in vitro* demonstrated that heterologous anti-IgE antibodies enhanced the killing ability of eosinophils and platelets (Capron *et al.*, 1986). Whether the continuous presence of low levels of auto-anti-IgE or IgE–anti-IgE complexes would have a similar effect will require further investigation.

It was clear, however, that at the time of secondary infection, a greater number of eosinophils was present in the small intestine of IR2-immunized rats than in controls. Even if the activity of individual eosinophils was not affected, the 47% increase in numbers could have been sufficient to enhance worm elimination.

Another factor that could bear on the results relates to the saturation of IgE receptors on cells in the IR2-immunized rat. Free Fc receptors on MMC and/or eosinophils in rats with low serum IgE levels could lead to more effective states of MMC/eosinophil sensitization once parasite-induced IgE synthesis was underway. Such cells could be more responsive to worm antigen in a secondary infection.

A major problem in interpreting the data is that the basic mechanism by which the rat rids itself of nematodes remains unclear. It is often assumed that worms are expelled by peristalsis initiated by an immediate-type hypersensitivity reaction (Askenase, 1980), although to our knowledge this mechanism remains unproven. There is clearly a massive degranulation of MMC prior to primary (Woodbury *et al.*, 1984) and secondary expulsion (Fig. 3; Miller *et al.*, 1983; Woodbury & Miller, 1982) and an increase in permeability of the gut lining (King & Miller, 1984). Two observations from the current experiments are worth highlighting. Firstly, by assaying RMCP II levels as a measure of MMC degranulation during a secondary infection, it was found that massive RMCP II release was first detected by 42 hr, and maximal by Day 4, periods well before there was any evidence of worm elimination. Furthermore, higher levels of RMCP II were observed in the control than in the auto-anti-IgE rats, which does not correlate with an augmented loss of worms in the latter group. Either the amount of MMC degranulation was not related to the effectiveness of eliminating the nematode, or an alternative mechanism was activated in the auto-anti-IgE rats. Thus, it would appear that the contents of MMC were at best indirectly involved in ridding the host of the nematode.

A second point arises from observing the distribution of worms along the small intestine of the control animals. The only substantial shift of worms between segments was recorded on Day 6 of the secondary infection—not, as one might expect, a movement distally, but rather towards the stomach, a well-

recognized pattern (Alphey, 1970). One would expect during 'expulsion' that the worm burden would redistribute in large numbers to lower segments. It was apparent while the worm counting was in progress that the control rats were very close to, if not in the middle of, the 'rapid expulsion' stage, yet such a redistribution was never seen. Either the passage of some 900 worms was so rapid as to be missed entirely, or an alternative mechanism of worm loss was operating. In view of the acute inflammatory response in progress, the potential activation of macrophages, platelets and eosinophils and the concomitant increase in permeability into the gut lumen, perhaps many of the worms were completely destroyed *in situ*. Experiments to examine this hypothesis are currently in progress.

The ability of auto-anti-IgE induction to influence the fate of a nematode highlights the delicate balance struck between parasite and host. It will be interesting to elucidate the mechanism of the anti-IgE autoantibodies and to see whether these are able to disrupt this relationship in favour of the host in other parasitic infections.

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