

Hypersensitivity reactions in the small intestine

I Thymus dependence of experimental 'partial villous atrophy'

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SUMMARY Rats infected with the intestinal nematode *Nippostrongylus brasiliensis* have crypt hyperplasia with villous atrophy in affected areas of the small intestine. In thymus-deprived (B) rats the course of infection is prolonged but, despite the presence of many worms in the intestinal lumen, villi and crypts appear largely normal. This suggests that the tissue damage associated with *N. brasiliensis* infection is caused, not by the worms, but by a local thymus-dependent immune reaction. There is some evidence to implicate lymphocytes rather than antibodies in this reaction.

It is already known that T-cell-associated damage to the small intestine, such as occurs in allograft rejection, produces subtotal villous atrophy. The present findings suggest that when T cells react locally with helminth antigens a similar type of damage occurs. The presence of a local cell-mediated immune reaction may be the common factor which causes villous atrophy and crypt hyperplasia in many small intestinal diseases, eg, viral enteritis, giardiasis, cow's milk allergy, and coeliac disease.

The terms partial and subtotal villous atrophy refer to a group of abnormal features of jejunal morphology—low or absent villi, long crypts of Lieberkühn, and increased lymphoid cell infiltration of the mucosa (Shiner and Doniach, 1960). These lesions are common to many different clinical and experimental conditions, including coeliac disease (Shiner and Doniach, 1960), cow's milk allergy (Savilahti, 1973), viral gastroenteritis (Schreiber, Blacklow, and Trier, 1973; Barnes and Townley, 1973), giardiasis (Zamchek, Hoskins, Winawer, Broitman, and Gottlieb, 1963), helminth infection (Symons, 1965; Barth, Jarrett, and Urquhart, 1966), and allograft rejection of small intestine (Holmes, Klein, Winawer, and Fortner, 1971; Ferguson and Parrott, 1973). This suggests that a single effector mechanism may cause the morphological changes in all of these situations, and, since the agents listed are capable of provoking local immune responses, the mechanism could be immunological. We have investigated this by using *Nippostrongylus brasiliensis* infection of rats as an experimental model and have studied small intestinal morphology in thymus-deprived rats infected with *N. brasiliensis*. The results described below support

the idea that the occurrence of crypt hyperplasia with villous atrophy may be incidental to an immune reaction which takes place in the small intestinal mucosa.

Materials and Methods

Outbred female hooded Lister rats were used (Animal Suppliers (London) Ltd). Thymus-deprived (B) rats were prepared by thymectomy at five weeks (Ferguson, 1973), followed four weeks later by 850r whole-body irradiation with shielding of the feet to allow autorepopulation of bone marrow (Parrott and Ferguson, 1974).

Nippostrongylus brasiliensis infection

Rats were infected between one and three months after irradiation. Normal and B rats were infected by a subcutaneous injection of 4000 *N. brasiliensis* larvae (from a culture maintained at the Wellcome Laboratories for Experimental Parasitology). Culture of larvae, faecal egg counts, and intestinal worm counts were performed by standard techniques (Jennings, Mulligan, and Urquhart, 1963; Jarrett, Jarrett, and Urquhart, 1968).

Animals were killed between six and 20 days after infection. Blocks of small intestine were taken at

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20 cm from the pylorus, were fixed in formol saline, paraffin embedded, and 6μ sections were cut along the long axis of the intestine. The sections were stained with haematoxylin and eosin and examined with a Leitz Ortholux microscope. Measurements of villous height and crypt depth were obtained with a micrometer eyepiece and the villus height : crypt depth (v/c) ratio is used below to indicate the extent of morphological abnormality in each specimen examined.

Experiments and Results

As part of a series of experiments on the thymus dependence of reagin production in rats (Jarrett and Ferguson, 1974), we infected several groups of normal (N) and thymus-deprived (B) rats with *N. brasiliensis*. This parasite produces villous atrophy, crypt hyperplasia, and oedema at the site of infection (Symons, 1965; Barth *et al*, 1966) (fig 1), worms are spontaneously expelled in an immunological reaction (the 'self-cure' phenomenon) starting on the eleventh to twelfth day after infection (Jarrett *et al*, 1968) and within a few days intestinal morphology returns to normal. T cell depletion of rats has been shown to prolong the course of infection (Ogilvie and Jones, 1967; Wilson, Jones, and Leskowitz, 1967), and in our experiments substantial numbers of worms were present in the small intestines of many B rats beyond the time when they would normally have been expelled. Thus, of 30 B rats killed and examined between 13 and 20 days after infection, 16 were found to have more than 500 worms in the small intestine. Histological examination of the jejunum in these 16 animals showed that in five cases the morphology was similar to that of infected normal

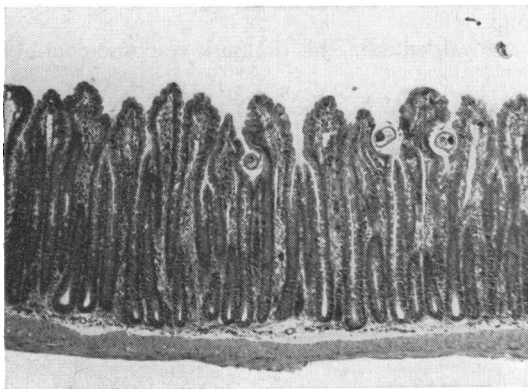


Fig 1 Morphology of the rat small intestine on the 10th day after infection with 4000 *N. brasiliensis* larvae. v/c ratio = 0.8. H & E \times 150.

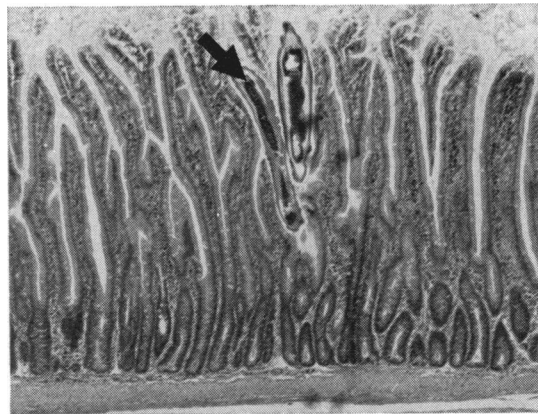


Fig 2 Completely normal morphology of the small intestine on the 19th day after infection of a thymus-deprived rat with 4000 *N. brasiliensis* larvae. Worms are present in close proximity to the epithelium (arrowed) but the v/c ratio is 2.2. H & E \times 150.

rats with v/c ratios of less than 1.2. However, in the remaining 12 animals, worms could be seen in proximity to the epithelium, but the morphological appearance of crypts, villi, enterocytes, and lamina propria appeared essentially normal (fig 2) and v/c ratios were in the normal range (fig 3).

In a further experiment six B and six normal (N) rats of the same age were each infected with 4000

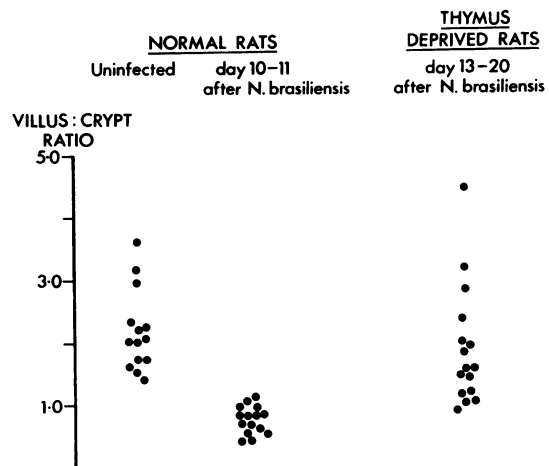


Fig 3 Ratios of villus height : crypt depth in the small intestines of 14 normal rats, 15 immunologically intact rats infected with *N. brasiliensis* and studied just before the onset of self-cure (day 10-11), and 16 thymus-deprived rats with impaired helminth immunity and delayed self-cure (worm counts > 500 per rat when examined 13-20 days after infection).

N. brasiliensis larvae. The animals were killed and examined at intervals from six to 15 days after infection. Results of worm counts and villus: crypt measurement are summarized in table I. Infection followed the usual course in the normal rats, with a sharp drop in worm counts between days 11 and 13, and with low v/c ratios. In contrast, worm counts in the B rats remained high up to day 15, and as in the previous experiments intestinal morphology appeared normal with v/c ratios in the normal range.

Since depletion of T-cells also leads to a marked depression of the production of IgE antibodies to the parasite (Jarrett and Ferguson, 1974; Ogilvie and Jones, 1967) a further experiment was performed in an attempt to assess the contribution, if any, by these antibodies to the enteropathic effect. Thus five normal and 13 B rats were each infected with 4000 *N. brasiliensis* larvae. Five of the B rats were passively immunized with hyperimmune anti-*Nippostrongylus* serum having a PCA titre of 1:2048, each rat receiving 1 ml of this serum intraperitoneally on days 5, 6, and 7 after infection. All rats were killed 10 days after infection. Details of worm

Days after Infection	Normal Rats		B Rats	
	Worm Count	V/C Ratio	Worm Count	V/C Ratio
6	2100	0.9	1080	1.3
8	1610	0.6	2730	1.9
11	1480	1.2	1690	2.3
13	11	1.1	2186	1.9
15	5	2.8	1140	1.7
15	23	2.6	2180	1.8
Untreated, uninfected rats (n = 14)	mean = 2.18 SD = 0.65			

Table I *Small intestinal worm counts and villus height: crypt depth (v/c) ratios in normal and thymus-deprived (B) rats infected with 4000 N. brasiliensis larvae*

Experimental Group	Number of Rats	Mean Worm Count	V/C Ratios
Normal rats	5	1186	0.63 0.77 0.85 1.00
Thymectomized irradiated (B) rats	8	1112	0.66 1.34 0.75 1.64 1.23 1.75 1.32 2.00
B rats, passively immunized with hyperimmune anti- <i>Nippostrongylus</i> serum	5	750	0.85 1.20 1.42 1.59 1.91

Table II *Worm counts and villus height: crypt depth (v/c) ratios in rats infected with 4000 N. brasiliensis larvae and killed 10 days later*

counts and histology are summarized in table II. The results indicate that passive immunization of B rats with hyperimmune serum did not restore the worm-associated tissue damage. The small intestinal morphological changes which are usually found in *N. brasiliensis* infection were present in all five normal rats, in two of the eight B rats, and in one of the passively immunized B rats. These results are entirely consistent with the previous findings, ie, in around 70% of B rats *N. brasiliensis* infection does not cause villous atrophy and crypt hyperplasia.

Discussion

There are striking changes in the rat small intestine at the site of infection with the nematode parasite *Nippostrongylus brasiliensis*. Crypts of Lieberkühn are elongated and there is increased crypt cell proliferation (Symons, 1965); examination of the mucosal surface shows that villi are short and fused, inter-villous ridges are prominent, and the luminal surface may appear convoluted or flat in heavily infected animals (Loehry and Creamer, 1969); cell loss from this abnormal mucosa is considerably increased (Loehry, Croft, Singh, and Creamer, 1969). However, in the experiments described above, small intestinal morphology was essentially normal in 70% of *N. brasiliensis*-infected, thymus-deprived rats, even though there were substantial numbers of worms in close proximity to the intestinal epithelium. This finding indicates that the crypt hyperplasia and villous atrophy of *N. brasiliensis* infection are associated with a thymus-dependent immune reaction in the small intestine, and are not simply due to toxic effects of the presence of worms. It is likely that the tissue damage which does occur in 30% of the B rats is due to residual T cell function, but further experiments will be needed to confirm this point.

A local 'enteropathic' immune response could be

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mediated by antibodies and/or lymphocytes, and indeed it is known that IgE antibodies, associated with helminth infection and immunity, are thymus-dependent (Ogilvie and Jones, 1967; Michael and Bernstein, 1973; Jarrett and Ferguson, 1974). However, our attempt to replace this latter component by passive immunization with hyperimmune anti-*Nippostrongylus* serum did not restore the capacity of worm infection to damage the small bowel in thymus-deprived rats. This provides limited evidence implicating cellular rather than humoral factors as the cause of the intestinal lesion in immunologically intact animals.

Villous atrophy and crypt hyperplasia have been shown to result from the local immune reactions of allograft rejection of small bowel (Holmes *et al.*, 1971; Ferguson and Parrott, 1973), and graft-versus-host disease (Reilly and Kirsner, 1965). In mice, allograft rejection is a thymus-dependent phenomenon (Ferguson and Parrott, 1973), and, since the morphological changes precede the appearance of serum antibodies to graft antigens (Elves and Ferguson, 1974), it is likely that the tissue damage is mediated by lymphocytes. In rejection of allografts, the antigens involved are those of the small intestinal tissues *per se*, and it is perhaps not surprising that this cell-mediated immune reaction causes rapid extrusion of enterocytes and crypt cell proliferation. We have now shown that clearly defined effects on crypts and villi also occur in association with the local immune response to the antigens of *N. brasiliensis*, and would like to suggest that hypersensitivity due to a local T-cell-mediated immune response may be the cause of villous atrophy and crypt hyperplasia in other situations. It remains to be shown whether this has its effect by means of cytotoxic T lymphocytes, by secretion of an enteropathic lymphokine, in association with antibody, eg, IgE, or even, in some instances, via thymus-dependent cells other than lymphocytes, such as mast cells (Miller, 1969).

Although no physiological role for intestinal T cells has yet been demonstrated, it is likely that T-cell-mediated immune reactions can be mounted in the intestine, either as part of a protective immune response to microorganisms, parasites, or tumours or as a result of allergy to drugs or foods. We suggest that this cellular immune reaction alters small intestinal morphology and probably impairs its functions. Such an effect would be transient and relatively harmless in situations where the antigen can be rapidly eliminated, as in viral gastroenteritis but would be intense, severe and longstanding where antigen exposure is continuous as in untreated cow's milk allergy and coeliac disease.

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