Enhancement of apoptosis with loss of cellular adherence in the villus epithelium of the small intestine after infection with the nematode *Nippostrongylus brasiliensis* in rats

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(*Received 14 December 1998; revised 2 March 1999; accepted 8 March 1999*)

SUMMARY

It has been reported that infection with *Nippostrongylus brasiliensis* induces villus atrophy with various histological alterations. In *N. brasiliensis*-infected rats, villus length in the jejunum was reduced significantly at day 10 p.i., when serum levels of rat mast cell protease (RMCP) II had increased significantly. To determine whether the villus atrophy is associated with enhancement of apoptosis, apoptotic nuclei were labelled using the nick end-labelling method. Numbers of labelled cells were markedly increased in the villus epithelium at 7–10 days p.i., while the numbers returned to normal 14 days p.i. when worms were rejected from the intestine and villus length became normal. Examination of the expression of the adhesion molecule E-cadherin showed granular immunoreactivity in the cytoplasm of atrophic villus epithelium with loss of normal localization to epithelial cell borders. In mast cell-deficient *Ws/Ws* rats, villus length was reduced as significantly as in +/+ counterparts at day 10 p.i. with marked increases in the numbers of apoptotic cells. These results suggested that villus atrophy was closely associated with enhanced apoptosis and loss of adhesion in epithelial cells. Mast cell activation appears not to be involved in these alterations.

Key words: nematode, apoptosis, E-cadherin, small intestine, *Ws/Ws* rat, mast cell.

INTRODUCTION

Intestinal villus atrophy with or without crypt hyperplasia has been reported in a number of intestinal diseases such as coeliac disease, giardiasis, intestinal helminth infection, autoimmune enteropathy, graft-versus-host disease, and during allograft rejection of transplanted small intestine (MacDonald, 1992). In the normal intestine, epithelial cells are derived from stem cells at the base of the crypt and migrate along the crypt-villus axis as they differentiate (Potten & Allen, 1977). On reaching the villus tip, epithelial cells detach and are shed via a form of apoptosis (Gavrieli, Sherman & Ben, 1992; Frisch & Francis, 1994). Therefore, it is of interest to determine whether apoptosis is up-regulated in the pathological states in which villus atrophy evolves. In fact, it was reported that apoptosis was enhanced significantly in active coeliac disease which is characterized by flat mucosa with marked villus atrophy (Moss *et al*. 1996). Infection of rodents with the nematode *Nippostrongylus brasiliensis* induces partial villus atrophy and crypt hyperplasia in the jejunal mucosa together with malabsorption before and around the time of immunological worm rejection from the intestine (Symons, 1965; Ferguson & Jarrett, 1975; Nolla, Bristol & Mayberry, 1985; Lunn *et al*. 1986; Perdue *et al*. 1989). Although it has been demonstrated that villus atrophy after *N. brasiliensis* infection was associated with detachment or separation of epithelial cells from the lamina propria (Perdue *et al*. 1989), the role of apoptosis in the progression of the pathological state has not been elucidated. Ferguson & Jarrett (1975) reported that villus atrophy did not develop in thymus-deprived rats after *N. brasiliensis* infection and suggested that the tissue damage was caused not by the worms, but by a local thymus-dependent immune reaction. It has also been postulated that mediators released from activated mast cells, such as rat mast cell protease type II (RMCP II), and leukotriene (LT) B4, LTC4 and LTE4, might result in epithelial damage (Woodbury *et al*. 1984; Perdue *et al*. 1989; D'Inca *et al*. 1990). On the other hand, no association has been observed between RMCP-II and the mucosal damage during anaphylaxis in rats sensitized by nematode infection and challenged with worm antigen (Scudamore *et al*. 1995).

In the present study, to determine whether villus atrophy induced by *N. brasiliensis* infection is associated with dissociation of cell–cell attachment

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https://doi.org/10.1017/S003118209900462X Published online by Cambridge University Press

and acceleration of apoptosis, we examined the occurrence of apoptosis in atrophic villus epithelium using a nick end-labelling method, and the expression of the epithelial cell adhesion molecule E-cadherin immunohistochemically. Further, the role of mucosal mast cells in the pathogenesis of villus atrophy was studied using genetically mast cell-deficient *Ws/Ws* rats and their +/+ counterparts. *Ws/Ws* rats show a 12-base deletion in the tyrosine kinase domain of c-*kit* cDNA, and are deficient in both mucosal and connective tissue-type mast cells (Tsujimura *et al*. 1991; Arizono *et al*. 1993).

MATERIALS AND METHODS

Animals and N. brasiliensis *infection*

Specific pathogen-free male Brown Norway/Sea (BN) rats were purchased from SLC Inc. (Shizuoka, Japan). *Ws/Ws* rats were produced as described previously (Arizono *et al*. 1993). Animals, 8–10 weeks of age, were injected subcutaneously with 2000 *N. brasiliensis* L3 larvae as described previously (Arizono *et al*. 1993). To evaluate adult worm burdens, the small intestine was removed on day 7, 10, 14 or 28 days p.i., opened longitudinally and incubated in saline at 37 °C for 3 h. The numbers of emerging worms were counted under a dissecting microscope.

Tissue preparation

Animals were sacrificed by overinhalation of ether at various time-points after infection. A segment of the jejunum 20 cm distal to the pyloric ring was removed, opened longitudinally, fixed in 4% buffered formalin overnight or in Carnoy's fluid for 3 h, and embedded in paraffin. Sections 4 μm thick were cut and mounted on poly-L-lysine-coated slides.

Histochemistry for alkaline phosphatase (*ALP*)

ALP activity in the brush border of the small intestine is well preserved in formalin-fixed paraffin-embedded sections (Lake, 1991). Histochemical demonstration of ALP was carried out on paraffin-embedded sections using the indoxyl-tetrazolium method (Borgers, Lewis & Stoward, 1991). Briefly, sections were dewaxed and incubated in medium containing 0·46 mM 5-bromo-4-chloro-3-indoxyl-phosphate toluidine salt, 0·37 mM Nitro-BT and 0·1 M Tris–HCl buffer, pH 9·2, for 15 min. Sections were rinsed in distilled water and mounted in glycerine–gelatine. For control studies, the enzyme substrate, 5-bromo-4-chloro-3-indoxylphosphate toluidine salt, was omitted from the reaction medium, or sections were pre-incubated with L-phenylalanine, an inhibitor of ALP, at concentrations of 50–200 mM for 30 min at 37 °C, followed by incubation with reaction medium containing 50–200 mM L-phenylalanine for 20 min.

Immunohistochemistry

Immunohistochemical staining for RMCP II was carried out on formalin-fixed sections, and that for proliferating nuclear antigen (PCNA) was performed on Carnoy's fluid-fixed sections. Sections were treated with 0·3% H_2O_2 for 20 min, followed by incubation with 10% normal goat serum for 10 min. Sections were then incubated with anit-RMCP II rabbit serum (1:500 dilution: Arizono *et al*. 1987), or with mouse monoclonal antibody against PCNA (PC10; 1:200 dilution; Jackson Lab. Inc., West Grove, USA) for 1 h. Slides for RMCP II were followed by incubation with peroxidase-labelled anti-rabbit IgG goat serum (Zymed Laboratories Inc.) for 1 h, and those for PCNA by incubation with goat anti-mouse immunoglobulins conjugated with peroxidase labelled-dextran polymer (Envision+®, peroxidase, mouse, Dako, Carpinteria, CA) for 30 min. For detection of E-cadherin, wet autoclave pre-treatment was employed for antigen retrieval according to the method described by Bankfalvi *et al*. (1994). Briefly, dewaxed sections were treated with 0·3% H_2O_2 for 20 min, then immersed in 0·01 M sodium citrate buffer, pH 6·0, in plastic Coplin jars and incubated in a YS-A-C105 autoclave (Yuyama Co., Toyonaka, Japan) at 121 °C for 10 min. After autoclaving, the slides were allowed to cool to room temperature for 20–30 min, followed by rinsing 3 times in PBS. Sections were then incubated with anti-E-cadherin mouse IgG2a (1:50 dilution, Transduction Laboratories, Lexington, KY, USA) for 30 min at 37 °C, followed by further incubation with goat anti-mouse immunoglobulins conjugated with peroxidase labelled-dextran polymer. The slides were finally incubated in 0·05 M phosphate buffer (pH 7·4) containing 0·2 mg/ml 3,3-diamino-benzidine tetrahydrochloride (DAB: Dojin Lab., Kumamoto, Japan) and 0·005% hydrogen peroxide. For control sections, primary antibody was omitted, or species- or isotype-matched normal antibodies were employed.

Detection of apoptosis

Apoptotic cells with DNA fragmentation were detected by the terminal deoxynucleotidyl transferase (TdT)-mediated deoxyribonucleoside triphosphate (dNTP)-biotin nick end-labelling method using a TdT-FragEL™ DNA fragmentation detection kit (Calbiochem, Cambridge, MA, USA). Briefly, paraffin-embedded sections were dewaxed, incubated with proteinase K, and treated with 3% H_2O_2. Subsequently, the sections were incubated with a reaction mixture containing TdT with

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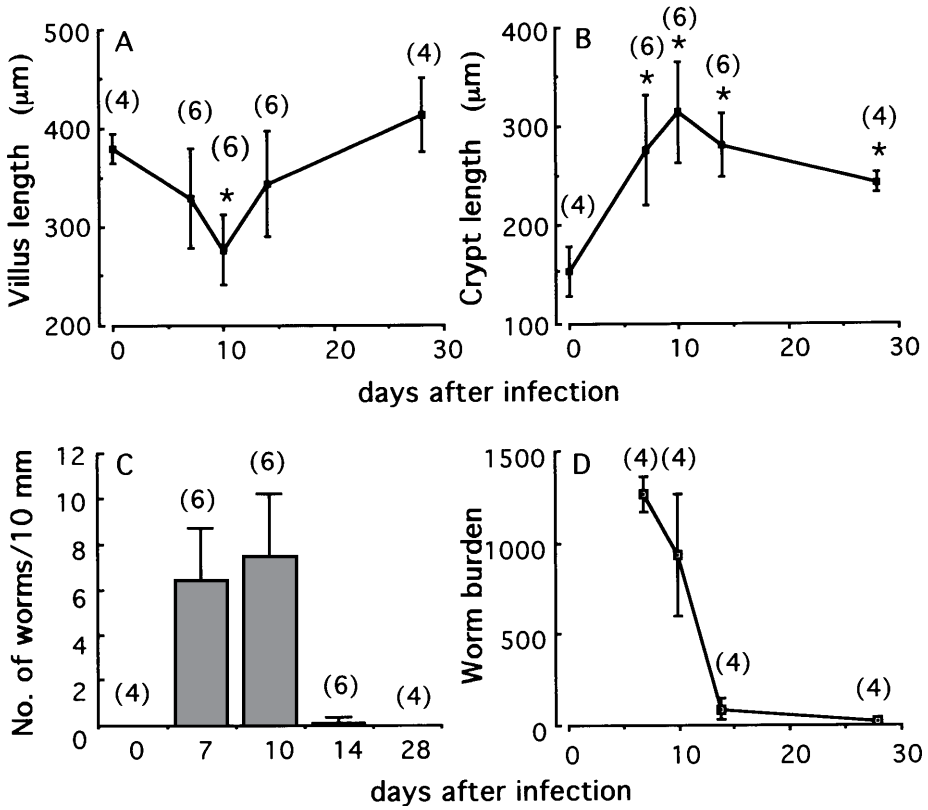

Fig. 1. Changes in villus length (A), crypt length (B) and numbers of worms (C) in sections of the jejunum, and worm burdens (D) in the small intestine of BN rats after *Nippostrongylus brasiliensis* infection. A, B and C were examined using the same sections. Worm burdens (D) were examined using a different group of rats. Numbers of animals examined are indicated in parentheses. Data represent means ± S.D. *Significantly different from day 0 ($P < 0{\cdot}05$).

biotinylated dNTP at 37 °C for 90 min, followed by incubation with streptavidin–peroxidase for 30 min. Finally, the DAB reaction was carried out.

Measurement of the lengths of jejunal villi and crypts

The lengths of villi and crypts were measured on haematoxylin and eosin-stained sections of the jejunum obtained 20 cm distal to the pyloric ring. Lengths of villi and crypts were defined as those from the villus tip to the ostium of the crypt and from the ostium to the bottom of the crypt, respectively. Twenty villus-crypt units, which were cut exactly perpendicularly, were selected from 1 section per animal, and lengths of villi and crypts were measured directly under a microscope using an ocular lens with a micrometer. The average length in each section was employed as the representative length in a given animal, and means ± S.D. of the average lengths of 4–6 animals were calculated.

Counts of worms in sections

Numbers of sections of worms lying close to the mucosa in haematoxylin and eosin-stained sections of the jejunum were counted under a microscope. The length of sections of the jejunum was measured using an electric digitizer (2-dimensional measuring software, Nikon cosmozone; Nikon, Tokyo, Japan), and the numbers of worm sections/10 mm length of tissue were determined. Jejunal sections corresponding to 43–60 mm were analysed in each animal.

Quantitative evaluations of staining of ALP, E-cadherin, PCNA and apoptosis

Intensities of ALP staining of each villus were graded subjectively as follows: 4, intense staining in the brush border of the whole of the villus; 3, staining slightly reduced at least in part of the villus; 2, staining moderately reduced at least in part of the villus; 1, complete absence of staining at least in part of the villus. Thirty villi in 1 section per animal were analysed, and the average grade in the section was designated as the ALP score of a given animal. Reduction of E-cadherin expression at epithelial cell borders with concomitant appearance of granular staining in the cytoplasm was observed in some villus tip cells. Thus, E-cadherin expression in each villus was graded as follows: 4, normal expression; 3, reduced expression in 1–5 cells in the villus tip; 2, reduced expression in 6–10 cells; 1, reduced expression in more than 11 cells. E-cadherin expression was analysed in 30 villi in 1 section per animal, and the average grade was designated as the E-cadherin score for a given animal. As an index of cell growth, numbers of PCNA-positive as well as negative cells

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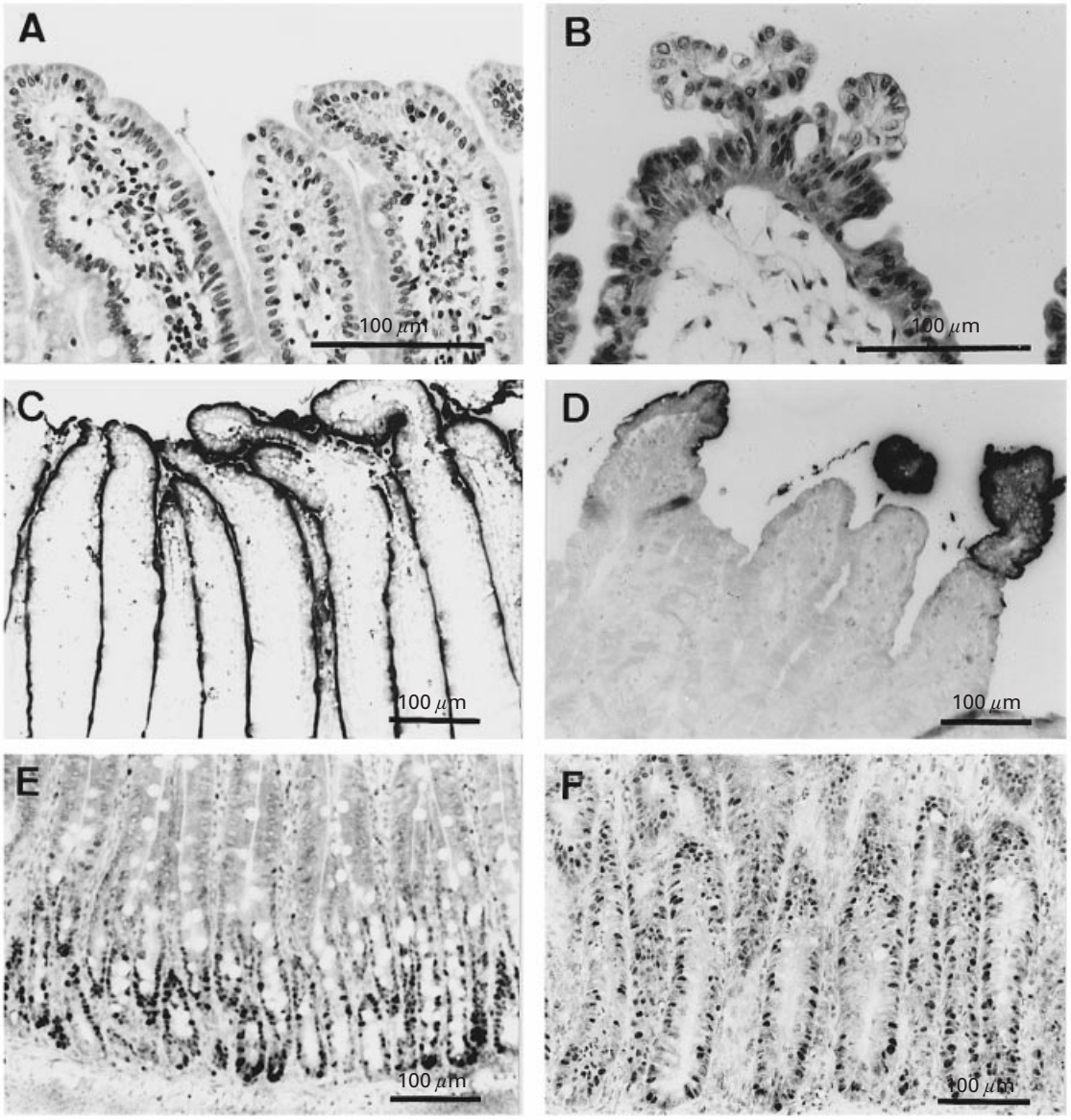

Fig. 2. Histological alterations in the jejunal epithelium before (A, C, E) and 10 days after (B, D, F) *Nippostrongylus brasiliensis* infection in BN rats. (A, B) Haematoxylin and eosin staining, showing the upper part of villi before (A) and after (B) infection. (C, D) Alkaline phosphatase histochemistry, showing intense activity in the brush border of uninfected animal (C) and marked reduction of the activity after infection (D). (E, F) PCNA immunohistochemistry, showing distribution of PCNA-positive nuclei in crypts before (E) and after infection (F).

in the epithelium were counted in 10 villus-crypt units in 1 section per animal, and the percentage of the total number of positive cells relative to the total epithelial cell number was employed as the PCNA score. As an index of apoptosis, the numbers of nick-end labelling-positive cells were counted in the epithelium of 10 villi in 1 section per animal without including crypt epithelial cells since enhancement of apoptosis appeared always in the upper part of the villi. The percentage relative to the total number of positive cells in total villus epithelial cell numbers was designated as the apoptotic score.

ELISA for RMCP II

Determination of the levels of RMCP II in sera was carried out using an ELISA kit (Moredun Animal Health Ltd, Edinburgh, UK), according to the manufacturer's instructions.

Statistical analysis

The Mann-Whitney *U*-test (two-tailed) was employed for statistical analysis, and a P value of less than 0·05 was considered significant.

RESULTS

Villus atrophy induced by N. brasiliensis *infection*

To examine the time-course of villus atrophy after *N. brasiliensis* infection, the length of the jejunal villi

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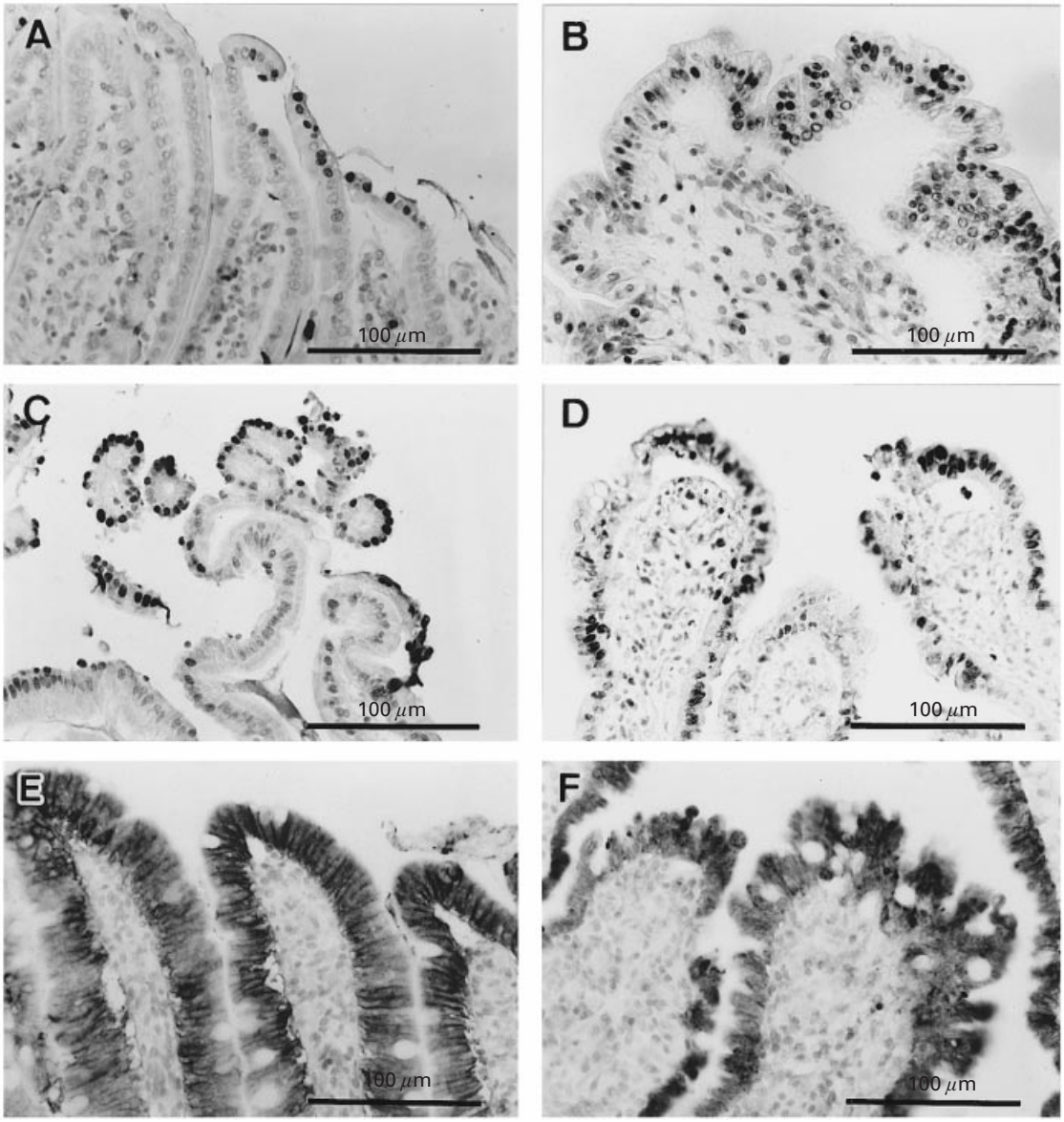

Fig. 3. Occurrence of apoptosis and expression of E-cadherin in the jejunal epithelium before (A, E) and 10 days after *Nippostrongylus brasiliensis* infection (B, C, D, F) in BN (A, B, C, E, F) and *Ws/Ws* rat (D). (A–D) Nick end-labelling method showing apoptotic nuclei. In control animals (A), a few cells were labelled at the villus tip, while at day 10 p.i. (B, C and D), numbers of labelled nuclei were markedly increased. (E, F) E-cadherin immunohistochemistry, showing localization at lateral cell borders in normal villi (E), and granular staining in the cytoplasm with loss of normal localization after infection (F).

obtained 20 cm from the pyloric ring of BN rats was measured 7, 10, 14 and 28 days p.i. Villus length was slightly decreased 7 days p.i. and significantly decreased 10 days p.i. (Fig. 1A). The villus atrophy was transient and returned to normal as early as 14 days p.i., when the numbers of worms in the same sections as well as worm burdens in the small intestine were markedly decreased (Fig. 1C, D). On the other hand, crypt length was increased significantly 7 days p.i., and the elongation of crypts was observed at least until 28 days p.i. (Fig. 1B).

A variety of histological alterations was observed in the villus epithelium of the jejunum at 7 and 10 days p.i. These included vacuolation and/or basophilic alteration of the cytoplasm and separation and shedding of the epithelium from the lamina propria mucosa together with a decrease in villus length (Fig. 2A, B).

The brush border enzyme ALP was examined histochemically on paraffin-embedded sections. Intense ALP activity was observed in the villus brush border of uninfected animals (Fig. 2C). Incubation in the absence of the substrate did not reveal any reaction product, and incubation with an ALP inhibitor, L-phenylalanine, showed marked suppression of the activity. ALP activity at 7–10 days p.i. was reduced partly, if not totally, especially in the middle to lower part of villi, while it was well preserved in the upper part of villi (Fig. 2D). Intensities of ALP activity in sections were graded

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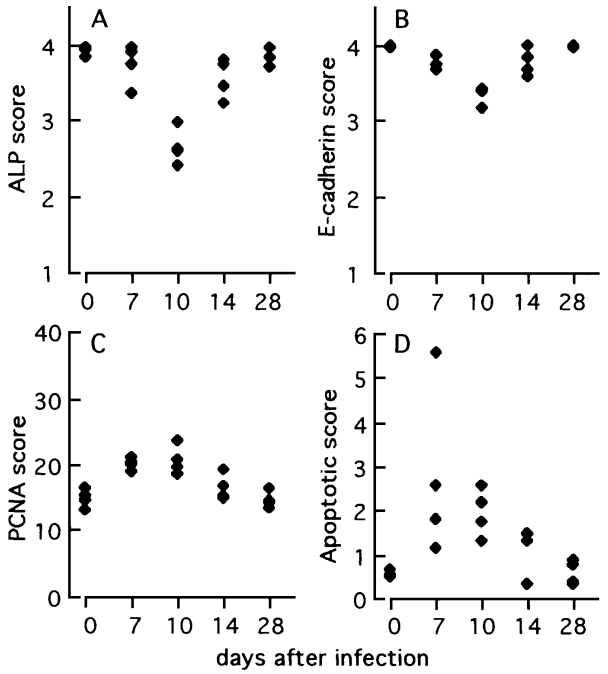

Fig. 4. Alterations of the activity of ALP (A), the expression of E-cadherin (B), and the percentages of PCNA-positive cells (C) and apoptotic cells (D) after *Nippostrongylus brasiliensis* infection. Each score was obtained as described in the Materials and Methods section. Scores in individual animals (4 rats at each time point) are presented in the figures.

subjectively. The scores were decreased markedly at day 10 p.i. and returned to normal 28 days p.i. (Fig. 4A).

To determine whether the numbers of proliferating cells were increased in the crypts after infection, immunohistochemical staining for PCNA, which is synthesized in proliferating cell nuclei mainly in the S phase of the cell cycle (Bravo & MacDonald-Bravo, 1985), was carried out. In uninfected rats, PCNA-positive nuclei were distributed in the lower three quarters of the crypts (Fig. 2E). At 7 and 10 days p.i., the distribution of PCNA-positive cells varied markedly between individual crypts. In some crypts, PCNA-positive cells were distributed throughout the whole of the elongated crypts (Fig. 2F), while in others PCNA-positive cells were found only in the lower half. PCNA scores, the percentage of PCNA-positive cells relative to the total epithelial cell number, were increased 7–10 days p.i., but returned to normal 28 days p.i. (Fig. 4C).

Apoptosis in villus epithelial cells after N. brasiliensis *infection*

Apoptosis in villus epithelial cells was examined using the nick end-labelling method. In normal villi, a few labelled cells were occasionally found at the villus tip (Fig. 3A). In contrast, labelled nuclei were more frequently encountered in the atrophic villus epithelium 7 and 10 days p.i. (Fig. 3B, C, D). Labelled nuclei were found almost exclusively in the epithelial cells of the upper part of villi, some partly detached from and others still attached to the lamina propria mucosa. Despite the increase in number of labelled nuclei, typical apoptotic bodies were rarely found in villus epithelial cells. Apoptotic scores, percentages of labelled cells in total villus epithelial cells, were increased at 7 and 10 days p.i. (Fig. 4D). Fourteen days p.i. and thereafter when villus length was normalized, numbers of labelled cells were markedly decreased.

E-cadherin expression in villus epithelial cells after N. brasiliensis *infection*

Since marked detachment of epithelial cells was observed in atrophic villi, the expression of the major epithelial adhesion molecule E-cadherin was examined immunohistochemically. E-cadherin immunoreactivity was localized mainly to the lateral cell borders of villus epithelial cells in uninfected rats (Fig. 3E). At 7 and 10 days p.i., expression of E-cadherin in the lateral cell borders was reduced in the upper part of some, but not all, atrophic villi. In the cells with loss of normal expression, unusual granular immunoreactivity appeared in the cytoplasm (Fig. 3F). In the lower part of villi, E-cadherin expression was always normal. Scores of E-cadherin expression returned to normal 28 days p.i. (Fig. 4B).

Villus atrophy in mast cell-deficient Ws/Ws *rats after* N. brasiliensis *infection*

To evaluate the role of mast cells in the development of villus atrophy, histological alterations in the jejunum were compared between mast cell-deficient *Ws/Ws* and normal +/+ rats at day 10 p.i. In the *Ws/Ws* rat jejunum, RMCP II-positive mast cells were absent either before or at day 10 p.i. In uninfected +/+ rats, RMCP II-positive mast cells were observed in the lamina propria mucosa of all crypt-villus units (Fig. 5A). However, at day 10 p.i., RMCP II-positive mast cells were reduced in number in the jejunal mucosa. This reduction in number of RMCP II-positive mast cells in +/+ rats occurred in a segmental pattern: in some segments including more than 10 villus-crypt units mast cells were totally undetectable, while in other areas, numbers of RMCP II-positive mast cells were normal or reduced only slightly (Fig. 5B). In Carnoy's solution-fixed alcian blue-stained sections, mast cells were also undetectable in large areas of the jejunum at day 10 p.i. To determine whether the disappearance of RMCP II-positive mast cells in the jejunum of +/+ rats after infection was due to the release of RMCP II, serum levels of RMCP II were examined. In +/+ rats, serum RMCP II levels were increased significantly at day 10 p.i. (Fig. 6).

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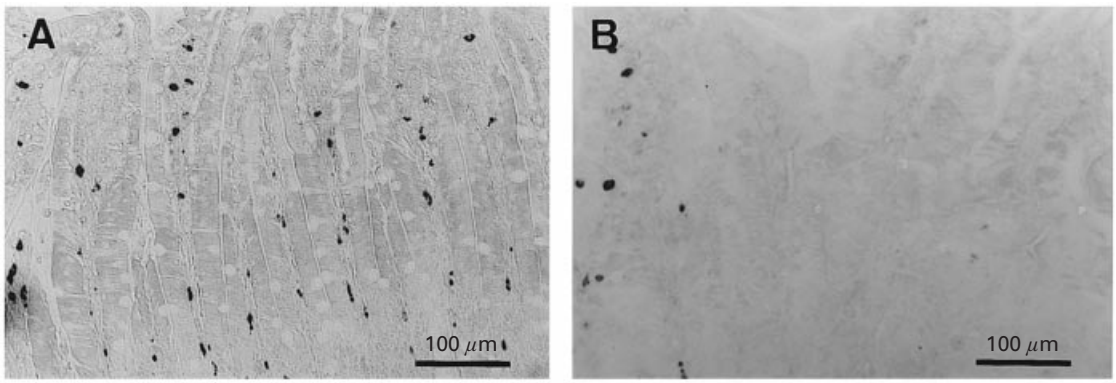

Fig. 5. Immunohistochemical staining of RMCP II in the jejunal propria mucosa before (A) and 10 days after *Nippostrongylus brasiliensis* infection (B) in +/+ rats. Note that numbers of RMCP II-positive mast cells were markedly reduced after infection.

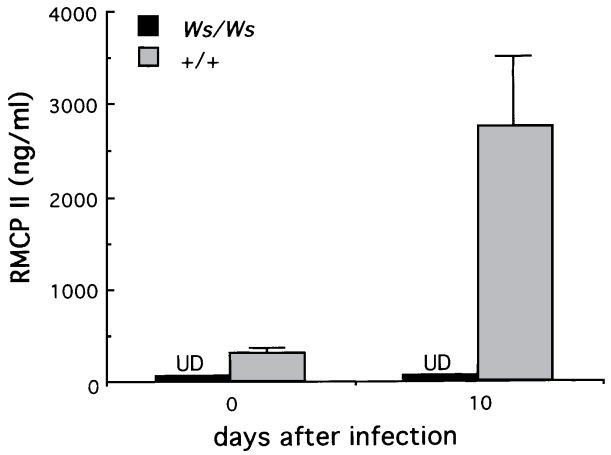

Fig. 6. Serum levels of RMCP II in *Ws/Ws* and +/+ rats. Data shown are means±S.D. of 4 rats. UD: undetectable.

Average length of villi was reduced as markedly in *Ws/Ws* as in +/+ rats. The lengths of crypts and the PCNA scores were increased significantly in both rat strains (Table 1). The nick end-labelling method also showed significant increases in number of apoptotic cells in the villus epithelium of *Ws/Ws* and +/+ rats after infection (Table 1).

DISCUSSION

The present results confirmed the previous report (Perdue *et al*. 1989) that villus atrophy developed after *N. brasiliensis* infection, and that once worms were rejected from the infestation site, villus size rapidly returned to normal. Villus atrophy might evolve either by enhancement of apoptosis and/or necrosis, decrease of proliferation, or imbalance of the two. The results of the present nick end-labelling method showed that in the period of villus atrophy apoptosis was enhanced in the epithelial cells of the upper part of the villi, while this became normal after worm rejection. On the other hand, crypts showed elongation together with an increase in the number of PCNA-positive cells after infection. These results are consistent with previous reports indicating marked elevation of thymidine kinase activity in the jejunal mucosa 7–10 days p.i. (Perdue *et al*. 1989), and faster turnover of villus epithelium after infection (Symons, 1965), suggesting that cell proliferation increases in crypts after infection. These results indicated that in nematode infection enhancement of apoptosis in villi and acceleration of cell proliferation in crypts occurred simultaneously, although it is not clear whether the latter developed secondarily to compensate for the accelerated apoptosis. The observation that villus atrophy did develop despite acceleration of cell proliferation after infection suggests that cell loss due to enhanced apoptosis might have exceeded the cell replacement by proliferation. It remains possible, however, that non-apoptotic cell death, if it occurred in nematode infection, might also contribute to the development of villus atrophy together with enhanced apoptosis. Marked enhancement of apoptosis has been reported in active coeliac disease (Moss *et al*. 1996). The pattern of occurrence of apoptosis observed in the present study, however, was not exactly the same as that described in active coeliac disease, in which apoptotic cells were distributed not only in the flattened mucosal surface epithelium but also deep in crypts (Moss *et al*. 1996). The reason for this difference is not clear at present, but it seems likely that the intensity of apoptosis was related to the severity of villus atrophy: active coeliac disease with total villus atrophy showed extensive apoptosis, while nematode infection in which apoptotic cell distribution was more restricted developed partial villus atrophy.

E-cadherin is expressed on most epithelia and some carcinoma cells of epithelial origin and mediates homophilic, calcium-dependent cell–cell adhesion (Takeichi, 1991). In normal villi, E-cadherin immunoreactivity was localized to lateral and basal borders of epithelial cells of villi. At the time when villus atrophy developed, E-cadherin expression was

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Table 1. Lengths of villi and crypts, PCNA scores, and apoptotic scores in the jejunum of *Ws/Ws* and +/+ rats before and 10 days after *Nippostrongylus brasiliensis* infection

(Data shown are means ± S.D. of 4 rats)

	Ws/Ws		+/+	
	Day 0	Day 10	Day 0	Day 10
Villus length (μm)	477·3 ± 11·3	376·4 ± 27·1*	448·9 ± 31·0	369·3 ± 36·1†
Crypt length (μm)	230·1 ± 24·8	295·4 ± 13·5*	193·7 ± 18·6	271·0 ± 12·0*
PCNA score	15·6 ± 2·6	26·3 ± 0·6*	15·6 ± 1·8	26·7 ± 2·8*
Apoptotic score	0·74 ± 0·10	2·28 ± 2·40*	0·51 ± 0·26	2·55 ± 2·45*

* Significantly different from day 0 from corresponding animals ($P < 0·05$).
† No significant difference from day 0 from corresponding animals.

reduced at cell borders at least in the upper part of some, but not all, villi, with concomitant granular expression of E-cadherin in the cytoplasm of these cells. In the medial and lower parts of villi, normal expression of E-cadherin was usually observed. The decreased expression of E-cadherin in the cell membrane in the upper part of villi suggested that cell–cell adhesion or cell-basement membrane attachment was impaired and shedding of epithelial cells was promoted. Thus, apoptosis and loss of E-cadherin expression appeared to occur simultaneously, although it is unclear whether apoptosis triggered the loss of E-cadherin or *vice versa*, or both developed independently.

It has been reported that the activities of the villus brush border enzymes such as sucrase, maltase and ALP were significantly decreased at day 10 p.i., the time of villus atrophy (Perdue *et al*. 1989). The present histochemical observations confirmed that marked reduction of ALP activity occurred in the atrophic villus epithelium, although the activity was usually preserved at the tips of blunt villi with a decrease of ALP activity in the lower part of the villi. These results suggested that the decrease in ALP activity might not necessarily have been caused by villus epithelial damage or apoptosis, but may reflect regeneration and/or dedifferentiation of epithelial cells.

The mechanisms that trigger enhancement of apoptosis and loss of normal expression of E-cadherin after nematode infection are not yet clear. Mediators from mast cells have been postulated as those that could trigger detachment of epithelial cells, since studies of intestinal anaphylaxis suggested that mast cell activation resulted in epithelial damage (Urquhart *et al*. 1964; Perdue *et al*. 1984; Lake *et al*. 1984; Freier, Eran & Goldstein, 1985; Sjolander & Magnusson, 1987; D'Inca *et al*. 1990). The present results confirmed those of Woodbury & Miller (1982) and Perdue *et al*. (1989) that serum RMCP II levels were elevated together with a decrease in the number of stained mast cells in the propria mucosa 7–10 days p.i., suggesting that mast cell activation occurred during this period. However, as shown in the present study *Ws/Ws* rats developed villus atrophy with accelerated apoptosis as significantly as in +/+ rats. These results indicated that mast cell-derived mediators are not the major factors involved in promotion of villus atrophy, at least in natural *N. brasiliensis* infection. On the other hand, Ferguson & Jarrett (1975) reported that in *N. brasiliensis*-infected thymus-deprived (B) rats, villi and crypts appeared largely normal despite prolonged infection, suggesting that T cell-mediated mechanisms are necessary in the development of villus atrophy. The involvement of T cells has been proposed in the induction of villus atrophy in graft-versus host disease and coeliac disease (MacDonald, 1992). Further, recent studies have shown that intraepithelial lymphocytes promote apoptosis and/or necrosis in the villus epithelium possibly through the Fas/Fas ligand system (Inagaki *et al*. 1997; Guy *et al*. 1998). The mechanisms that trigger increased apoptosis and villus atrophy in nematode infection should be clarified in future studies.

This work was supported by grants from the Ministry of Education and Culture, Japan, and the Toray Research Institute.

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