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Goblet cell mucins as the selective barrier for the intestinal helminths: T-cell-independent alteration of goblet cell mucins by immunologically 'damaged' *Nippostrongylus brasiliensis* worms and its significance on the challenge infection with homologous and heterologous parasites

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SUMMARY

The aim of this study was to examine the role of T cells on the alteration of terminal sugars of goblet cell mucins in the small intestinal mucosa of parasitized rats and to clarify the biological significance of the altered mucins in the mucosal defence against intestinal helminths. For this purpose, *Nippostrongylus brasiliensis* adult worms obtained from donor rats at 7 ('normal' worms) or 13 days ('damaged' worms) post-infection were implanted intraduodenally into euthymic and hypothymic (*rnu/rnu*) rats. Expulsion of implanted normal worms and associated goblet cell changes were extremely delayed in hypothymic recipients compared with euthymic recipients. In contrast, intraduodenally implanted damaged worms were expelled by day 5 regardless of the strains. Around the time of expulsion of implanted damaged worms, euthymic recipients showed both goblet cell hyperplasia and alteration of mucins, whereas hypothymic rats showed only the latter. Dexamethasone treatment completely abolished goblet cell changes of both strains of recipients. To clarify the importance of the constitutional changes of goblet cell mucins in mucosal defence, euthymic rats were primed by implantation of damaged worms to induce goblet cell changes, and then 3 or 5 days later they were challenged by implantation with normal worms. The results show that when goblet cell changes were induced by priming with damaged worms, recipient rats could completely prevent the establishment of normal worms. When hypothymic rats were primed and challenged in the same manner, a similar but slightly less preventive effect was observed. Such a protective effect of altered mucins seems to be selective because priming of euthymic rats with damaged *N. brasiliensis* did not affect the establishment of *Strongyloides venezuelensis*. These results suggest that: (1) once *N. brasiliensis* adult worms are 'damaged' by the host's T-cell-dependent immune mechanisms, they can induce alteration of sugar residues of goblet cell mucins via host-mediated, T-cell-independent processes; (2) the expression of such altered mucins is highly effective not only in causing expulsion of established damaged worms but also in preventing establishment of normal worms; and (3) the preventive effect of altered mucins is selective against parasite species.

INTRODUCTION

Mucosal defence against intestinal helminths such as *Nippostrongylus brasiliensis* and *Strongyloides* species seems to be mediated by different systems, because after concurrent infection in rats they were expelled separately with their own kinetics.[1] Moreover, Mongolian gerbils were able to expel *N. brasiliensis* but were unable to expel *S. ratti*[2] or *S. venezuelensis*.[3] This selective protection seems to be related to the peculiar phenotypes of mucosal mast cells of Mongolian gerbils.[4] The possible importance of interleukin-3 (IL-3)-dependent hyperplasia of mucosal mast cells in the expulsion of *Strongyloides* species but not of *N. brasiliensis* has been repeatedly demonstrated by using mast cell-deficient W/W^v mice[5,6] and nude mice.[7,8] Thus, mucosal mast cells are considered to be the selective effector of expulsion of *Strongyloides* species, whereas other cells seem to be required for the expulsion of *N. brasiliensis*. Goblet cells in the jejunal mucosa have been considered as the most likely candidate of the effector cells against *N. brasiliensis*, because goblet cell hyperplasia[9,11] and qualitative/quantitative changes of the mucins[12] were observed in *N. brasiliensis*-infected rats around the time of expulsion. Furthermore, not only worm expulsion but also goblet cell hyperplasia were hastened by adoptive transfer of immune thoracic duct lymphocytes.[10] Recently we have found that, by using lectin histochemistry, terminal sugars of goblet cell mucins altered drastically around the time of expulsion of *N. brasiliensis*,[13] and that such constitutional changes of goblet cell mucins

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were, one way or another, under the regulation of the host's local immune system.[14] The present study was, therefore, designed to evaluate the role of T cells in worm expulsion and goblet cell changes, and to elucidate the significance of altered mucins in mucosal defence against *N. brasiliensis*. For this purpose, *N. brasiliensis* worms harvested at different times from donor rats with primary infection were transferred into euthymic and hypothymic rats. The results show that once *N. brasiliensis* worms were immunologically 'damaged' in donor rats, they were expelled even from hypothymic rats within 5 days in association with alteration of goblet cell mucins. Histological study revealed that goblet cell hyperplasia was dependent on T cells, whereas alteration of terminal sugars of goblet cell mucins was not dependent on T cells. Furthermore, when goblet cell mucins were altered by implantation with 'damaged' worms, establishment of subsequently implanted 7-day-old ('normal') worms was completely inhibited in euthymic rats, with about 85% inhibition in hypothymic rats. Such protective effect of altered mucins seems to be selective because challenge infection with *S. venezuelensis* was not affected.

MATERIALS AND METHODS

Animals

Inbred male Lewis rats (LEW/Sea; Seiwa Experimental Animals Ltd, Yoshitomi, Fukuoka, Japan), 7–8-weeks old, were purchased and kept in clean conventional conditions in the Animal Center of the Miyazaki Medical College, Miyazaki, Japan. They were fed and watered *ad libitum* and used at 9–11 weeks old at the start of each experiments. In addition, hypothymic F344-*rnu*/*rnu* and their normal counterpart +/+ rats (Clea Japan Co. Ltd, Tokyo, Japan) were purchased. They were kept in filter-capped cages and fed autoclaved food chow and water *ad libitum*.

Dexamethasone treatment

Dexamethasone treatment was carried out according to the methods of *Ogilvie*.[15] In brief, 2 mg/kg of dexamethasone solution was given daily by intramuscular injection into hind legs of rats, each leg being injected alternately.

Parasitological technique

The strain of *N. brasiliensis* has been maintained in our laboratory by serial passage in Lewis rats over 7 years by subcutaneous inoculation with infective third-stage larvae (L_3) prepared by the charcoal culture method. Adult worms were obtained from the intestine of Lewis rats at 7 ('normal' worms) or 13 days ('damaged' worms) post-infection with 3000–4500 L_3. The strain of *S. venezuelensis* was originally isolated from wild rats in Okinawa, Japan,[16] established as a laboratory strain,[17] and has been maintained in our laboratory for about 2 years by serial passage in Wistar rats by subcutaneous inoculation with L_3 prepared by the filter paper culture method. Adult worms of both species were washed several times with physiological saline (0·85%), adjusted to a required number in 0·5 ml saline, and were introduced surgically into the proximal duodenum of recipient rats with a 1-ml syringe fitted with 18 G needle.[14] Control rats were given an equal volume of saline alone in a similar manner. Rats were killed by overdose of ether anaesthesia on the designated days after infection. Immediately after removal of the histological specimen (about 2 cm in length) from the jejunum 20 cm behind the pylorus, the remaining small intestine was cut open longitudinally, incubated at 37° with saline for 2 hr, and then the worms counted. The worms remaining in the histological specimens were counted under a dissecting microscope before fixation and included in worm burden.

General histology and lectin histochemistry

Tissue segments obtained as above were fixed in Carnoy's fluid, dehydrated through an ascending series of ethanol, embedded in paraffin wax, and sectioned at 4 μm. Sections were stained with alcian blue and periodic acid Schiff (PAS) for routine screening, or with lectin histochemistry using *Helix pomatia* agglutinin (HPA) (Sigma Chemical Co., St Louis, MO) which specifically recognizes the terminal GalNAc residue.[18] For lectin histochemistry, paraffin sections were rehydrated and immersed in 0·3% H_2O_2 in methanol for 20 min to quench endogenous peroxidase activity. They were rinsed with 0·01 M phosphate buffer, pH 7·4, containing 0·15 M NaCl (PBS), incubated in PBS supplemented with 1% bovine serum albumin for 10 min to prevent non-specific protein binding, and then rinsed with PBS. Specimens were placed in a moist chamber and incubated in a solution containing 25 μg/ml of biotinylated HPA in PBS for 1 hr. After incubation with biotinylated HPA, the specimens were washed three times with PBS for 5 min each and then treated with streptavidin–horseradish peroxidase conjugate (Gibco BRL, Gaithersburg, MD) at a dilution of 1:300 in PBS for 60 min. After rinsing with PBS three times, each for 5 min, the sections were immersed in 0·02% diaminobenzidine in 0·05 M Tris–HCl, pH 7·6, containing 0·003% H_2O_2, for 5–10 min. Nuclei were stained with methyl green. All procedures were performed at room temperature. As control experiments for lectin histochemistry, a mixture of 0·2 M (final concentration) haptenic sugar GalNAc (Nakalai Tesque, Kyoto, Japan) and biotinylated HPA solutions was used. The mixture was incubated for 2 hr and applied to sections instead of biotinylated HPA solution.

Scanning electron microscopy (SEM)

The jejunums of euthymic rats pretreated with damaged worms for 5 days and challenged by intraduodenal implantation of normal worms were removed 1 hr after the challenge and were cut into several pieces of approximately 1 cm in length. Specimens of control rats pretreated with saline alone and challenged by normal worms were also taken in a similar manner. Those specimens were immediately frozen in isopentane cooled with liquid nitrogen and were cracked with a chisel and a hammer. The cracked samples were fixed with acetone containing 1% glutaraldehyde at −80° for 2 days and then −20° for 2 hr. They were then left at 4° for 2 hr and washed with cold acetone at 4° for 3 × 1 hr and subsequently post-fixed with acetone containing 1% OsO_4 at 4° for 2 hr. After rinsing with acetone for 2 × 30 min at room temperature, they were routinely processed for scanning electron microscopy (Hitachi S-800).

Statistical analysis

The results were analysed by using unpaired Student's *t*-test. Significance was accepted when $P < 0·05$.

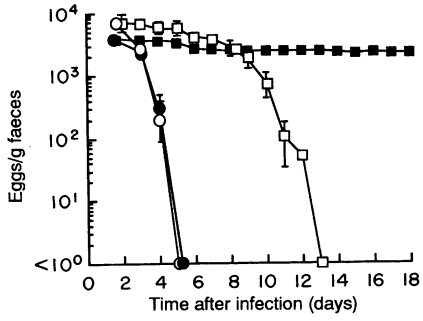

Figure 1. Kinetics of daily faecal egg count after implantation of 300 normal or damaged *N. brasiliensis* adult worms into euthymic and hypothymic rats. Euthymic rats were given normal (□) or damaged worms (○). Hypothymic rats were given normal (■) or damaged worms (●). Vertical bars represent SD of the mean from five animals.

Table 1. Goblet cell response after implantation of 300 damaged *N. brasiliensis* adult worms into euthymic and hypothymic rats

		Goblet cells/10 villi	
Animals	Days	Alcian blue–PAS	HPA
+/+	0	290·4±5·3	0
	5	561·7±12·1*	280·0±5·1*
rnu/rnu	0	290·9±3·8	0
	5	300·1±9·5**	105·4±6·3*

* $P<0{\cdot}01$; ** not significant, against uninfected control (day 0).

RESULTS

Fate of implanted normal and damaged worms in euthymic and hypothymic rats

When 300 normal *N. brasiliensis* worms obtained from donor rats 7 days after a primary infection were implanted intraduodenally into naive euthymic and hypothymic recipients, faecal egg count in hypothymic rats remained stable even 18 days after implantation, while that in euthymic rats remained stable for 7 days and then rapidly decreased to below detection level by day 13 (Fig. 1). These results were consistent with our previous observations.[14] In contrast, after intraduodenal implantation with 300 damaged worms, which were obtained from donor rats 13 days after a primary infection, into euthymic and hypothymic recipients, faecal egg count of both groups decreased rapidly, to below detection level by day 5 (Fig. 1). When these euthymic and hypothymic recipients implanted with damaged worms were autopsied on day 5, about 95% or more of implanted damaged worms were already expelled in both groups, although the number of residual worms in hypothymic rats (15·7±2·5; 94·8±0·9% reduction) was slightly but significantly higher than that in euthymic rats (2·3±1·5; 99·2±0·9% reduction).

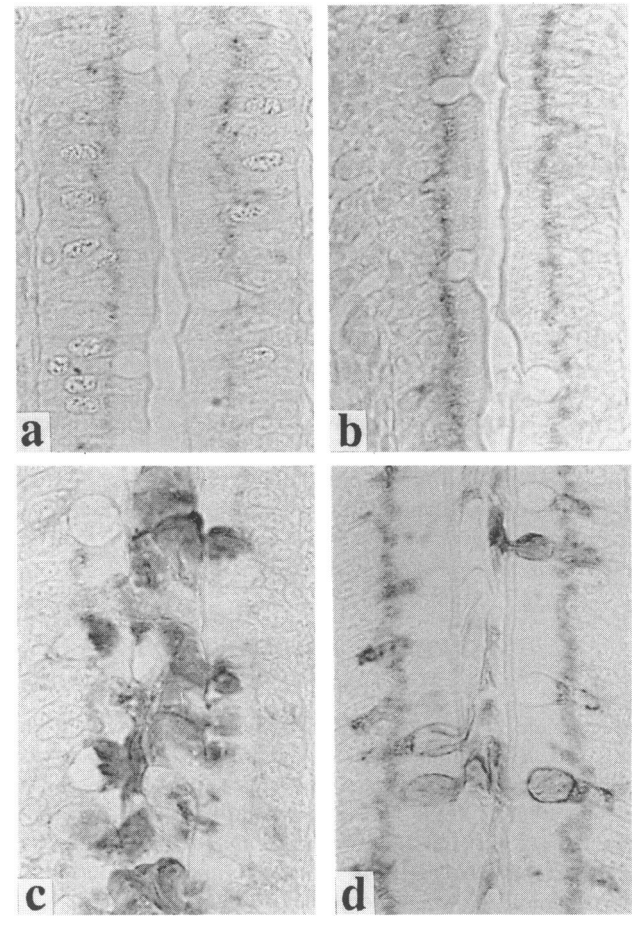

Figure 2. Light micrographs showing HPA stainability of the jejunum 5 days after implantation of 300 damaged *N. brasiliensis* adult worms into euthymic and hypothymic rats. Euthymic rats (a, c); hypothymic rats (b, d). Goblet cell mucins were negative to HPA staining before infection (a, b), but became strongly positive 5 days after implantation (c, d) in both euthymic and hypothymic rats. Note the significant increase in number of goblet cells in euthymic but not in hypothymic rats. Magnification: ×400.

Goblet cell responses of euthymic and hypothymic rats after implantation of damaged worms

Histochemical examination using alcian blue–PAS staining and lectin histochemistry are summarzied in Table 1 and Fig. 2. Before infection, the number of goblet cells was comparable between euthymic and hypothymic rats (Table 1) and HPA-staining was negative for mucin droplets of goblet cells in both groups (Fig. 2a,b). In uninfected animals, HPA-staining was positive only in the Golgi apparatus of goblet cells and of absorptive epithelial cells. Five days after implantation of damaged worms, significant goblet cell hyperplasia and alteration of the sugar terminals of goblet cell mucins was observed in euthymic rats (Fig. 2c). In the case of hypothymic rats, after implantation with damaged worms, about 30% of goblet cells became strongly positive to HPA staining (Fig. 2d) without significant rise in number of goblet cells (Table 1). In both strains, HPA-positive mucins were sometimes seen lying on goblet cells having normal HPA-negative cytoplasmic mucin droplets (data not shown), indicating rapid and transient secretion of HPA-positive mucins.[14]

Table 2. Effects of dexamethasone treatment on worm burden and goblet cell changes after implantation of 300 damaged *N. brasiliensis* adult worms into euthymic and hypothymic rats

Animals	Dexamethasone treatment	Worm burden	Goblet cells/10 villi	
			Alcian blue–PAS	HPA
+/+	No	7·3±4·2	589·4±14·4	298·4±4·7
+/+	Yes	178·7±8·6*	295·9±5·0*	0*
rnu/rnu	Yes	170·0±4·2*	265·4±5·0*	0

Three rats per group were intraduodenally implanted with 300 damaged *N. brasiliensis* adult worms and were treated with daily intramuscular injection of dexamethasone (2·0 mg/kg) or saline for 5 days from the day of infection.

* $P<0·01$ against control.

Table 3. Effect of priming by implantation of damaged *N. brasiliensis* adult worms on challenge infection by implantation of normal *N. brasiliensis* adult worms

Recipient	Priming with	Timing (days)	Worm burden	Goblet cells/10 villi	
				Alcian blue–PAS	HPA
+/+	Saline	−5	200·3±13·7	264·5±8·1	0
+/+	D-worms†	−3	0*	563·4±27·9*	257·9±14·2*
+/+	D-worms	−5	0*	347·2±17·8**	163·9±23·1*
+/+	Saline	−5	195·3±10·1	259·9±16·4	0
+/+	D-worms	−5	0*	343·7±6·6**	177·5±7·6*
rnu/rnu	D-worms	−5	30·0±9·5*	253·5±5·0‡	85·7±8·8*

Rats were primed by implantation of 300 damaged *N. brasiliensis* adult worms on the designated days and then they were challenged by implantation of 300 normal *N. brasiliensis* adult worms. Worm burden and goblet cell changes in the small intestine were examined 48 hr after challenge. Each figure represents mean±SD from three rats.

† D-worms, damaged *N. brasiliensis* adult worms.

* $P<0·01$, ** $P<0·05$, ‡ not significant, against respective saline-treated control rats.

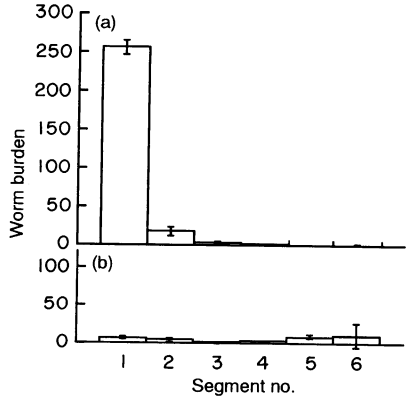

Figure 3. Distribution of implanted normal *N. brasiliensis* adult worms in the small intestine of euthymic rats pretreated with saline (a) or 300 damaged worms (b) 5 days before. Small intestines were obtained 4 hr after implantation of normal worms and were divided into six segments of equal length. Vertical bars represent SD of the mean from four animals.

Effects of dexamethasone treatment on the fate of intraduodenally implanted damaged worms and on goblet cell responses in euthymic rats

To determine whether alteration of terminal sugars of goblet cell mucins induced by the transfer of damaged worms requires host T-cell independent cellular responses or is mediated by the damaged worms themselves, effects of dexamethasone treatment on worm expulsion and goblet cell changes were examined. As shown in Table 2, dexamethasone treatment daily for 5 days from the day of worm implantation caused significant suppression of worm expulsion and of goblet cell changes in both euthymic and hypothymic recipients. Control euthymic rats implanted with 300 damaged worms and treated with saline could expel implanted worms by day 5, in association with goblet cell hyperplasia and alteration of terminal sugars of goblet cell mucins.

Effects of goblet cell changes on the establishment of subsequently implanted normal worms

To see whether altered mucins are in fact protective against *N. brasiliensis* adult worms, two groups of euthymic rats (five each)

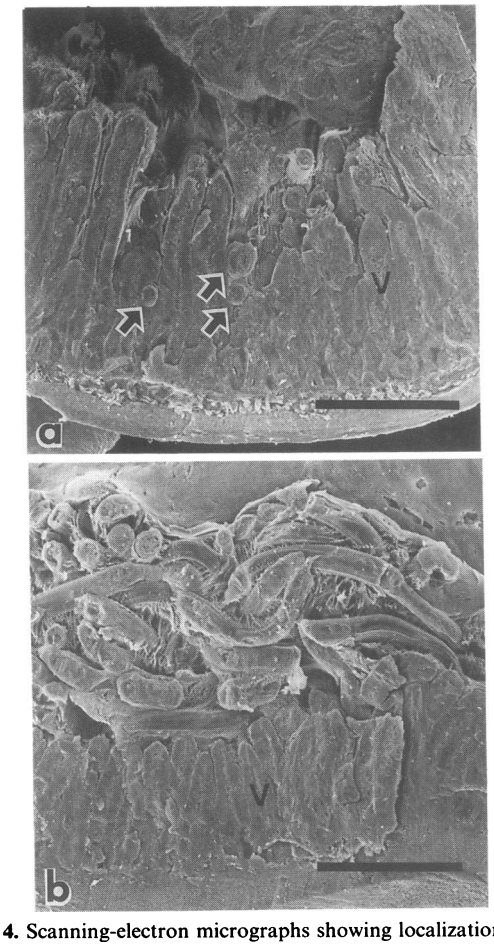

Figure 4. Scanning-electron micrographs showing localization of normal *N. brasiliensis* adult worms in the intestine 1 hr after implantation in euthymic rats pretreated with saline (a) or with 300 damaged worms (b) 5 days previously. Scale bar, 500 μm. V, villus.

were primed by intraduodenal implantation of 300 damaged worms 3 and 5 days prior to the intraduodenal challenge implantation with 300 normal worms, which were obtained from donor rats 7 days after a primary infection. Control rats pretreated by intraduodenal injection with saline were also challenged by intraduodenal implantation with 300 normal worms. The number of worms residing in the intestine was determined 48 hr after intraduodenal implantation of normal worms. As shown in Table 3, about 2/3 of implanted normal worms established in the intestine of saline-treated control rats, whereas implanted normal worms were completely expelled from the intestine of rats pretreated with damaged worms 3 or 5 days previously. When hypothymic rats were primed by intraduodenal implantation of damaged worms and, 5 days later, challenged by intraduodenal implantation of normal worms, about 85% of implanted normal worms were expelled within 48 hr (Table 3). Although goblet cell hyperplasia and alterations of terminal sugars of mucins were observed in euthymic rats, only the latter phenomenon was seen in hypothymic rats (Table 3).

Mode of action of altered mucins on implanted normal worms

From the results shown above, once alteration of the terminal sugars of goblet cell mucins was induced by implantation of damaged worms, subsequently implanted normal worms were expelled within 48 hr even from T-cell deficient hosts. To see the mode of action of altered mucins on normal worms, euthymic rats were primed with damaged worms 5 days prior to intraduodenal implantation with normal worms, and then the distribution of implanted normal worms along the intestine was examined 4 hr later. As shown in Fig. 3, while almost all implanted normal worms remained concentrated in the upper 1/6 of the small intestine of control rats, more than 90% of the normal worms were expelled and only less than 10% of the worms were seen scattered in the whole length of the small intestine of rats primed with damaged worms. Those rapidly expelled normal worms were not immunologically or physically damaged, because when they were harvested from the terminal ileum of primed rats 4 hr after implantation and retransferred into a naive recipient, they could parasitize in the new host for over 1 week (data not shown). Scanning electron microscopic study revealed that, even 1 hr after intraduodenal implantation, normal worms implanted into saline-treated control rats had already migrated into the intervillous space and become firmly attached, winding around villi at about 10 cm distal from the pylorus (Fig. 4a). On the other hand, those implanted into primed rats formed tangles surrounded by thick mucus on the surface of distal ileum (Fig. 4b), indicating that the altered mucins prevented migration and attachment of normal worms.

Effect of altered mucins on another species of intestinal helminth, *S. venezuelensis*

Since priming by intraduodenal implantation with damaged *N. brasiliensis* worms caused rapid expulsion of subsequently implanted normal worms regardless of the T-cell functions of the host, we examined whether or not such a protective effect was selective against homologous species. For this purpose, euthymic rats were primed by implantation with damaged *N. brasiliensis* worms, and 5 days later were challenged with either normal *N. brasiliensis* worms or *S. venezuelensis* adult worms, and the worm burden was determined 48 hr after the challenge. As shown in Table 4, the establishment of intraduodenally implanted *S. venezuelensis* adult worms was not affected by priming with damaged *N. brasiliensis*.

DISCUSSION

Expulsion of *N. brasiliensis* and associated cellular responses, such as intestinal mastocytosis and goblet cell hyperplasia, are somehow mediated by T cells, because these phenomena are transferred by sIg^- immune thoracic duct lymphocytes.[10,11,19] In the present study, about 95% of intraduodenally implanted damaged worms were expelled from hypothymic (*rnu/rnu*) rats within 5 days in association with alteration of the expression of terminal sugars of goblet cell mucins, whereas intraduodenally implanted normal worms parasitized the intestine of hypothymic rats over 18 days without causing significant changes of goblet cells. These results suggest that once a host's T-cell mediated immunity causes the damage of *N. brasiliensis* adult worms, the subsequent process of expulsion can occur without T-cell help. Some reservation is needed, however, before definite conclusions are drawn. Although hypothymic rats lack functional T cells,[20] their mucosal immune activation and maturation of the small intestine at weaning are comparable with

Table 4. Effect of priming by implantation of damaged *N. brasiliensis* adult worms on challenge infection by homologous and heterologous parasites

Pretreatment	Challenged with	Worm burden	Goblet cells/10 villi	
			Alcian blue–PAS	HPA
Saline	Nb†	183·7±7·5	285·7±11·2	0
D-worms‡	Nb	0·3±0·5*	342·0±9·1**	172·7±15·8*
Saline	Sv§	168·3±5·1	279·0±16·8	0
D-worms	Sv	165·3±11·4¶	353·4±13·0**	171·0±6·3*

Euthymic rats were primed by implantation of 300 damaged *N. brasiliensis* adult worms, and 5 days later they were challenged by implantation of 300 normal *N. brasiliensis* adult worms or 500 *S. venezuelensis* adult worms. Worm burden and goblet cell changes in the small intestine were examined 48 hr after challenge. Each figure represents mean±SD from four rats.

† Nb, normal *N. brasiliensis* adult worms.

‡ D-worms, damaged *N. brasiliensis* adult worms.

§ Sv, *S. venezuelensis* adult worms.

* $P<0·01$, ** $P<0·05$, ¶ not significant, against respective saline-treated control rats.

those of euthymic rats.[21] Even they could produce IgE antibody after *N. brasiliensis* infection.[22] Concerning the mechanisms of expulsion of *N. brasiliensis*, Ogilvie and Love[23] proposed a two-step theory in which a specific antibody causes the damage of worms and, subsequently, a lymphocyte-mediated non-specific inflammatory response causes the expulsion. Although our results basically support their concept of a two-step mechanism of expulsion, the second step seems to be mediated by T-cell independent non-specific inflammatory responses.

Histological examinations 5 days after intraduodenal implantation of damaged worms into euthymic and hypothymic rats revealed that alterations of terminal sugars of goblet cell mucins was observed in both rats, although goblet cell hyperplasia was noted only in euthymic rats. In association with goblet cell changes, both euthymic and hypothymic rats could expel 95% or more of implanted damaged worms by day 5, though the efficacy was slightly higher in euthymic than hypothymic rats. Thus, T-cell independent qualitative changes of goblet cell mucins seem to be critical for the expulsion, whereas T-cell dependent quantitative changes determines the rapidity of expulsion. In the present study, dexamethasone treatment completely suppressed goblet cell change and worm expulsion in euthymic and hypothymic rats. These results suggest that changes in terminal sugars of goblet cells are host-mediated but not worm-mediated phenomena. Conversely, expulsion of damaged worms is not merely due to the nature of the worms but is mediated by the host's cellular responses.

Although the precise regulatory mechanisms remain unclear, goblet cell changes during expulsion of *N. brasiliensis* are, as mentioned above, regulated by at least two separate systems: T-cell dependent proliferation and T-cell independent alterations of sugar terminals of mucins. Related to this, several cytokines such as IL-1[24] and tumour necrosis factor,[25] of which major sources are non-T cells, are known to have regulatory effects on mucosal epithelial cells, including goblet cells. As for the parasite-derived signals to induce goblet cell changes, *N. brasiliensis* adult worms produce/release a signal(s) only when they are damaged by the host's immunity, or, even if normal worms also produce/release a stimulatory signal(s), they may simultaneously produce/release an inhibitory signal(s) until they are damaged by the host's immunity. Inhibition of intestinal epithelial cell growth was induced by *N. brasiliensis* infection[26] and such an inhibitory effect was not observed when normal worms were implanted into immunized recipients.[14] Since neither stimulatory nor inhibitory effects were induced when the parasites were killed by heating or freezing (N. Ishikawa *et al.* unpublished data), excretory/secretory (ES) products of the parasites seem to contain both signals. To this end, qualitative or quantitative differences of ES products between normal and damaged worms should be explored in future.

In the present study, regardless of euthymic or hypothymic, once rats were primed to express altered terminal sugars of goblet cell mucins by intraduodenal implantation with damaged worms, they could expel subsequently implanted normal worms within 48 hr. In the following experiment, the majority of implanted normal worms were purged from the intestine within 4 hr. This expulsion was extremely rapid compared with the requirement of 3–5 days for the expulsion of implanted normal worms from immune rats. Thus, such a rapid expulsion implys that altered mucins act as the physical barrier, but do not cause damage, against normal worms to prevent attachment/migration of the parasites to the intestinal mucosa. In fact, purged normal worms could parasitize in the naive host with normal kinetics. Furthermore, scanning electron microscopic study revealed that implanted normal worms were surrounded by mucins and could not attach on nor migrate into the mucosa expressing altered mucins. These results were similar to the previously reported rapid expulsion and mucus trapping of implanted immature *N. brasiliensis* worms in immune rats.[27-29]

In spite of an extremely potent preventive effect against *N. brasiliensis*, altered mucins did not affect the establishment of intraduodenally implanted *S. venezuelensis* worms. Although mucins have been considered as a non-specific defence mechanism of the host,[30-33] the present results together with our series of previous experiments clearly demonstrate that the protective role of goblet cell mucins in worm expulsion is highly selective.

As an overall conclusion, although expulsion of *N. brasiliensis* is a T-cell mediated phenomenon, alteration of sugar terminals of goblet cell mucins is induced by the signal from damaged worms as a T-cell independent process, and the altered mucins seem to act as a selective physical barrier against *N. brasiliensis* worms.

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