TRICHURIASIS : LOCALIZED INFLAMMATORY RESPONSES IN THE COLON

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Abstract. Most patients with trichuriasis have light worm burdens. Data regarding the inflammatory response to *Trichuris* worms in the colon of lightly infected persons are scant. Nine patients whose *Trichuris* infection was found by colonoscopy had biopsies taken from a site adjacent to visible worms and from a second site some 20 cm distally. The biopsies were studied by routine and immunohistochemical methods. None of the biopsies showed mucosal ulceration, significant congestion, fibrosis, gland distortion or goblet cell mucin depletion. There was no difference between worm and worm-free sites in terms of edema, lymphoid follicles or epithelial slough. Worm sites had higher numbers of eosinophils, neutrophils and total inflammatory cells and lower numbers of plasma cells. However there was no difference in lymphocyte, mast cell, and B- and T-cell counts between the two sites. This suggests that the *T. trichiura* worm incites a local inflammatory response involving eosinophils and neutrophils, even when the colon has only a light burden of worms.

INTRODUCTION

Trichuris trichiura is an intestinal nematode. Commonly known as the whipworm, it is distributed worldwide but is more common in the tropics. It resides in the human cecum and large bowel. There is little information on the inflammatory response to *Trichuris* worms in the colon of patients with light worm burdens, who constitute the majority of those infected with *Trichuris*. The available information has been derived largely from patients with heavy worm burdens associated with the *Trichuris* Dysentery Syndrome (TDS) (Cooper *et al*, 1990; 1991; MacDonald *et al*, 1991; Grencis and Cooper, 1996). *Trichuris* worms are often detected incidentally in the cecum of patients referred for colonoscopy at our institution; this was exploited to enable a study aimed at gaining an insight into the inflammatory response in the vicinity of the worm in lightly infected patients.

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MATERIALS AND METHODS

Each of nine subjects whose *Trichuris* infection was identified during colonoscopy had a biopsy taken from a site adjacent to a visible worm and a biopsy of another site some 20 cm distal to the first. The patients had been referred for colonoscopy for a variety of reasons. Patients with overt colitis were excluded. The biopsies were fixed in 10% formalin and processed by routine histopathological methods. Serial sections were stained with: hematoxylin and eosin (H&E); combined periodic acid Schiff-Alcian blue (PAS-AB) to assess mucin depletion; and toluidine blue which highlights mast cells. Immunohistochemical stains for B- (L26) and T-lymphocytes (UCHL/CD 45 RO) (DAKO, Denmark) were performed.

A single pathologist, blind to the biopsy site, assessed the histopathology slides. Histological architectural changes were noted: these included the presence of mucosal ulcer, congestion, edema, fibrosis, crypt or gland distortion, goblet mucin depletion, lymphoid follicles, cryptitis, epithelial slough, and worms. A calibrated micrometer eyepiece (Leica) was used to quantify the various inflammatory cells. The

total number of inflammatory cells per 0.5 mm length and 0.25 mm depth of mucosa was counted. The total number of lymphocytes, plasma cells, eosinophils and neutrophils in the lamina propria were counted per 500 cells; the total number of mast cells was assessed per 500 cells in the toluidine-blue stained sections, while the total number of B- and T-cells per 200 cells were determined in the appropriate immunohistochemically-stained slides. Lymphoid aggregates were avoided during counts. Each quantitative measurement was made twice and a mean was calculated. The study was approved by the Research and Ethics Committee of Universiti Sains Malaysia.

Statistical analysis

The data were analysed by the McNemar test for categorical data, and by a paired *t*-test for normally distributed continuous data. The tests were conducted in two-tailed fashion and the level of significance was set at 0.05.

RESULTS

There were nine *Trichuris* infected subjects. Seven of the nine patients were male with a mean age of 54 years (range 24 - 75 years). All were ethnic Malays. The worm burden was low in all cases; no patient suffered from the *Trichuris* Dysentery Syndrome. The indications for colonoscopy are summarized in Table 1.

The number of biopsies ranged from 2 to 10 per patient. None showed evidence of mucosal ulceration, significant congestion, fibrosis, gland distortion or goblet cell mucin depletion. Focal cryptitis involving 3 glands was seen in one biopsy only at a worm-free site. There was no difference between worm and worm-free sites in terms of edema, lymphoid follicles or epithelial slough (Table 2).

Worm sites had higher numbers of eosinophils, neutrophils and inflammatory cells but lower number of plasma cells. There was no difference of lymphocyte, mast cell, B- and T-lymphocyte counts between the two sites (Table 3). The pattern of lymphocyte distribution within the mucosa showed no difference between worm and worm-free sites.

Trichuris worms were detected by microscopy in two colonic biopsies from worm sites.

Table 1
Indications for colonoscopy.

Patient	Indication for colonoscopy
1	Anemia with positive stool occult blood
2	Abdominal discomfort for 6 months
3	Abdominal pain, diarrhea, appendicular mass
4	Tenesmus, past history of colonic tubular adenoma
5	Recurrent abdominal pain and anemia
6	Bowel incontinence for 5 years
7	Bleeding per rectum, past history of colonic polyp
8	Adult intussusception
9	Bleeding per rectum with hypokalemia

Table 2
Histological architecture.

Histological architecture	Frequency at worm site	Frequency at worm-free site	[a]p-value
Edema	4/9 (44%)	7/9 (78%)	0.25
Lymphoid follicle	7/9 (78%)	6/9 (67%)	1.00
Epithelial slough	3/9 (33%)	2/9 (22%)	1.00

[a]McNemar test

Table 3
Inflammatory cell counts.

Variable	Worm site	Worm-free site	Test statistics[a]	p-value
Lymphocytes/500 cells	200.64 ± 43.25	189.17 ± 41.15	0.63	0.54
Plasma cells/500 cells	223.28 ± 41.35	269.56 ± 47.58	-4.66	0.002
Neutrophils/500 cells	3.06 ± 2.40	0.22 ± 0.44	3.71	0.006
Eosinophils/500 cells	73.02 ± 36.40	41.06 ± 17.77	2.44	0.04
Mast cells/500 cells	18.83 ± 9.50	19.89 ± 8.29	-1.23	0.25
B-cells/200 cells	1.80 ± 0.94	1.61 ± 1.58	0.29	0.78
T-cells/200 cells	111.98 ± 50.30	93.72 ± 39.74	1.38	0.20
Total cells/0.5 mm length, 0.25 mm depth	514.22 ± 153.72	409.50 ± 157.83	3.16	0.013

[a]Paired *t*-test

The worms were seen adjacent to but not invading the colonic mucosa; some were associated with epithelial slough.

DISCUSSION

Our study shows that there is a local inflammatory response around *Trichuris* worms in the colon of lightly infected individuals. Although there was no significant histological architectural distortion, there were increased numbers of eosinophils and neutrophils in biopsies adjacent to the worm compared with biospies of worm-free sites. There was no difference in the numbers of lymphocytes, mast cells, B- and T- lymphocytes and plasma cells between the two sites.

It is acknowledged that optimum controls would have been biopsies from completely healthy asymptomatic subjects; however, based on the experience of the pathologist in the study, the biopsies taken 20 cm distal to the worm sites would in usual practice have been reported as normal and therefore were reasonable controls. The pathologist could not be completed blinded as adult worm fragments were noted in two biopsies from the worm site.

To date, studies of the intestinal histopathology of *Trichuris* infected patients have shown minimal changes even in subjects with the *Trichuris* Dysentery Syndrome (Cooper *et al*, 1999; MacDonald *et al*, 1991; Grencis and Cooper, 1996). Minimal epithelial damage in the immediate vicinity of the worm may be seen: this damage may induce a compensatory increase in crypt cell proliferation (Cooper *et al*, 1991; MacDonald *et al*, 1991). This is in keeping with our findings, which showed no evidence of mucosal ulceration, significant congestion, fibrosis, gland distortion or goblet cell mucin depletion. There was no difference between worm and worm-free sites in terms of edema, lymphoid follicles or epithelial slough.

Various kinds of effector cells are thought to control the multiplication and spread of parasites in the human host. However, there is little information on the exact role and mechanism of each of these cells in the context of *Trichuris* infection. Eosinophils are characteristically associated with worm infections. The IgE-dependent mast cell reaction localizes eosinophils through release of eosinophilic chemotactic factor by mast cells. Parasite-derived chemotactic factors and antigen-stimulated T-cells also play a role in attracting eosinophils towards the worm. Eosinophils destroy and kill worms by antibody-dependent cell-mediated cytotoxicity (ADCC) and release of their granule contents onto the surface of the worms (Tavern and Bradley, 1996). Our findings suggest that eosinophils may play a role as they were found in higher numbers in the vicinity of the worm. There are no other recent studies on the

function and importance of eosinophils in human *Trichuris* infection; an animal study suggested the role of eosinophils and ADCC in resistance to *Trichuris muris* infection was not likely to the great (Betts and Else, 1999).

Neutrophils are phagocytic and can kill a variety of parasites. They produce an intense oxidative burst and their secretory granules contain highly cytotoxic proteins. Receptors on the membranes of neutrophils render them effective participants in ADCC reactions. The increased number of neutrophils within the lamina propria adjacent to worms, as found in our study, is of significance. The absence of active cryptitis concurs with the findings of a previous study (MacDonald *et al*, 1991) *Trichuris* Dysentery Syndrome patients.

An IgE-mediated immune mucosal response to *Trichuris* does occur in human beings but is thought to be insufficient to cause appreciable parasite expulsion. Eosinophils and mast cells act together as effector cells via the ADCC mechanism in the destruction of worms. Mast cells and subepithelial cells with surface IgE in rectal biopsies were found to be significantly increased in children with *Trichuris* Dysentery Syndrome (Cooper *et al*, 1991). However, a few recent animal studies have undermined the importance of mast cells in resistance to *Trichuris muris*, an intestinal nematode that affects mice (Betts and Else, 1999; Koyama and Ito, 2000); this is contradictory to the traditional view of those major effector mechanisms thought to be critical. Our study did not show a significant difference in mast cell numbers between worm and worm-free sites and supports the view that a localized mast cell response may not be critical in the resistance of *Trichuris* infection.

Many parasites induce specific antibody production as well as non-specific hypergammaglobulinemia. Antibodies can act directly on parasites to damage or neutralize them. Antibodies may enhance phagocytosis and play an important role in the ADCC mechanism. Cecal biopsies from children with TDS revealed a slight increase in IgM plasma cells without any change in IgA and IgG plasma cell numbers (MacDonald *et al*, 1991). However, the role of plasma cells is debatable because our study showed a significantly lower number of plasma cells in the vicinity of the worm: one of the reasons for this is merely the displacement of plasma cells by other inflammatory cells specifically recruited to the worm site, namely eosinophils and neutrophils. In our experience, though subjective, the number of plasma cells in the worm-free sites does not appear to be increased compared with colonic biopsies taken from patients with other non-inflammatory conditions. The *Trichuris* worm triggers production of certain cytokines by resident T-lymphocytes and macrophages, which amplify local inflammatory responses and induce the formation of metabolites toxic to parasites. Animal experiments have shown that T-helper cells have an important role in defence against parasitic nematodes. In our study there was no significant difference in lymphocyte, T- and B- cell counts between worm and worm-free sites. The higher total number of inflammatory cells in the vicinity of the worm is a reflection of the increase in eosinophils and neutrophils.

The study shows evidence of a local inflammatory reaction to the *Trichuris* worm. It is possible that the cumulative inflammatory response is proportional to the worm burden and may contribute to the adverse effects of heavy infection, such as growth stunting.

In a recent case (a 13-year-old boy with TDS seen at our institution), the inflammatory cells were counted in the manner advanced by this study and significantly higher numbers of eosinophils and total inflammatory cell were found, especially in the area adjacent to worms. This anecdotal evidence requires confirmation.

In conclusion, our findings show that *Trichuris* infection, even in low worm burdens, causes a local inflammatory response in those areas of the colon that are adjacent to worms. The cells that are increased significantly in the lamina propria are eosinophils and neutrophils.

REFERENCES

Betts CJ, Else KJ. Mast cells, eosinophils and antibody-mediated cellular cytotoxicity are not critical in resistance to *Trichuris muris*. *Parasite Immunol* 1999; 21: 45-52.

Cooper ES, Bundy DAP, MacDonald TT, Golden MHN. Growth suppression in the *Trichuris* dysentery syndrome. *Eur J Clin Nutr* 1999; 44: 285-91.

Cooper ES, Spencer J, Whyte-Alleng CAM, *et al*. Immediate hypersensitivity in colon of children with chronic *Trichuris trichiura* dysentery. *Lancet* 1991; 338: 1104-7.

Grencis RK, Cooper ES. *Enterobius, Trichuris, capillaria* and hookworm including *Ancylostoma caninum. Gastroenterol Clin North Am* 1996; 25: 579-97.

Koyama K, Ito Y. Mucosal mast cell responses are not required for protection against infection with the murine nematode parasite *Trichuris muris*. *Parasite Immunol* 2000; 22: 13-20.

MacDonald TT, Choy M-Y, Spencer J, *et al*. Histopathology and immunohistochemistry of the caecum in children with the *Trichuris* dysentery syndrome. *J Clin Pathol* 1991; 44: 194-9.

Tavern J, Bradley JE. Immunity to protozoa and worms. In: Roitt I, Brostoff J, Male D, eds. Immunology, 4th ed. London: Mosby, 1996: 18.1-18.19.