Paneth and intermediate cell hyperplasia induced in mice by helminth infections

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SUMMARY

Hyperplasia of Paneth and intermediate cells is a recently described component of the response of the small intestine of mice to infection with the nematode *Trichinella spiralis*. To investigate whether this hyperplasia is parasite specific or represents a generic intestinal response to infection, mice were infected with *T. spiralis*, *Nippostrongylus brasiliensis*, *Heligmosomoides polygyrus* or *Schistosoma mansoni* and tissue samples taken at various time-points post-infection to determine Paneth and intermediate cell numbers. All infections induced Paneth and intermediate cell hyperplasia, but the patterns of response varied between the parasite species concerned, reflecting differences in their relationships with the host. Increases in the numbers of these cells appeared to correlate with known patterns of T-helper-2 immune responses.

Key words: Paneth cells, intermediate cells, T cell immunity, *Trichinella spiralis*, *Nippostrongylus brasiliensis*, *Heligmosomoides polygyrus*, *Schistosoma mansoni*.

INTRODUCTION

Paneth cells are a recognized component of the intestinal epithelial layer and produce a number of biologically active materials. However, little is known of the way their numbers are regulated, and their relationship to infection and particularly parasitic infection has been largely ignored. We recently reported for the first time that parasitic infection (with *Trichinella spiralis*) induces Paneth and intermediate cell hyperplasia in all regions of the small intestine of mice (Kamal *et al*. 2001). This response is T cell-dependent and evidence suggests that thymus-independent T cells may also be involved (Kamal *et al*. 2001).

Paneth cells are found in the base of the small intestinal crypts of Lieberkuhn in many species including humans (Satoh *et al*. 1990). They are characterized by eosinophilic, zinc-binding, protein-containing granules in the cytoplasm (Sawada *et al*. 1994). The *in vivo* function of Paneth cells has not been elucidated, but they contain tumour necrosis factor (TNF)-α mRNA (Keshav *et al*. 1990), guanylin (DeSauvage *et al*. 1992), lysozyme (Peeters & Vantrappen, 1975), epidermal growth factor (Poulsen *et al*. 1986) and secretory phospholipase A_2 (Mulherkar *et al*. 1991). Murine Paneth cells also produce and secrete antimicrobial peptides called cryptdins (Ouellette *et al*. 1989), which are analogous to the alpha-defensins found in human Paneth cells (reviewed by Lehrer & Ganz, 1999). Although it is known that Paneth cell secretions are functionally anti-microbial *in vitro*, the observation that their numbers increased during infection with *T. spiralis* was a novel finding relating Paneth cell responses to parasitic infection *in vivo*.

Intermediate cells appear to be transitional between goblet cells and Paneth cells as they contain globules that not only consist of mucus, but also dense granules resembling those found in Paneth cells (Merzel & Leblond, 1969). The cells are normally found in the lower mid-crypt, so they lie above the Paneth cells which line the base of the crypts, but lie below the goblet cells on the villi (Cheng & Leblond, 1974). An unexpected finding was that infections with *T. spiralis* in mice also induce hyperplasia of intermediate cells, resulting in them being found on the lateral aspects of villi as well as in the upper crypts (Kamal *et al*. 2001). These intermediate cells stained with phloxine and were positive for cryptdin but negative for intestinal trefoil factor, suggesting they are functionally more like Paneth cells than goblet cells. In this study phloxine-tartrazine positive Paneth and intermediate cells have been treated as a single population.

T. spiralis is a powerful inducer of inflammatory and pathological changes in the host's intestine, the majority of which result from T cell-mediated events

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(Ishikawa, Wakelin & Mahida, 1997; Garside *et al*. 2000). In order to evaluate the significance of Paneth cell hyperplasia as a response to intestinal infection *per se* and to understand more about its control, it is important to know whether the response is parasite specific. This paper describes Paneth and intermediate cell responses in infections with 3 other parasites that locate in or pass through the intestine, namely *Nippostrongylus brasiliensis* and *Heligmosomoides polygyrus*, which provide a direct comparison with *T. spiralis*, and *Schistosoma mansoni*, the eggs of which elicit a marked intestinal inflammation (Domingo & Warren, 1969). Mice infected with *T. spiralis* were included as a positive control. In each case, tissue samples were taken from the small intestines of experimental mice on days during infection corresponding to the different phases of disease and the total number of phloxine-tartrazine positive cells was quantified.

MATERIALS AND METHODS

Animals

Eight-week-old, age-matched specific pathogen-free NIH and C57BL/6 inbred mice (Harlan-Olac Ltd, Bicester, UK) were maintained in a conventional animal house. Each experimental group consisted of 3–5 animals. All procedures were carried out in accordance with UK Home Office Regulations.

Parasites

T. spiralis infections were maintained in BKW stock mice and infective muscle-stage larvae recovered as previously described (Wakelin & Lloyd, 1976). NIH mice were infected by oral administration of 300 larvae. *N. brasiliensis* was maintained in Wistar rats and infective larvae produced from faecal cultures using standard methods (Ishikawa, Horii & Nawa, 1993). NIH mice were infected with 500 L_3 larvae by subcutaneous injection. *H. polygyrus* infections were maintained in BKW stock mice and infective larvae produced from faecal cultures using standard methods (Jenkins & Behnke, 1977). NIH mice were infected with 200 L3 larvae by oral administration. Confirmation of infection with each species was obtained by observation of worms during *post-mortem* examination. After each infection, the mice were sacrificed and intestinal tissue samples were taken from the proximal small intestine (10 cm distal to the pylorus) on various days post-infection (p.i.) between 0 and 12. Tissue was taken from uninfected NIH mice to provide baseline data.

Intestinal tissues were taken from C57BL/6 mice infected percutaneously with approximately 30 cercariae of *S. mansoni* (Smithers & Terry, 1965). Samples for histological analyses were taken during the early appearance of eggs (day 35, 40 and 46 p.i.), at peak (day 50, 60 p.i.) and during the chronic (day 115 p.i.) phase of the infection. Eggs were clearly visible in the intestinal tissues.

The time-course of infections with *T. spiralis*, *N. brasiliensis* and *H. polygyrus* in NIH mice is known from many previous experiments in this laboratory. *T. spiralis* worms establish in the intestine for 7–8 days before being rapidly expelled. *N. brasiliensis* adults are present in the intestine for a short period between the 3rd and 8th days of infection. In contrast, *H. polygyrus* establishes chronic infections, adult worms persisting for many weeks.

Histological techniques

Intestinal tissue samples were fixed in 10% buffered formalin, dehydrated in ethanol and embedded in paraffin wax. Sections were stained with phloxine-tartrazine to visualize Paneth and intermediate cells (Kamal *et al*. 2001; Subbuswamy, 1973), counts of which were combined. The sections were examined blind and numbers of phloxine-tartrazine positive cells were counted between the tip of one intact longitudinally sectioned villus and the tip of the next adjacent villus to give the number of cells in one villus crypt unit (VCU). Data are expressed as mean ± S.E.M. and were analysed by one-way analysis of variance and Student's *t*-test. A value of $P < 0{\cdot}05$ was taken as statistically significant.

RESULTS

Changes in Paneth and intermediate cell numbers in NIH mice infected with intestinal nematodes

Trichinella spiralis. The time-course of changes was similar to that reported previously (Kamal *et al*. 2001), showing that the mice used were responding normally (Fig. 1). On days 2 and 4 p.i. the numbers of Paneth and intermediate cells were similar to those in naïve NIH mice. After this time the number of cells steadily increased, reaching a peak at day 10 ($P < 0{\cdot}001$). By day 12 numbers had fallen slightly, but were still significantly higher than on days 2, 4, 6, and 8 p.i. The increase in numbers of Paneth cells was accompanied by the expected change in staining of the granules – i.e. from predominantly yellow (immature) to red (mature). Hyperplasia of the intermediate cells was associated with their appearance on the lateral aspects of the villi.

Nippostrongylus brasiliensis. Infected mice showed a gradual increase in Paneth and intermediate cells (Fig. 2). By day 4 p.i. there was a statistically significant increase compared with uninfected controls ($P < 0{\cdot}05$). The increase continued for at least another 8 days, numbers reaching their highest value

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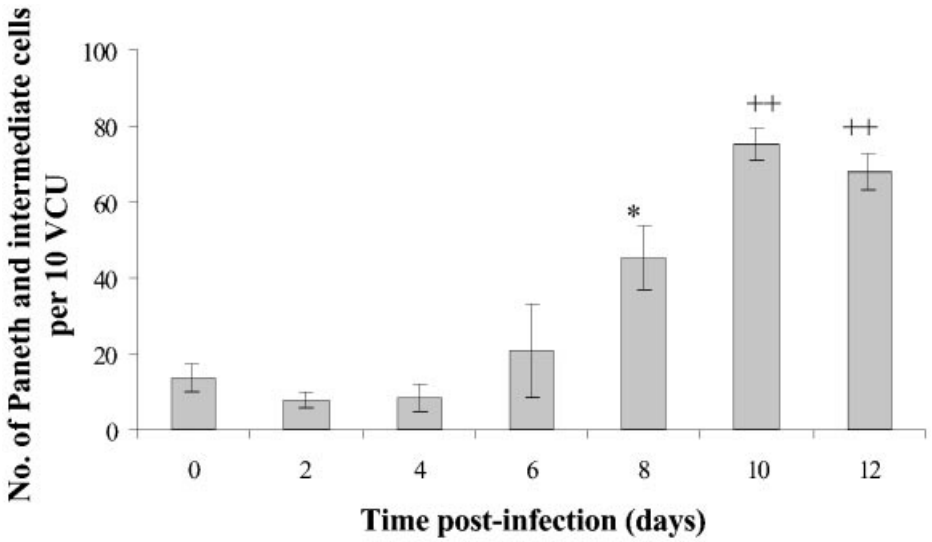


Fig. 1. Mean numbers of Paneth and intermediate cells (±S.E.M.) per 10 villus crypt units (VCU) in the duodenum of uninfected (day 0) and *Trichinella spiralis*-infected NIH mice on days 2, 4, 6, 8, 10, 12 and 14 p.i. $*P < 0{\cdot}05$ *vs* uninfected control (day 0), $^{++}P < 0{\cdot}001$ *vs* uninfected control (day 0).

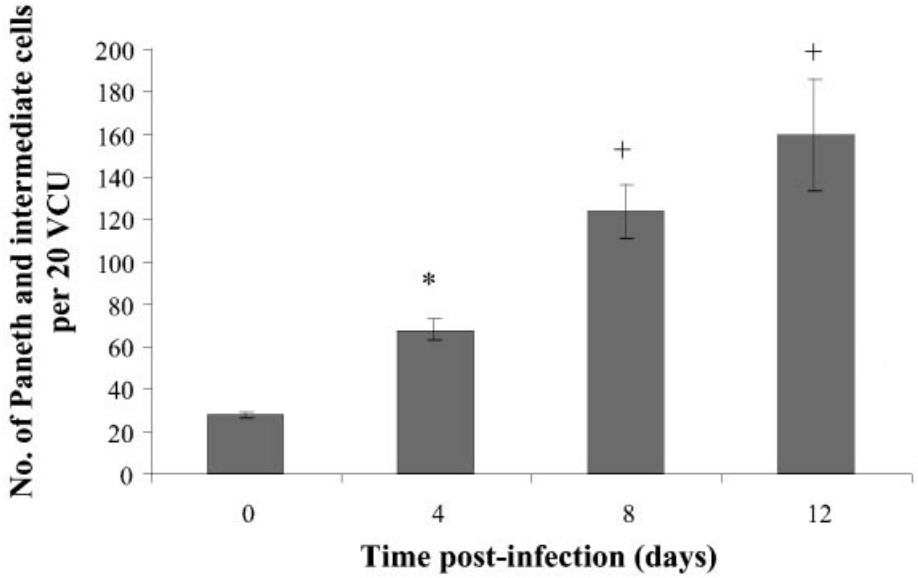


Fig. 2. Mean numbers of Paneth and intermediate cells (±S.E.M.) per 20 villus crypt units (VCU) in the duodenum of uninfected (day 0) and *Nippostrongylus brasiliensis*-infected NIH mice on days 4, 8 and 12 p.i. $*P < 0{\cdot}05$ *vs* uninfected control (day 0), $^{+}P < 0{\cdot}01$ *vs* uninfected control (day 0).

at day 12 p.i. As with *T. spiralis*, hyperplasia was associated with altered staining properties of the Paneth cells and altered distribution of intermediate cells.

Heligmosomoides polygyrus. In infected mice there was a gradual increase in Paneth and intermediate cells up until day 8 p.i. At day 4 the number of cells was significantly greater than in uninfected controls ($P < 0{\cdot}05$). The peak number of cells was found at day 8 p.i. At day 12, Paneth and intermediate cell numbers were similar to those found at day 4 p.i. (Fig. 3). Granule and distribution changes were as seen in *T. spiralis* and *N. brasiliensis*.

Changes in Paneth and intermediate cell numbers in C57BL/6 mice infected with Schistosoma mansoni

Mice infected with *S. mansoni* displayed a gradual increase in Paneth and intermediate cells (Fig. 4). Eggs were clearly visible in the intestinal tissues from day 40 onwards. By day 46 p.i. there were significantly more cells compared with uninfected naïve controls ($P < 0{\cdot}05$) and peak values were reached at day 50 p.i. (Fig. 5A, B). Paneth cells showed mature (red-staining) granules. After day 50 the numbers of cells declined, although they remained significantly increased compared with uninfected controls at day 115 p.i. (Fig. 5C).

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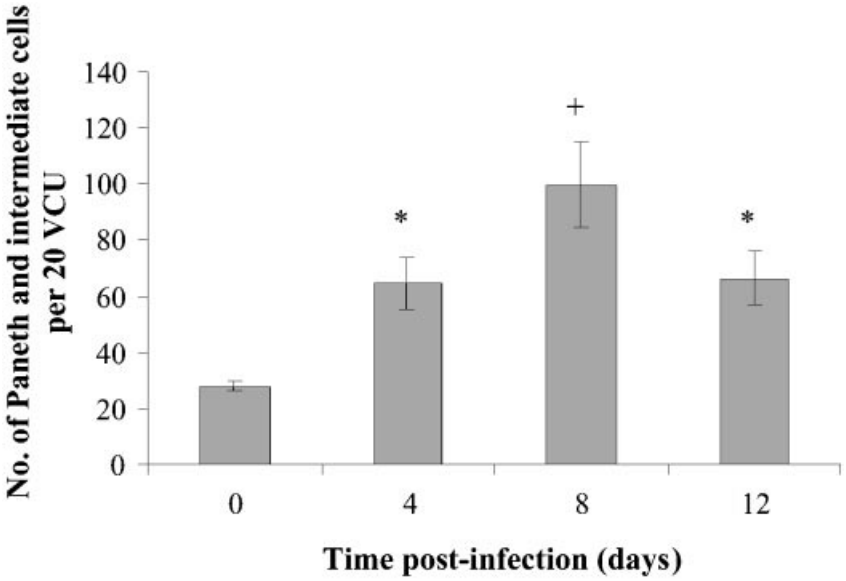


Fig. 3. Mean numbers of Paneth and intermediate cells (±S.E.M.) per 20 villus crypt units (VCU) in the duodenum of uninfected (day 0) and *Heligmosomoides polygyrus*-infected NIH mice on days 4, 8 and 12 p.i. $^{*}P < 0{\cdot}05$ *vs* uninfected control (day 0), $^{+}P < 0{\cdot}01$ *vs* uninfected control (day 0).

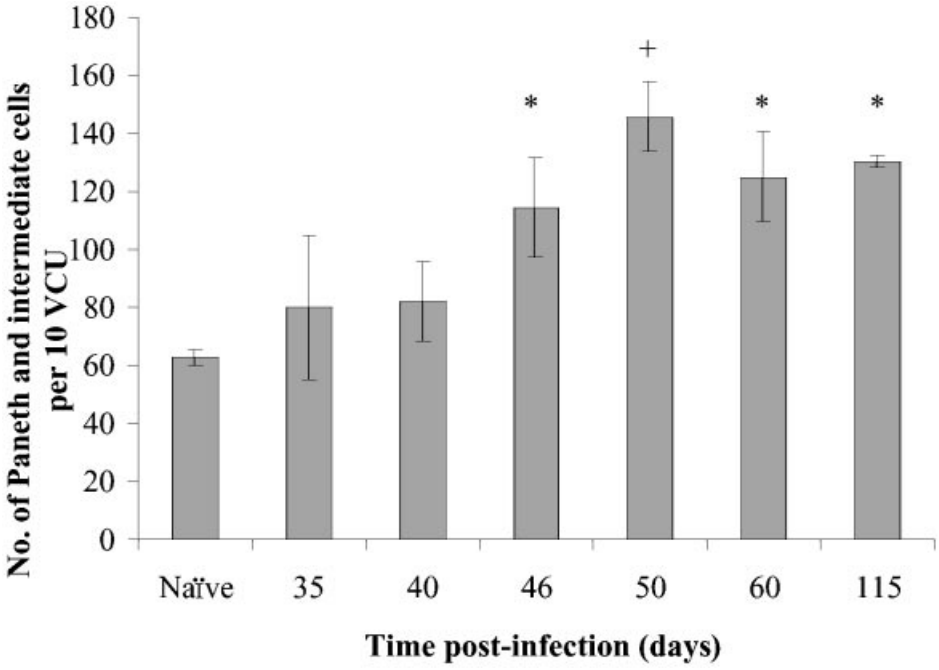


Fig. 4. Mean numbers of Paneth and intermediate cells (±S.E.M.) per 10 villus crypt units (VCU) in the small intestine of uninfected (naïve) and *Schistosoma mansoni*-infected mice on days 35, 40, 46, 50, 60 and 115 p.i. $^{*}P < 0{\cdot}05$ *vs* naïve uninfected controls, $^{+}P < 0{\cdot}01$ *vs* naïve uninfected controls.

DISCUSSION

Although many components of the intestinal inflammatory response to gastrointestinal nematodes in mice have been intensively studied, the involvement of Paneth cells in this response has only recently been recognized (Kamal *et al*. 2001). Mice infected with *T. spiralis* show not only a marked increase in the number of these cells, there is also an accompanying increase in intermediate cells, which share many characteristics with Paneth cells. In NIH mice the increase in cell number is most obvious after the first week of infection, reaching a peak at day 10. The data presented here show that hyperplasia of Paneth and intermediate cells occurred with all of the host–parasite combinations studied, although the patterns of response varied between species; hyperplasia is therefore not a phenomenon unique to infections with *T. spiralis*. This raises a number of important questions about the observed infection-

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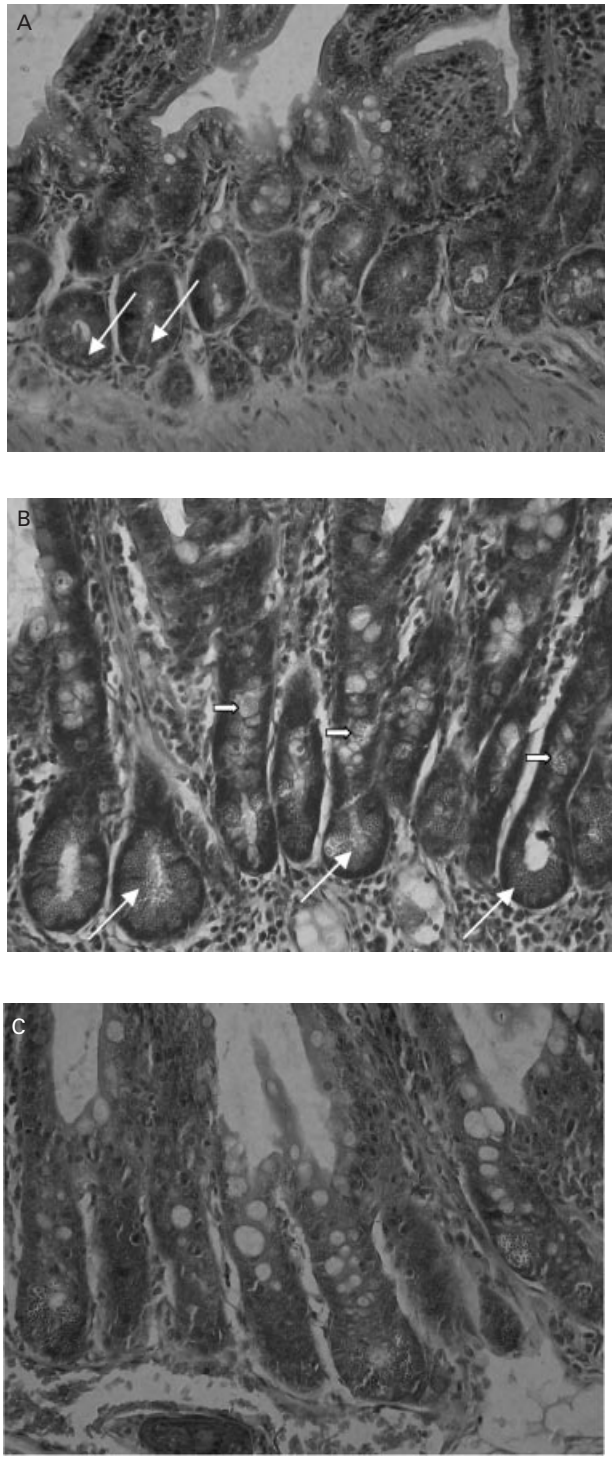


Fig. 5. Paneth cell and intermediate cell responses in C57BL/6 mice infected with *Schistosoma mansoni*. (A) Small intestinal tissue from an uninfected mouse, showing the crypts of Lieberkühn containing small numbers of Paneth cells (arrowed). No intermediate cells can be seen in this uninfected sample. (B) Small intestinal tissue taken 50 days p.i. with *S. mansoni*, showing larger crypts of Lieberkühn containing more Paneth cells (long arrows). Intermediate cells can be seen in the upper regions of the crypts (short arrows). (C) Small intestinal tissue taken 60 days p.i. with *S. mansoni*. This section shows the crypts are larger than in the uninfected controls, but the number of Paneth and intermediate cells are fewer than on day 50 p.i.

induced increase in these cells, both in relation to the biology of the parasites concerned and regarding the underlying cellular control mechanisms.

Mice infected with *N. brasiliensis* had increased numbers of Paneth and intermediate cells at day 4 p.i, i.e. shortly after worms reach the intestine, and this increase continued until at least day 12 p.i. In this host–parasite relationship, worm expulsion begins on about day 6 p.i. and by day 10 there are no worms left in the intestine. NIH mice infected with *N. brasiliensis* develop a type 2 cytokine response in the mesenteric lymph node (measured by *in vitro* release of IL-5) from day 4 (Ishikawa *et al*. 1998) and other workers have shown that levels of IL-3, IL-4 and IL-9 mRNA are sharply increased in the node at this time (reviewed by Artis & Grencis, 2001). These cytokine levels are maintained until at least day 12 p.i., except for IL-9 which decreases from its peak level by day 6. Therefore, the increase in Paneth and intermediate cell numbers seen here in response to *N. brasiliensis* occurs when there is an increase in the levels of Th2 cytokine expression. This picture is very similar to that seen with *T. spiralis*, where type 2 responses are also elevated from day 4 (Ishikawa *et al*. 1998).

In both *T. spiralis* and *N. brasiliensis* Paneth and intermediate cell numbers were markedly increased at about the time of worm expulsion. However, whilst this may suggest a causal correlation, we have previously shown that Paneth and intermediate cell responses occurred in both T cell-sufficient and athymic mice infected with *T. spiralis* (Kamal *et al*. 2001) yet the latter are known not to expel adult worms (Ruitenberg & Steerenberg, 1974).

A different pattern of Paneth cell response was seen in mice infected with *H. polygyrus*. Cell numbers peaked at day 8 p.i., and decreased, values on day 12 p.i. being similar to those on day 4. Larval *H. polygyrus* invade the intestinal mucosa immediately after infection and develop there before entering the gut lumen to mature into adult worms approximately 8–10 days later. The expression of mRNA for IL-3, IL-5 and IL-9 has been found to be considerably increased in Peyer's patches by day 2 p.i., and in the mesenteric lymph node, together with IL-4, IL-10 and IL-6, between days 3 and 4 p.i. (Urban *et al*. 1992). Therefore, the hyperplasia of Paneth and intermediate cells observed here coincides with a known increased expression of Th2 cytokines and development of larvae in the intestinal mucosa. A major difference in the response of mice to *H. polygyrus* compared with *T. spiralis* and *N. brasiliensis* is that, whereas the mucosal larval stages generate protective immunity, the adult worms interfere with the expression of immunity, and this has been associated with altered Th2-associated cytokine activity (Pleass & Bianco, 1994; Wahid *et al*. 1994). The decrease in Paneth and intermediate cell numbers in *H. polygyrus*-infected mice on day 12

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p.i. may therefore reflect this adult worm-induced down-regulation of the expression of Th2 cytokines, which would concur with the data of Dehlawi, Wakelin & Behnke, (1987), who showed that adult *H. polygyrus* suppress intestinal mast cell responses.

In mice infected with *S. mansoni* there appeared to be a slight although not significant, increase in the number of Paneth and intermediate cells at days 35 and 40 p.i., which is about the time at which eggs began to appear in the tissues. The increase continued and was statistically significant at day 46 p.i. Th1 cells play a critical role during the time when schistosomula are present in the lungs of infected mice (Wynn *et al*. 1994) and this response persists until egg production commences, between days 34 and 40 p.i., when Th1 responses are downregulated and Th2 responses upregulated (Grzych *et al*. 1991). Thus, the rise observed in intestinal Paneth and intermediate cells in C57BL/6 mice coincides with the onset of the Th2 phase of the host response described by other workers. Numbers peaked at day 50 and then declined, but remained significantly increased compared to controls. As the disease becomes chronic, i.e. day 115 p.i., the Th2 response weakens and this may correlate with the decrease in Paneth and intermediate cells seen after day 50 p.i.

The results presented here show that infection with 4 species of helminth elicited a Paneth and intermediate cell hyperplasia in the small intestine, and that in each case the response observed occurred at a time when Th2 responses are known to be operative. It seems, then, that Paneth and intermediate cell responses conform to other Th2-dependent aspects of parasite-induced intestinal inflammation, such as the mucosal mast cell and goblet cell response. However, we have previously shown that Paneth and intermediate cell responses occurred in both T cell-sufficient and athymic mice infected with *T. spiralis* but not in infected TCRβ^{-}xδ^{-} knockout mice (Kamal *et al*. 2001). As the latter lack all T cells with functional T cell receptors, whereas the athymic mice lack thymically-derived T cells; it was suggested that thymic-independent T cells may also regulate Paneth and intermediate cell hyperplasia. If confirmed this would be a novel observation and one with some interesting implications for the regulation of inflammatory responses induced by intestinal parasites.

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REFERENCES

ARTIS, D. & GRENCIS, R. K. (2001). Helper cell cytokine responses during intestinal nematode infection: induction, regulation and effector function. In *Parasitic Nematodes Molecular Biology, Biochemistry and Immunology* (ed. Kennedy, M. W. & Harnett, W.), pp. 331–371. CABI Publishing, Wallingford.

CHENG, H. & LEBLOND, C. P. (1974). Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. II. Mucous cells. *The American Journal of Anatomy* **141**, 481–502.

DEHLAWI, M. S., WAKELIN, D. & BEHNKE, J. M. (1987). Suppression of mucosal mastocytosis by infection with the intestinal nematode *Nematospiroides dubius*. *Parasite Immunology* **9**, 187–194.

DESAUVAGE, F. J., KESHAV, S., KUANG, W., GILLETT, N., HENZEL, W. & GOEDDELL, D. V. (1992). Precursor structure, expression, and tissue distribution of human guanylin. *Proceedings of the National Academy of Sciences, USA* **89**, 9089–9093.

DOMINGO, E. O. & WARREN, K. S. (1969). Pathology and pathophysiology of the small intestine in murine schistosomiasis mansoni including a review of the literature. *Gastroenterology* **56**, 231–240.

GARSIDE, P., KENNEDY, M.W., WAKELIN, D. & LAWRENCE, C. E. (2000). Immunopathology of intestinal helminth infection. *Parasite Immunology* **22**, 605–612.

GRYCH, J. M., PEARCE, E., CHEEVER, A., CAULADA, Z. A., CASPAR, P., HEINY, S., LEWIS, F. & SHER, A. (1991). Egg deposition is the major stimulus for the production of Th2 cytokines in murine schistosomiasis mansoni. *Journal of Immunology* **146**, 1322–1327.

ISHIKAWA, N., GOYAL, P. K., MAHIDA, Y. R., LI, K.-F. & WAKELIN, D. (1998). Early cytokine responses during intestinal parasitic infections. *Immunology* **93**, 257–263.

ISHIKAWA, N., HORII, Y. & NAWA, Y. (1993). Immune-mediated alteration of the terminal sugars of goblet cell mucins in the small intestine of *Nippostrongylus brasiliensis*-infected rats. *Immunology* **78**, 303–307.

ISHIKAWA, N., WAKELIN, D. & MAHIDA, Y.R. (1997). Role of T helper cells in intestinal goblet cell hyperplasia in mice infected with *Trichinella spiralis*. *Gastroenterology* **113**, 542–549.

JENKINS, S. N. & BEHNKE, J. M. (1977). Impairment of primary expulsion of *Trichuris muris* in mice concurrently infected with *Nematospiroides dubius*. *Parasitology* **75**, 71–78.

KAMAL, M., WAKELIN, D., OUELLETTE, A. J., SMITH, A., PODOLSKY, D. K. & MAHIDA, Y. R. (2001). Mucosal T cells regulate Paneth and intermediate cell numbers in the small intestine of *T. spiralis*-infected mice. *Clinical and Experimental Immunology* **126**, 117–125.

KESHAV, S., LAWSON, L., CHUNG, P., STEIN, M., PERRY, V. H. & GORDON, S. (1990). Tumor necrosis factor mRNA localised to Paneth cells of normal murine intestinal epithelium by *in situ* hybridisation. *Journal of Experimental Medicine* **171**, 327–332.

LEHRER, R. I. & GANZ, T. (1999). Antimicrobial peptides in mammalian and insect host defence. *Current Opinion in Immunology* **11**, 23–27.

MERZEL, J. & LEBLOND, C. P. (1969). Origin and renewal of goblet cells in the epithelium of the mouse small intestine. *The American Journal of Anatomy* **124**, 281–306.

MULHERKAR, R., DESAI, S. J., RAO, R. S., WAGLE, A. S. & DEO, M. G. (1991). Expression of enhancing factor gene

https://doi.org/10.1017/S0031182002002068 Published online by Cambridge University Press

and its localization in mouse tissues. *Histochemistry* **96**, 367–370.

OUELLETTE, A. J., GRECO, R. M., JAMES, M., FREDERICK, D., NAFTILAN, J. & FALLON, J. T. (1989). Developmental regulation of cryptdin, a corticostatin/defensin precursor mRNA in mouse small intestinal crypt epithelium. *Journal of Cell Biology* **108**, 1687–1695.

PEETERS, T. & VANTRAPPEN, G. (1975). The Paneth cell: a source of intestinal lysozyme. *Gut* **16**, 553–558.

PLEASS, R. J. & BIANCO, A. E. (1994). The role of adult worms in suppressing functional protective immunity to *Heligmosomoides polygyrus baeri* challenge infections. *Parasite Immunology* **16**, 619–628.

POULSEN, S. S., NEXO, E., OLSEN, P. S., HESS, J. & KIRKGAARD, P. (1986). Immunohistochemical localization of epidermal growth factor in rat and man. *Histochemistry* **85**, 389–394.

RUITENBERG, E. J. & STEERENBERG, P. A. (1974). Intestinal phase of *Trichinella spiralis* in congenitally athymic (nude) mice. *Journal of Parasitology* **60**, 1056–1057.

SATOH, Y., YAMANO, M., MATSUDO, M. & ONO, K. (1990). Ultrastructure of Paneth cells in the intestine of various mammals. *Journal of Electron Microscopy Technique* **16**, 69–80.

SAWADA, M., HORIGUCHI, Y., ABUJIANG, P., MIYAKE, N., KITAMURA, Y., MIDORIKA, O. & HIAI, H. (1994). Monoclonal antibodies to a zinc-binding protein of rat Paneth cells. *Journal of Histochemistry and Cytochemistry* **42**, 467–472.

SMITHERS, S. R. & TERRY, R. J. (1965). The infection of laboratory hosts with cercariae of *Schistosoma mansoni* and the recovery of adult worms. *Parasitology* **55**, 695.

SUBBUSWAMY, S. G. (1973). Paneth cells and goblet cells. *Journal of Pathology* **111**, 181–189.

URBAN, J. F. Jr., MADDEN, K. B., SVETIC, A., CHEEVER, A., TROTTA, P. P., GAUSE, W. C., KATONA, I. M. & FINKELMAN, F. D. (1992). The importance of Th2 cytokines in protective immunity to nematodes. *Immunological Reviews* **127**, 205–220.

WAHID, F. N., BEHNKE, J. M., GRENCIS, R. K., ELSE, K. J. & BENSMITH, A. W. (1994). Immunological relationships during primary infection with *Heligmosomoides polygyrus*: Th2 cytokines and primary response phenotype. *Parasitology* **108**, 461–471.

WAKELIN, D. & LLOYD, M. (1976). Immunity to primary and challenge infections of *Trichinella spiralis* in mice: a re-examination of conventional parameters. *Parasitology* **72**, 173–182.

WYNN, T. A., OSWALD, I. P., ELTOUM, I. A., CASPAR, P., LOWENTEIN, C. J., LEWIS, F. A., JAMES, S. L. & SHER, A. (1994). Elevated expression of Th1 cytokines and nitric oxide synthase in the lungs of vaccinated mice after challenge infection with *Schistosoma mansoni*. *Journal of Immunology* **153**, 5200–5209.

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