MUCOSAL RESPONSES TO INFECTION WITH *TRICHINELLA SPIRALIS* IN MICE

KAMAL M.*, WAKELIN D.* & MAHIDA Y.**

Summary :

Infections with *T. spiralis* in mice elicit strong inflammatory responses. The nature and control of these responses, and their relationship to the process of worm expulsion, have been debated for many years. Many components of inflammation are, like worm expulsion, T cell-dependent, but some are not. The paper describes novel observations on Paneth cell responses to infection in immunologically normal mice and in a variety of T cell-deficient mice. Responses occurred normally in *nu/nu* and *scid/scid* mice but not in β/δ knock out mice incapable of generating cells with functional TCR. However all of these mice failed to expel worms in the pattern seen in immunologically normal controls. These data are incorporated into a discussion of the causal relevance of intestinal inflammatory changes to the process of worm expulsion.

KEY WORDS : *Trichinella spiralis*, intestine, worm expulsion, mucosa, epithelium, inflammation, mast cells, goblet cells, Paneth cells, T cells, cytokines.

It has been known for many years that infections with *Trichinella spiralis* (*T. spiralis*) in the mouse induce an acute intestinal inflammatory response (Larsh, 1975). The kinetics of this response vary between inbred strains, but in general the peak inflammatory response coincides with adult worms expulsion from the intestine, after which inflammation subsides quite rapidly. In immunologically normal mice, inflammation is characterised by villous atrophy, crypt hyperplasia, mucosal mastocytosis, goblet cell hyperplasia and increased altered mucosal permeability (Garside, Grencis & Mowat, 1992). These changes reflect increased stem cell activity and reduced epithelial cell survival. They are much reduced in T cell deficient mice, indicating a T cell dependency, and there is an associated increase in adult worm survival, although worm loss does eventually occur. Results such as these have led to the view that the inflammatory response is T cell-mediated and also that inflammation *per se* plays an important role in worm expulsion. This latter view has recently been challenged as a result of work in a variety of cytokine and cytokine-receptor knockout mice (Lawrence *et al.*, 1998). This paper summarises some recent research on the induction and control of intestinal inflammation and reviews the relation between inflammation and protective immunity.

* School of Life and Environmental Sciences and
** School of Medical and Surgical Sciences, University of Nottingham, Nottingham NG7 2RD, UK.
Correspondence: D. Wakelin.
Tel.: (115) 9513232 – Fax: (115) 9513252.
E-mail: D.Wakelin@nottingham.ac.uk

MATERIALS AND METHODS

Mice were infected with the London isolate of *T. spiralis* (ISS-25) using standard methods for maintenance, infection and worm recovery (Wakelin & Lloyd, 1976). The data presented here relate to infections in inbred BALB/c and C57/BL6 mice (Harlan Olac, UK), outbred euthymic and thymus-deficient (*nu/nu*) CD1 mice (Charles River, UK), inbred T and B cell-deficient CBA-17 *scid/scid* mice (kindly donated by Dr K Else, University of Manchester, UK) and β/δ TCR chain knockout mice (kindly donated by Dr A Smith, IAH, Compton, UK). Intestinal tissues for histology (taken 10 cm from the stomach) were fixed in Carnoy's fixative, and 5 mm sections stained with Haematoxylin/Eosin (H/E) for general histology, Alcian Blue/Safranin for mast cells or Alcian Blue/PAS for goblet cells. Tissues were fixed in formol saline and sections stained in Phloxine/Tartrazine for Paneth cells. Paneth cells were also identified in 2 mm sections cut from glutaraldehyde-fixed material stained with Toluidine Blue.

RESULTS

Immunologically normal (BALB/c) mice infected with 300 *T. spiralis* muscle larvae showed the characteristic pattern of intestinal inflammatory response that has been described previously. Changes in the villus-crypt ratio (reflecting villous atrophy and crypt hyperplasia) were most marked at eight days post infection (pi) and mucosal mast cell (MMC) and goblet cell numbers rose rapidly, also peaking between day 8 and 14 of infection.

Examination of H/E- and Alcian Blue/Safranin-stained sections from Carnoy's fixed intestinal tissue taken

from infected BALB/c showed striking changes in the numbers of granulated cells located at the base of the crypts. These were presumed to be Paneth cells, but definitive identification required fixation in formol-saline and more specific staining with Phloxine/Tartrazine. When this was carried out it was clear that there was an infection-related increase in the number of cells, in the size and staining properties of their granules, and in their distribution. Data for Paneth cells and goblet cells are shown in Table I. The increase in cell numbers peaked at day 14 pi and the Paneth cells at this time had much larger granules, which stained strongly with phloxine. Phloxine-stained cells were also present high up in the crypts and even on the villus (Figs 1*a*, 1*b*).

	Day after infection				
	0	8	14	21	27
Goblet cells	133.4	193.1	256.9	158.6	148.6
Paneth cells	5.1	79.9	106.9	73.7	36.7

Numbers represent mean counts per 10 villus-crypt units from the duodenum.

Table I. – Numbers of Paneth cells in BALB/c mice infected with 300 larvae of *Trichinella spiralis*.

	Numbers of Paneth cells					
	WTCD1	*nu/nu* CD1	BALB/c	*scid/scid*	C57/BL6	βδ KO
Group						
Infected	104.4	109.5	106.9	134.8	77.7	63.0
Control	25.9	33.6	5.1	36.5	38.2	57.2

Numbers represent mean counts per 10 villus-crypt units from the duodenum.

Table II. – Numbers of Paneth cells in immunologically normal, mutant and knockout mice infected with 300 larvae of *Trichinella spiralis*.

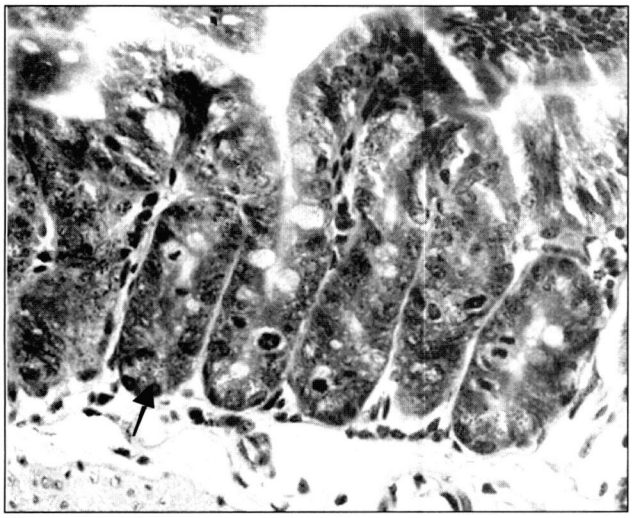

Fig 1*a*. – Phloxine-tartrazine-stained section of jejunal tissue from a control, uninfected mouse. Paneth cells, with small, pale granules, are visible at the base of a crypt (indicated by the arrow), (× 200).

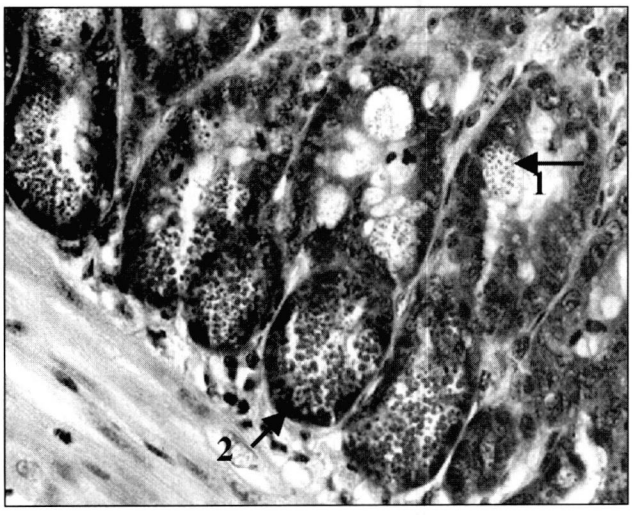

Fig 1*b*. – Phloxine-tartrazine-stained section of jejunal tissue from a mouse infected with 300 muscle larvae of *Trichinella spiralis* 14 days previously. Paneth cells with large dark granules are shown at the base of the crypt (arrow 2) and further up on the villus (arrow 1) (× 200).

To gain information on the induction and control of Paneth cell changes, infections were established in *nu/nu*, *scid/scid* and β/δ knockout mice. Numbers of MMC were also counted as a measure of the T cell dependency of inflammatory responses. Infection related increases in Paneth cells, with corresponding changes in granularity and distribution, were seen in *nu/nu* and *scid/scid* mice, but not in the β/δ knockout animals (Table II). Euthymic CD1 showed a significant increase in MMC by day 8, but no response was seen in *nu/nu, scid/scid* or bd knockout mice. Worm count data showed that worm expulsion occurred at the expected time (between days 8 and 14 of infection) in euthymic CD1 mice, BALB/c (*scid/scid* controls) and C57 BL/6 (bd knockout controls) mice but expulsion did not occur over this time period in *nu/nu, scid/scid* or bd knockout mice.

DISCUSSION

The inflammatory responses elicited by *T. spiralis* in the mouse intestine are initiated by the penetration of larvae into the epithelial cells. *In vitro* studies show that this results in the rapid release (at five hours) of pro-inflammatory mediators such as IL-1β, IL-8 and ENA-78 (Li *et al.*, 1998). Within 48 hours there has been a change in the cells of the mesenteric

lymph node (MLNC), such that mitogenic (but not antigenic) stimulation generates release of type 1 cytokines such as IFN-γ (Ishikawa *et al.*, 1998). At a later time point both mitogenic and antigenic stimulation of MLNC elicit type 2 cytokine release, showing that parasite specific Th2 cells are present. The generation of mastocytosis and goblet cell hyperplasia is regulated by type 2 cytokines, and Th2 activity is necessary for the characteristic pattern of worm expulsion (Grencis, 1997). The data presented here show that infection induces profound changes in Paneth cells, resulting in increases in their number, altering their state of maturation and their distribution. Paneth cells contain a variety of mediators (including TNFα, lysozyme, phospholipase A_2) and antimicrobial peptides known as cryptdins (Ouellette & Selsted, 1996). Although implicated in antimicrobial defence, until recently little was known of Paneth cell responses to parasitic infection or of their control. Data from infection with *Trichinella* show that control of these cells differs markedly from that of mast cells and goblet cells. Whereas both of the latter are heavily T cell-dependent, Paneth cell responses occur normally in mice deficient in conventional T cells, but not in b/d knockout mice. Paneth cells may therefore be controlled by intestinal T cells that develop, under the stimulus of infection, from progenitors located in gut cryptopatches (Kanamori *et al.*, 1996). These progenitors are present in *nu/nu*, *scid/scid* and β/δ knockout mice, and in the first two can mature into cells bearing the αβ or γδ TCR; this would not be possible in β/δ knockouts.

The occurrence of Paneth cell hyperplasia in *nu/nu* and *scid/scid* mice, which fail to show the worm expulsion pattern shown by controls, implies that, in these mice at least, Paneth cells have no major influence on worm loss. This conclusion raises the broader question of correlations between observed inflammatory changes in the intestine and expulsion of *T. spiralis* – a question that has been debated for at least 50 years! Much evidence supports the view that inflammation is coincident with worm expulsion and that specific changes, e.g. mastocytosis (Grencis, 1997; Urban *et al.*, 2000) and T cell-induced changes in smooth muscle function (Vallance & Collins, 1998), are functionally correlated with this process. However, this view has been challenged by Lawrence *et al.* (1998). They found that IL-4 deficient mice failed to expel worms in the normal (BALB/c) time frame and showed neither villous atrophy/crypt hyperplasia nor mastocytosis, which is consistent with earlier data, but that TNFR-1 deficient mice, which did expel worms, also failed to show villous atrophy and mastocytosis (interestingly crypt hyperplasia did occur). These data imply that at least two major components of *T. spiralis* induced pathology (villous atrophy and mastocytosis), mediated through TNF-α- and IL-4 dependent events, are not required for worm expulsion. What then is the relationship between intestinal pathology and expulsion?

It is clear that expulsion is not a single process with a single cause. Immunologically normal mice show a characteristic expulsion pattern that is genetically determined and strain variable (Wakelin, 1980). Immunologically deficient mice do not show this pattern but in most cases can expel worms slowly. Overall, immunologically normal mice respond to infection with a similar array of intestinal immune and inflammatory responses, some of which are T cell-dependent while others (e.g. the Paneth cell response) are not, but there is considerable strain-dependent variation in the timing, degree and sequence of these responses. One conclusion that can be drawn is that the intestine has a multi-component and non-selective "default" response to infection with *T. spiralis* (and, indeed, other nematodes), and that several of these components, alone or in combination, can bring about worm expulsion – i.e. there is considerable redundancy in the system. Even the absence of major elements of the response (villous atrophy/mastocytosis) may not prevent expulsion from taking place. What does seem to be essential for the normal pattern of expulsion is an antigen-specific Th2 response, the release of type 2 cytokines (and specifically IL-4) and fundamental changes in a number of mucosal regulatory mechanisms (reflected in altered stem cell proliferation, epithelial and vascular permeability and neuroendocrine control). Whatever the precise event/s that cause worms to leave the gut it seems generally agreed that these do not include antigen-specific effector mechanisms causing direct and irreversible worm damage. "Inflammation" in its broadest sense still seems, therefore, to be the primary cause of *Trichinella* worm loss.

REFERENCES

GARSIDE P., GRENCIS R.K. & MOWAT A.M. T lymphocyte dependent enteropathy in murine *Trichinella spiralis* infection. *Parasite Immunology*, 1992, *14*, 217-225.

GRENCIS R.K. Th2-mediated host-protective immunity to intestinal nematode infections. *Philosophical Transactions of the Royal Society of London B*, 1997, *352*, 1377-1384.

KANAMORI Y., ISHIMARU K., NANNO M., MAKI K., IKUTA K., NARIUCHI H. & ISHIKAWA H. Identification of novel lymphoid tissues in m,urine intestinal mucosa where clusters of c-kit$^+$ IL-7$^+$ Thy1$^+$ lympho-hemopoietic progenitors develop. *Journal of Experimental Medicine*, 1996, *184*, 1449-1459.

LARSH J.E. Allergic inflammation as a hypothesis for the expulsion of worms from tissues: a review. *Experimental Parasitology*, 1975, 251-266.

LAWRENCE C.E., PATERSON J.C.M., HIGGINS L.M., MACDONALD T.T., KENNEDY M.W. & GARSIDE P. IL-4 regulated enteropathy in an intestinal nematode infection. *European Journal of Immunology*, 1998, *28*, 2672-2684.

LI C.K.F., SETH R., GRAY T., BAYSTON R., MAHIDA Y.R. & WAKELIN D. Production of proinflammatory cytokines and inflammatory mediators in human intestinal epithelial cells after invasion by *Trichinella spiralis*. *Infection and Immunity*, 1998, 2200-2206.

OUELLETTE A.J. & SELSTED M.E. Paneth cell defensins: endogenous peptide components of intestinal host defense. *FASEB Journal*, 1996, *10*, 1280-1289.

URBAN J.F., SCHOPF L., MORRIS S.C., OREKHOVA T., MADDEN K.B., BETTS C.J., GAMBLE H.R., BYRD C., DONALDSON D., ELSE K. & FINKELMAN F.D. Stat6 signalling promotes protective immunity against *Trichinella spiralis* through a mast cell- and T cell-dependent mechanism. *Journal of Immunology*, 2000, *164*, 2046-2052.

VALLANCE B.A. & COLLINS S.M. The effect of nematode infection upon intestinal smooth muscle function. *Parasite Immunology*, 1998, *20*, 249-253.

WAKELIN D. Genetic control of immunity to parasites. Infection with *Trichinella spiralis* in inbred and congenic mice showing rapid and slow responses to infection. *Parasite Immunology*, 1980, *2*, 85-98.

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