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Early cytokine responses during intestinal parasitic infections

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SUMMARY

Infections with gastro-intestinal nematodes elicit immune and inflammatory responses mediated by cytokines released from T-helper type-2 (Th2) cells. *In vitro* assays of cells from the mesenteric lymph nodes (MLN) of experimentally infected rodents confirm that, after about 1 week, the dominant cytokine responses to mitogens and antigens are those associated with this Th-cell subset. Polarization of the Th response in this way implies an initial local cytokine enviroment that favours Th2 development. However, experimental infections with *Trichinella spiralis* and *Nippostrongylus brasiliensis* show that, within 2 days of worms reaching the intestine, MLN cells (MLNC) respond with a Th1 rather than a Th2 response [i.e. there is an increase in mRNA for the type 1 cytokine interferon-γ (IFN-γ), and mitogen-stimulated MLNC release IFN-γ rather than interleukin-5 (IL-5)]. Antigen stimulation at this time does not elicit IFN-γ release and the MLNC cannot adoptively transfer immunity. Within a few days the MLNC phenotype changes. There is a Th2 response (IL-5 release) to both mitogen and antigen stimulation and MLNC can adoptively transfer immunity. Early release of IFN-γ is T-cell dependent, with $CD4^+$ T cells playing the major role. The data are discussed in relation to factors regulating the mucosal response to invasion by parasites.

INTRODUCTION

The response of the intestine to invasion by pathogens represents a complex interaction between non-specific inflammatory mechanisms and immunologically specific adaptive events. Infections with gastro-intestinal (GI) nematodes in mice have provided simple and easily quantifiable models to study the induction and regulation of mucosal T-cell-dependent responses.[1] Data from many sources, using a variety of techniques and a variety of inbred strains, show that these infections typically elicit T-cell responses that are biased towards the production of type 2 cytokines.[2] This is consistent with the characteristic association of GI nematode infections with inflammatory phenomena such as eosinophilia, mastocytosis and elevated immunoglobulin E (IgE). The reason for this bias is still unexplained.

The majority of studies have focused attention on responses occurring in the draining mesenteric lymph node (MLN) some days after the infections have become established in the intestine. A general picture is that, from about 6 to 7 days after infection, when restimulated with antigen or mitogens *in vitro*, MLN cells (MLNC) from infected mice release type 2 cytokines (e.g. interleukin (IL)-4, -5, -9 and -10) but little or no interferon-γ (IFN-γ).[3–7] With one species, *Trichuris muris*, there is a strong host genetic influence on the cytokine profile, the response of resistant strains being T helper type 2 (Th2)-dominated, while that of susceptible strains switches to a Th1 response.[8,9]

Th-subset polarization following infection with parasites has been described in several systems[10] and reflects the influence of the initial, local cytokine environment. Thus, development of a Th2 response is promoted by the presence of type 2, and the absence of type 1, cytokines. The reduced levels of IFN-γ seen in most GI nematode infections is consistent with this interpretation. Indeed, experiments using *Nippostrongylus brasiliensis* show that administration of rIFN-γ or rIL-12 before infection can prevent the development of Th2-associated protective immune responses.[11,12] However, few papers have analysed cytokine profiles at early time points after infection when Th-subset responses might be determined. With *Trichinella spiralis* it has been shown that cells taken within 2 or 3 days of infection and stimulated *in vitro* can produce type 1 rather than type 2 cytokines.[13–15] In contrast, a detailed study of cytokine mRNA in mice infected with *Heligmosomoides polygyrus*[16] showed that the earliest MLN response to infection was seen primarily in message for IL-9 (12 hr) and IL-3 (2 days), little or no change occurring in IFN-γ mRNA. The available data therefore show an interesting discrepancy, one that may affect interpretations of influences upon Th polarization as well as illuminating the initial response of the intestine to invasion by an infectious organism.

The present paper examines the production and *in vitro* release of cytokines from MLNC taken from mice infected

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with two different nematodes to: (a) define initial cytokine responses; (b) determine whether these are parasite specific; (c) define the relationship between intestinal invasion and cytokine response, and (d) identify the role of T-cell populations in this response.

MATERIALS AND METHODS

Animals

Specific pathogen-free male NIH inbred mice (Harlan-Olac Ltd, Bicester, UK) aged 8 weeks, were used throughout. Experimental groups consisted of five animals.

Parasitological techniques

The nematodes used were *Nippostrongylus brasiliensis* and *Trichinella spiralis*. The methods used for infections with these worms have been described previously.[17,18] Mice were infected orally with 300 first-stage larvae (L1) of *T. spiralis*, or subcutaneously (s.c.) with 500 third-stage larvae (L3) of *N. brasiliensis*. For experiments involving infection with fourth stage larvae (L4) of *N. brasiliensis,* Wistar rats were given 3000 L3 s.c. and killed after 2 days for recovery of L4 from the lungs. Mice were then infected orally with 300 of these larvae. Groups of mice were killed at intervals after infection, the intestines were processed for worm recovery and MLNC prepared for cytokine assays or for adoptive transfer.

Parasite antigens

Antigens of adult *N. brasiliensis* and larval *T. spiralis* were prepared using methods described previously.[19]

Preparation of MLNC suspensions

Suspensions of MLNC were prepared in RPMI-1640 medium (Gibco, Basle, Switzerland) and allowed to settle for 5 min. The supernatant, containing a suspension of single cells, was removed, washed by centrifugation and the cells resuspended in RPMI with 10% fetal calf serum (FCS) at 1×10^8 cells/ml. Cell viability was assessed using fluorescein diacetate (FDA, Sigma Chemical Co., Ltd, Poole, UK) and was normally >90%.

Preparation of T-cell depleted and T-cell-subset enriched MLNC

MLNC taken 2 and 8 days after infection with *T. spiralis* were treated *in vitro* with anti-Thy1.2 antibody (Serotec, Oxford, UK) plus complement to deplete T cells, using standard techniques. $CD4^+$ and $CD4^-$ fractions were prepared from MLNC suspensions taken at day 8 after infection. Purified $CD4^+$ cells were separated using anti-mouse CD4 (L3T4) Dynabeads and DETACHaBEAD mouse CD4 (Dynal (UK) Ltd, Bromborough, UK) according to the manufacturer's instructions. $CD4^+$ depleted and $CD8^+$ depleted fractions were prepared using anti-mouse CD4 and anti-mouse CD8 Dynabeads. Fractions were washed and resuspended in RPMI–FCS. The efficiency of depletion and purification was confirmed by fluorescence-activated cell sorting (FACS) analysis. Treatment with anti-Thy1.2 antibody removed >90% of T cells. CD4 and CD8 depletion resulted in cell populations with >95% $CD8^+$ or >95% $CD4^+$ cells, respectively.

For transfer of unseparated cells MLNC were prepared on days 2 and 8 from *T. spiralis*-infected mice and days 4 and 8 from *N. brasiliensis* L3-infected donors. For transfer of separated cells, MLNC were prepared from *T spiralis*-infected donors only on day 8. Recipient mice were given 2×10^7 cells intravenously. 24 hr later, recipients and controls were challenged with *T. spiralis* L1 or *N. brasiliensis* L3. Mice were killed on day 6 or 8 after challenge for worm recovery and their MLNC assessed for cytokine production.

Evaluation of cytokine production in vitro

MLNC or CD4/CD8-depleted MLNC were diluted to 5×10^6 (MLNC) or $1{\cdot}25 \times 10^6$ (depleted MLNC) cells/ml in RPMI–FCS. Volumes of 2 ml were placed into wells of 24-well plates (Costar, Cambridge, MA) and incubated with 5 μg/ml of concanavalin A (ConA; Sigma) or 50 μg/ml parasite antigen for 48 hr at 37° and 5% CO_2. The supernatants were then collected and stored at −20°. Cytokines in the supernatants were measured by sandwich enzyme-linked immunosorbent assay (ELISA).[8] IFN-γ and IL-5 were used as candidate type1 and type 2 cytokines, respectively. The pairs of cytokine-specific monoclonal antibodies (mAb) used were: IFN-γ–R46A2/biotinylated XMG1.2; IL-5–TRFK-5/biotinylated TRFK-4 (Pharmingen, San Diego, CA). Cytokine levels were quantified against recombinant standards (Pharmingen).

Reverse-transcription polymerase chain reaction (RT–PCR)

RT–PCR was carried out as described previously.[20] Briefly, total RNA was obtained by lysing MLNC in RNAzol B (Biogenesis Ltd, Poole, UK), extracting with chloroform and precipitating with isopropanol. After washing and drying the RNA pellet was resuspended in 50 μl diethyl pyrocarbonate-treated H_2O. Random hexamer primed reverse transcription was carried out with 200 U Moloney murine leukaemia virus reverse transcriptase (Gibco). Control tubes without transcriptase were set up with half of the RNA from each sample. Primers and probes for IFN-γ and reduced glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (as a reference marker) were kindly designed by Drs R. Seth and R. A. Robins (Immunology, QMC Nottingham) and the primers synthesized in house (IFN-γ upper – CAG CAA CAA CAT AAG CGT CAT and lower –CCG AAT CAG CAG CGA CTC). Specific oligonucleotide probes to detect PCR products were synthesized and labelled with digoxigenin (DIG) by R & D Systems Europe Ltd (Abingdon, UK) PCR products were quantified by enzyme-linked oligonucleotide chemoluminescent assay (ELOCA)[20] using the labelled probes to identify PCR products after blotting onto nylon membranes. After incubation with rabbit anti-DIG antibody labelled with alkaline phosphatase (Boehringer Mannheim, Leeds, UK) the membranes were incubated with chemiluminescent substrate (CSP-Star™, Boehringer Mannheim) and the photons emitted measured by a microplate scintillation spectrophotometer. The signal for the cytokine PCR product was recorded as counts per second (c.p.s.) and expressed as a ratio of the c.p.s. for the GAPDH product.

Statistical analysis

Worm numbers and cytokine levels are expressed as mean ± SE Data were analysed using an unpaired Student's *t*-test. Significance was accepted when $P < 0{\cdot}05$.

RESULTS

Cytokine production after infection with *T. spiralis*

Groups of mice infected with 300 larvae were killed after 2, 4 and 8 days. Worm recoveries were 123·4±10·8, 156·5±16·3 and 96·5±10·5, respectively. MLNC were stimulated *in vitro* with Con A or *T spiralis* antigen and levels of IFN-γ and IL-5 measured (Fig. 1). Release of IFN-γ above control values was seen only when day 2 cells were stimulated with Con A; IL-5 was released later in infection from both Con A- and antigen-stimulated cells.

The choice of 50 μg/ml antigen used for *in vitro* stimulation had been determined by prior experiment, using cells taken from day 4 to day 8 after infection (data not shown). To establish whether the failure of day 2 cells to release IFN-γ in response to antigen *in vitro* was dose related, cells taken at 2 or 8 days after infection with *T. spiralis* were stimulated *in vitro* with Con A or with concentrations of antigen from 6·25–100 μg/ml. Cells taken after 2 days did not release IFN-γ when pulsed with antigen at any concentration. In contrast, day 8 cells released IL-5 even at the lowest antigen concentration used (Table 1).

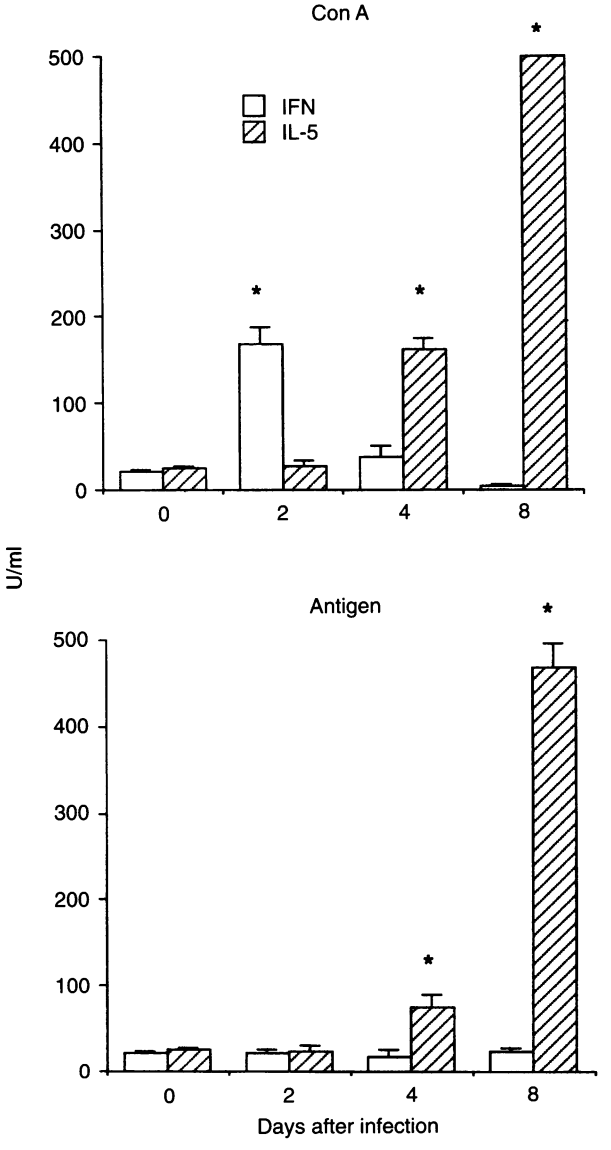

Figure 1. Release of the cytokines IFN-γ (□) and IL-5 (▨) from MLNC of mice infected with 300 *Trichinella spiralis*. MLNC were taken at intervals after infection and stimulated *in vitro* with Con A or with *T. spiralis* antigen. Cytokines in culture supernatants were measured by an antigen capture ELISA and the data expressed as U/ml. Each data point shows the mean (±SE) of five animals; an asterisk indicates values that are significantly different from day 0.

Cytokine production after infection with *N. brasiliensis*

In the first experiment mice were infected s.c. with 500 L3 and killed after 2, 4 and 8 days when worm recoveries were 14·6±15·3, 190·7±7·5 and 64·4±15·8, respectively (L3 migrate from the initial site of infection to the skin via the lungs to reach the intestine on about day 3/4). Levels of cytokines produced by MLNC *in vitro* are shown in Fig. 2. Increased release of IFN-γ was obtained only from day 4 cells stimulated with Con A, when worms had established in the intestine; IL-5 was released by day 8 cells after both Con A and antigen stimulation.

In the second experiment mice were infected s/c with 500 L3 or orally with 300 L4. With these infection levels comparable numbers of worms became established in the intestine. As L4 establish directly in the small intestine, peak values following L4 infection were obtained on day 2 (mean 188·6±9·3) and on day 4 after L3 infection (mean 187·6±12·2). Levels of IFN-γ released by MLNC stimulated *in vitro* with Con A are shown in Fig. 3. In each case increased release of IFN-γ occurred in cells taken when worms had established in the intestine – i.e. day 2 in L4-infected mice and day 4 in L3-infected mice. A significant increase in IL-5 release was recorded on day 4 in L4-infected mice but not until day 8 in L3-infected mice (data not shown).

Levels of IFN-γ mRNA induced by the early stages of infection

MLNC were prepared from mice infected with 300 *T. spiralis*, 500 *N. brasiliensis* L3 or 300 *N. brasiliensis* L4, extracted for RNA and processed for RT–PCR. Splenocytes stimulated with phytohaemagglutinin (PHA) (5 mg/ml for 24 hr) were used as a positive control. The results (Table 2) show that increased levels of IFN-γ mRNA were present at 24 hr after infection in mice infected with *T. spiralis* and 24 hr and 48 hr in those

Table 1. Cytokine release from MLNC taken 2 or 8 days after infection with *T. spiralis* and cultured *in vitro* with varying concentrations of parasite antigen/ml (U/ml mean±SE)

In vitro treatment	Day 2 cells IFN-γ	Day 8 cells IL-5
None	12·4±4·1	96·4±4·4
Con A	321·5±34·6	821·4±20·1
Antigen (100 μg)	18·8±6·4	623·4±12·3
Antigen (50 μg)	13·4±4·1	592·1±18·6
Antigen (25 μg)	13·2±4·2	428·6±24·5
Antigen (12·5 μg)	11·2±4·7	391·4±13·1
Antigen (6·25 μg)	14·6±3·6	300·6±19·1

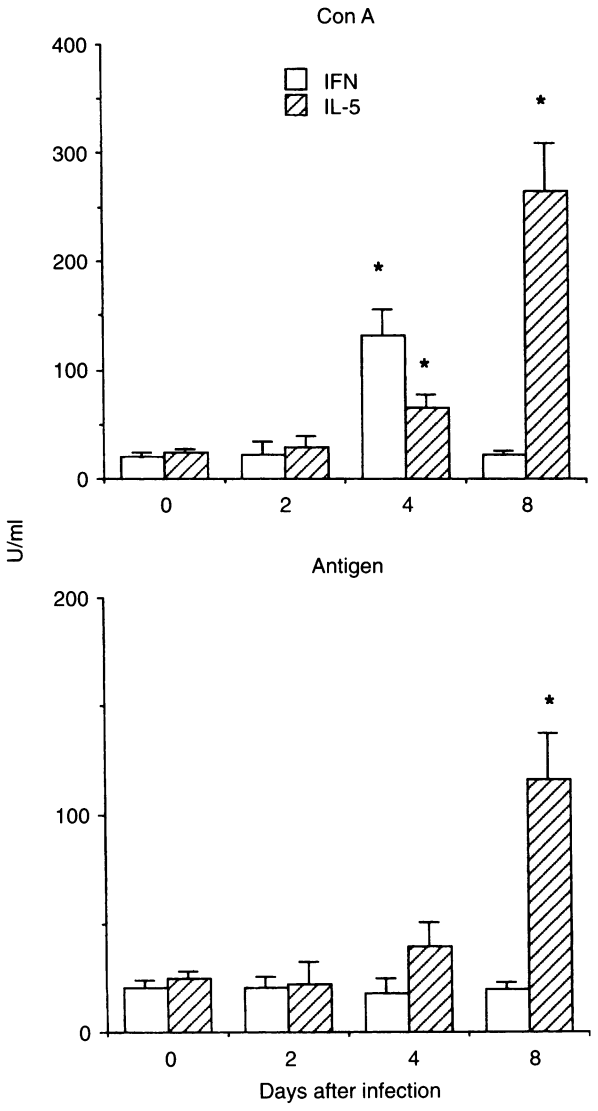

Figure 2. Release of the cytokines IFN-γ (□) and IL-5 (▨) from MLNC of mice infected with 500 *Nippostrongylus brasiliensis*. MLNC were taken at intervals after infection and stimulated *in vitro* with Con A or with *N. brasiliensis* antigen. Cytokines in culture supernatants were measured by an antigen capture ELISA and the data expressed as U/ml. Each data point shows the mean (±SE) of five animals; an asterisk indicates values that are significantly different from day 0.

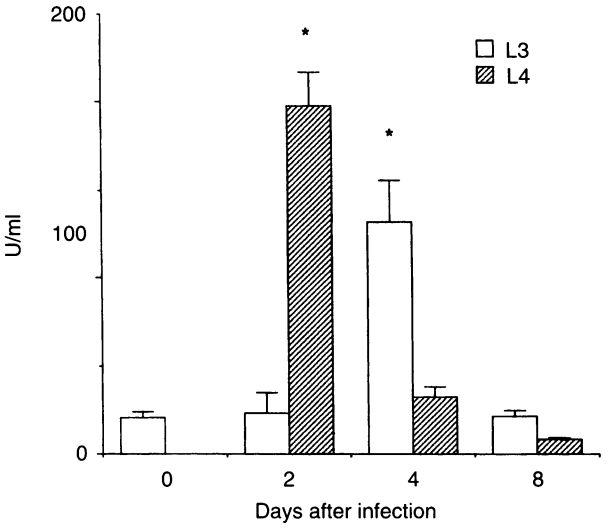

Figure 3. Release of the cytokine IFN-γ from MLNC of mice infected with 500 L3 (□) or 300 L4 (▨) of *Nippostrongylus brasiliensis*. MLNC were taken at intervals after infection and stimulated *in vitro* with Con A. Cytokines in culture supernatants were measured by an antigen capture ELISA and the data expressed as U/ml. Each data point shows the mean (±SE) of five animals; an asterisk indicates values that are significantly different from day 0.

Table 2. Early changes in levels of IFN-γ mRNA in mice infected with infective larvae (L1) of *Trichinella spiralis* and L3 or L4 *Nippostrongylus brasiliensis*

Infection	Hours after infection	IFN-γ/GAPDH ratio	
		Mean	SD
None		1·27	0·08
T. spiralis	12	0·99	0·17
N. brasiliensis L3	12	0·82	0·64
N. brasiliensis L4	12	0·98	0·14
T. spiralis	24	2·56	0·21
N. brasiliensis L3	24	1·32	0·16
N. brasiliensis L4	24	1·83	0·06
T. spiralis	48	1·40	0·09
N. brasiliensis L3	48	1·32	0·17
N. brasiliensis L4	48	2·15	0·20
Positive control		3·98	0·01

The IFN-γ/GAPDH ratio was determined after enzyme-linked oligonucleotide chemiluminescent assay (ELOCA) of the PCR products (see text).

infected with L4 of *N. brasiliensis*. No increase was seen after infection with *N. brasiliensis* L3.

Source of cytokines in *T. spiralis*- and *N. brasiliensis*-infected mice

The MLN of NIH mice contain 60–70% $CD4^+$ and $CD8^+$ T cells, 20–30%, B cells and small numbers of non-T-non-B cells. As IFN-γ is produced by many cells other than T cells, production of cytokines by MLNC taken 2 days after infection with *T. spiralis*, and 4 or 2 days after infection with L3 or L4 *N. brasiliensis*, was assessed after T-cell depletion, using anti-Thy1.2 antibody plus complement, prior to *in vitro* culture with Con A. The results (Table 3) show that removal of >90 of T cells reduced IFN-γ release to very low levels, implying that these cells were the primary source of the cytokines. Treatment of day 8 cells similarly removed the capacity to release IL-5 (data not shown).

To determine whether early IFN-γ production was associated with $CD4^+$ or $CD8^+$ T cells, $CD4^+$- or $CD8^+$-depleted populations were stimulated *in vitro* with Con A. The results showed that both $CD4^+$ and $CD8^+$ T cells released IFN-γ under these conditions, although production was greater when $CD4^+$ cells were present. Mean IFN-γ levels were: MLNC,

Table 3. Contribution of T cells to production of cytokines by Con A-stimulated MLNC taken from mice infected with *T. spiralis* or *N. brasiliensis* (U/ml mean ± SE)

In vitro treatment	Day 2 cells IFN-γ	Day 8 cells IL-5
T. spiralis		
None	185·4 ± 21·4	895·4 ± 32·2
Complement	168·3 ± 13·9	887·1 ± 24·1
anti-Thy-1	11·4 ± 3·6	34·4 ± 11·3
N. brasiliensis		
None	181·3 ± 21·0	684·3 ± 32·5
anti-Thy-1 + C′	38·4 ± 12·4	101·8 ± 21·5

130 U/ml; CD4-depleted MLNC, 33 U/ml; CD8-depleted MLNC, 140 U/ml

Functional properties of cells taken at intervals after infection

Correlation between the *in vitro* cytokine profiles of MLNC taken at different times after infection with their ability to express anti-parasite activity *in vivo* was assessed by adoptive transfer into naive recipients. Activity was measured by the degree to which worm survival was reduced in recipients. The levels of IL-5 produced *in vitro* by MLNC from these mice were also determined. It is clear, with both *T. spiralis* and *N. brasiliensis*, that effective transfer of immunity with unseparated cells was associated only with the populations that showed a type 2 cytokine profile, i.e. those taken day 8 after infection (Table 4). Cells taken earlier after infection, when a type 1 profile was evident, were ineffective. Recipients of cells taken at day 8 showed a higher level of IL-5 production when tested at day 6 after challenge than the controls. Effective transfer of immunity was also seen when purified day 8 $CD4^+$ cells were used (Table 5).

DISCUSSION

GI nematode infections are typically associated with Th2 activity and Th2-mediated responses. The majority of studies have concentrated on the development and expression of these responses rather than on their induction. Current understanding of T-cell activity, and in particular of Th subsets, has emphasized the importance of early events in determining the outcome of the immune response, polarization of Th subsets being heavily influenced by the local cytokine environment in which they develop.[21]

Table 4. Transfer of immunity against *Trichinella spiralis* or *Nippostrongylus brasiliensis* with mesenteric lymph node cells (MLNC) taken from donors at intervals after infection (*T. spiralis*: days 2 and 8; *N. brasiliensis*: days 4 and 8). Recipient and control mice were killed 6 days after challenge with 300 *T. spiralis* or 500 *N. brasiliensis*

Group of mice	No of worms (mean ± SE) *T. spiralis*	*N. brasiliensis*
2×10^7 Day 2/4 MLNC	137·8 ± 7·2	142·1 ± 8·9
No cells	134·2 ± 6·8	138·1 ± 16·3
2×10^7 Day 8 MLNC	88·3* ± 9·9	61·1* ± 9·3
No cells	141·0 ± 6·5	149·4 ± 18·2

*Mean significantly lower than no cells control ($P < 0·05$).

Table 5. Transfer of immunity against *T. spiralis* with unseparated or with $CD4^+$ or $CD4^-$ mesenteric lymph node cells (MLNC) taken from donors 8 days after infection. Recipient and control mice were killed 6 days after challenge with 300 *T. spiralis*. Levels of IL-5 were measured in MLNC of recipients on day 6

Group of mice	No of worms (Mean ± SE)	IL-5 (U/ml) (Mean ± SE)
No cells	133·6 ± 6·8	162·0 ± 11·9
Unseparated MLNC	74·6* ± 6·7	581·6* ± 36·7
$CD4^+$ MLNC	48·4* ± 9·5	804·8* ± 50·0
$CD4^-$ MLNC	130·0 ± 10·9	224·0 ± 28·8

*Mean significantly lower than no cells control ($P < 0·05$).

The data described here show that infection with GI nematodes induces rapid changes in the predominant phenotype of T cells in the MLN which drains the small intestine. Somewhat unexpectedly, the initial phenotype expressed was associated with type 1, rather than type 2 cytokine release. When stimulated *in vitro* with Con A, MLNC from uninfected mice released little or no IFN-γ or IL-5, the two cytokines assessed here as indicators of Th1 and Th2 activity, respectively. In contrast, MLNC taken two days after infection with *T. spiralis* released large amounts of IFN-γ but little IL-5 when stimulated with mitogen. RT–PCR studies showed a significantly increased level of IFN-γ mRNA at 24 hr after infection.

The IFN-γ released came primarily from the T cells in the MLN, both $CD4^+$ and CD8, although the former made the greater contribution. Within two days the capacity of MLNC to release IFN-γ *in vitro* declined rapidly and by day 8 Con A stimulation elicited release of IL-5 but not IFN-γ When MLNC were stimulated with parasite antigen no cytokine release was seen at day 2, but at day 8 IL-5 was released. These data show that, as infection progresses, there is a switch in Th phenotype which is accompanied by the acquisition of antigen specificity. Cells that are antigen-specific and which release IL-5 are functionally active and have the capacity to transfer protective immunity into naive recipients.

Other workers[13] have shown a somewhat similar change in cytokine profiles in *T. spiralis*-infected mice, but this change was restricted to cells from the spleen; cells from the MLN released little IFN-γ initially (day 3) but large amounts of IL-5 at day 7. There is evidence that the cytokine responses of $CD4^+$ cells show a degree of compartmentalization in the body, although data are sometimes inconsistent. For example, MLNC stimulated *in vitro* with immobilized anti-CD3 antibody released more IFN-γ but less IL-4 than cells taken from peripheral nodes or spleen;[22] significant release of IFN-γ occurred only after 5 days of culture. In contrast, MLNC taken from mice infected with *T. spiralis* and stimulated *in vitro* with parasite antigen contained more IL-5 and fewer IFN-γ-secreting cells than did the spleen,[13] data that agree with results using mitogen stimulation of cells from uninfected animals.[23] None of these results provides a basis for explaining the rapid infection-induced shift to a Th1 phenotype described here.

The data obtained using infections with L3 and L4 *N. brasiliensis* show that the early IFN-γ-dominated MLNC response is not parasite specific and coincides with the entry of worms into the intestine; it must therefore reflect some interaction between parasites and the mucosa. *N. brasiliensis* is closely associated with the epithelial surface but does not penetrate the mucosa, whereas *T. spiralis* actively invades enterocytes. Nevertheless, both parasites elicit a similar pattern of cytokine release. The nature of the interaction that leads to this response and the mediators involved are not clear at this stage. In many systems early production of IL-12 from phagocytic cells results in the development of a Th1 polarized response.[24] Administration of IL-12 to mice infected with *N. brasiliensis* inhibited Th2-dependent responses and altered the day 8 cytokine profile in MLNC from one dominated by IL-4, IL-5 and IL-9 to one dominated by IFN-γ.[12] The present data show a different situation in that the initial Th1 polarization was then reversed. It is also not clear how phagocytic cell responses may be induced and achieve Th1 activation at such an early stage after worms enter the intestine. Cytokine release from cells more immediately involved with the invading parasites must therefore also be considered. Among the epithelial cells that may influence local cytokine responses are the intra-epithelial lymphocytes, many of which carry the γδ T-cell receptor (TCR) and produce a variety of cytokines. Little is known of the responses of intestinal intra-epithelial lymphocytes (IEL) to nematode infection; however, γδ T cells in the spleen and peritoneal exudate (PEC) of mice infected with *N. brailiensis* responded by producing IL-4, rather than IFN-γ, although there was an early, low level production of IFN-γ 1 day after infection in PEC.[25] γδ T cells are only a small percentage of cells in the MLN[26] and are unlikely to make a significant contribution to the cytokines released *in vitro*.

The observations described in this paper suggest that the intestinal mucosa and associated lymphoid tissues may have an initial 'default' Th1 response[27] to invasion by pathogens such as nematode parasites. Preliminary experiments indicate that this response is shown after infection of NIH mice with two other nematodes (*Heligmosoides polygyrus* and *Trichuris muris*) and is also shown by other strains of mice infected with *T. spiralis*. The trigger for this default MLNC response is the initial interaction of parasites with the mucosa and presumably involves cytokine-mediated signals, although these remain undetermined. Subsequent exposure to antigens released by the nematodes must then result in a switch to a type 2 phenotype, again, presumably, as a consequence of an altered cytokine milieu. These cytokines may have a T-cell or, possibly, non-T-cell (?mucosal mast cell) source. Recognition of this sequence of events, and analysis of the underlying mechanisms, is important, because in certain cases, in hosts of particular genotypes, nematodes fail to induce host-protective Th2-mediated mechanisms[8] and long-lasting infections result, a situation that may underlie the chronicity of GI nematode infections in human hosts.

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