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Development of T-cell immune response in experimental murine trichinellosis

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Summary

Cellular response of mice BALB/c to the infection with 400 larvae of *Trichinella spiralis* and the parasite recovery in the host were examined during 180 days post infection (p.i.). Numbers of *T. spiralis* adults in small intestine were gradually reduced from the beginning of the infection (304.5 ± 40.5 per mouse) up to day 30 p.i. (20.3 ± 3.6 per mouse). Muscle larvae were present from day 20 p.i. in numbers of 15908.3 ± 2420.3 per mouse. The rapid increase and high numbers of larvae were found from day 30 p.i. (114520.0 ± 15419.9) till the end of the examination (100139.3 ± 4026.6). Both splenic T and B lymphocytes responded to nonspecific mitogens (concanavalin A and lipopolysaccharide) with increased proliferative activity even at the beginning of the infection, with the maximum on days 30 and 45 p.i., respectively. However, B lymphocytes were more stimulated during the muscle phase of the infection (after day 20 p.i.). Splenic CD4+ T subpopulation was reduced till day 20 p.i. and their numbers increased after day 30 p.i. The subpopulation of CD8+ T cells was already increased on day 15 p.i. The enhancement of serum IFN-γ concentration (Th1 type) till day 45 p.i. was changed to the decline until day 90 p.i. The production of serum cytokine IL-5 (Th2 type) was biphasic, reaching peaks on days 10 and 90 p.i.

Results suggest that the *T. spiralis* infection induced the increase of function activity of immunocompetent T and B cells, as well as the presence of splenic CD4+ and CD8+ lymphocytes. Th2 immune response was predominant during the intestinal (till day 30 p.i.) and chronic phase of the infection (after day 90 p.i.), but Th1 type reaction was activated during the migratory phase of larvae till their encapsulation.

Key words: *Trichinella spiralis*; mice; splenic CD4+ and CD8+ T lymphocytes; IFN-γ; IL-5

Introduction

Trichinella spiralis is the intestinal nematode parasite with worldwide distribution and which causes trichinellosis – a serious zoonosis. The complete life cycle of *T. spiralis* occurs in one host, which means that the host can exert its immune attack at several points in the life cycle. Adult worms reproduce in the small intestine, newborn larvae migrate through the blood and lymphatics to the skeletal muscle cells, and encapsulated larvae (L1) represent the infective stage of the parasite (Despommier, 1993). The immunological hallmarks of helminthosis including trichinellosis – eosinophilia, mastocytosis, and hypergammaglobulinemia IgE, are induced by cytokines from Th2 subset (Finkelman *et al.*, 1997). Studies of the biological features of the host-parasite relationship, which are based on immune response of the host or molecular techniques to characterize the parasite are important for diagnostics or therapy of trichinellosis in men (Nöckler, 2003) and understanding the fine balance between survival of parasite and its expulsion.

Every stage of the life cycle of *T. spiralis* can evoke a stage-specific protective host immune response due to the uniqueness in both the cuticular antigens and the excretion/secretion antigens of each stage (Wang, 1997). Larval cuticular fragments (Bruschi *et al.*, 1992) and metabolites may act as antigens stimulating both specific and non-specific defense reactions during the infection. *T. spiralis* secretes potent glycoprotein antigens that elicit a strong, systemic host immune response, yet local cellular infiltrates are limited (Reason *et al.*, 1994). The mechanism of expulsion of worms is dependent on a Th2 type of response (involving IL-4, IL-13, and IL-9) what leads to the activation of mucosal mast cells (Faulkner *et al.*, 1997; Urban *et al.*, 2000; Khan *et al.*, 2001). Muscle infection with *T. spiralis* elicited a focal cellular immune response. Parasites survive in nurse cells in close association with macrophages, CD8+ and CD4+ T lymphocytes, and B lymphocytes (Beiting *et al.*, 2004). B lymphocytes secreting antibodies, particularly IgG and IgE, may lead to an effective antibody-dependent cell mediated cytotoxic reaction against *T. spiralis* newborn larvae (Moloney & Denham, 1979; Wang & Bell,

1988).
CD4+ T helper type 2 cells are critical in host protective immune and inflammatory responses during *T. spiralis* intestinal infection (Wakelin & Goyal, 1996). CD4+ T cell activation and differentiation require their interaction with antigen-presenting cells. Secondary lymphoid organs (the spleen, lymph nodes, and mucosal lymphoid tissue) are thought to provide the proper environment for antigen-presenting cells to interact with and activate naive T and B cells. T cell-derived cytokines, such as IL-2, IFN-γ, IL-4, and IL-5 have been shown to be correlated with an effective immune response to *T. spiralis* infection in a murine system (Zhu & Bell, 1990; Pond *et al.*, 1992). It was proved that during the intestinal phase of the infection Th1 response is suppressed (Hogaboam *et al.*, 1996). Activated Th2 cells in mesenteric lymph nodes appear to be critical for host protection; however, Th1 cytokine (IFN-γ) is also produced at the first week of infection (Ishikawa *et al.*, 1998). Th1 and Th2 cytokines act antagonistically and mutually regulate each other (Abbas *et al.*, 1996). One of the distinctive features of trichinellosis is its chronicity, and the resulting persistent antigenic stimulation could lead to polarization of T cell subset populations and modification of immunoregulatory states. There is little known about cell-mediated immunity during the course of the chronic infection.
Our study was aimed at investigation of the regulatory role of T cells in modulation of immune response during long-lasting experimental murine trichinellosis in relation to the parasite burden.

Materials and Methods

Experimental schedule
The experiment was carried out on inbred BALB/c mice, 7 week old males. Animals were housed under the standard conditions (20 – 21°C, 50 – 60 % relative humidity, 12hr light regime) and fed ad libitum on a commercial diet. The experimental protocol was approved by the Parasitological Institute Animal Care Commitee.
Mice were divided into two groups:
Group 1 – mice (n=39) without the infection = control
Group 2 – mice (n=52) infected per os with 400 *T. spiralis* larvae per mouse on day 0 of the experiment.
The mice were sacrificed by cervical dislocation. The samples of blood and cells were isolated from three mice of control group and four mice of infected group on days 0 (before the infection) and 5, 10, 15, 20, 30, 45, 60, 75, 90, 120, 150 and 180 p.i.

Infective larvae of T. spiralis
The *Trichinella spiralis* (T1, ISS004, Maryland, USA, *Sus scrofa*) isolate used in the present experiments was maintained by serial passage in outbred ICR mice at the Parasitological Institute SAS and originally obtained and assigned codes from the Trichinella Reference Centre in Rome. Larvae were released by artificial digestion (1 % pepsin, 1 % HCL for 4 h at 37 °C) of tissue following the standard protocol and kept saline solution until inoculation of experimental mice.

Intestinal worm burdens
The intestinal phase of infection was investigated on days 5, 10, 15, 20 and 30 p.i. The recovery of intestinal worms was attempted using a standard method (Campbell, 1983). Small intestine was cut into 5 – 10 cm long pieces, placed into a sieve and incubated in conical pilsner glasses in 37°C NaCl (0.9 % saline) overnight. After incubation, gut pieces were discarded and the sediment was counted under stereomicroscope at 60 x magnification. Differentiation of males and females was based on size and the absence of copulatory appendages.

Isolation of muscle larvae T. spiralis
The muscle phase of infection was examined on 20, 30, 45, 60, 75, 90, 120, 150, and 180 d.p.i. Whole eviscerated carcasses were minced and artificially digested (1 % pepsin, 1 % HCL for 4 h at 37°C) according to Kapel and Gamble (2000). Samples were allowed to settle for 20 min before the supernatant was discard and the sediment was poured through a 180 µm sieve into a conical glass and washed with tap water. The sediment was finally transferred to a gridded Petri dishes and counted using a stereomicroscope at 40 x magnification. Depending on the density of larvae either a sub-sample or the whole sample was counted.

T and B lymphocyte proliferation assay
The splenic cells were aseptically isolated, washed twice with Dulbecco-PBS medium (pH 7.2) and finally with RPMI 1640 medium (Sigma). The contamination of erythrocytes was removed by lysis in hypotonic solution (0.85 % NH_4Cl) and lymphocytes were diluted so as to obtain a final concentration of 5 x 10^6 cells /ml. The proliferation assay was carried out in 96 wells plates (Nunc) and cells were incubated in RPMI 1640 medium (200 µl) containing 10 % bovine fetal serum and 20 µg/ml of gentamycine. Polyclonal activators Concanavalin A (Con A) (T cells) and lipopolysaccharide (LPS) (B-cells) (Sigma) at the final concentration: 3 µg/ml and 6 µg/ml, respectively, were added to the cell suspensions and incubated at 37°C in 5 % CO_2 and 85 % humidity for 72 hours. The stimulation indices (SI) were determined using colorimetric assay where 20 µl aliquots of 3,4-dimethylthiazolyl 2,5-diphenyltetrazolium bromide (Sigma) (0.1 % solution) were added to the cell pellets, incubated for 4 h at 37°C and 5 % CO_2 followed by the centrifugation of plates at 800 x g for 5 min. Reaction was terminated with DMSO (200 µl/cell sample) and DNA synthesis was measured using spectrophotometer (Multiskan Plus, Labsystem, Finland) at OD 540 and 680 nm. The stimulation indices (SI) were calculated according to the formula of Pagé *et al.* (1988):
$SI = E_{540} - E_{680}$ (stimulated cells) / $E_{540} - E_{680}$ (unstimulated cells)
Proliferative responses were measured for lymphocytes isolated from each mouse per group separately.

Numbers of CD4+ and CD8+ T cell
Lymphocytes isolated from the mice spleens and depleted of erythrocytes were re-suspended in PBS (pH 7.2) at a final concentration 1x 10^6 cells /ml. After incubation of cell samples with directly-labelled rat anti-mouse CD4+ (FITC) and rat anti-mouse CD8+ (PE) monoclonal antibodies (PharMingen) at the concentration 0.4 µg/10^6 cells at 4°C for 30 min, cells were then washed three times with PBS (pH 7.2) containing 0.1 % NaN_3 and analysed by the Flow cytometry method. Data for 10^4 cells/sample, falling within FSC and SSC gates set specific for lymphocytes, were collected with the FACScan flow cytometer (Becton Dickinson Biosciences). All data files were analysed with CellQuest software. Cells from each mouse per group were analysed individually. The final numbers of both cell populations were calculated as proportion from the total isolated lymphocytes per spleen/mouse.

Concentration of serum IL-5 and IFN-γ
The sandwich ELISA was employed to determine concentration of cytokines IL-5 and IFN-γ in serum of mice from all experimental groups after method of Šoltýs and Quinn (1999). IFN-γ and IL-5 were used as marker cytokines for Th1 and Th2 response, respectively. The two cytokine-specific monoclonal antibodies for IFN-γ were R4-6A2 and XMG1.2 and for IL-5 were TRFK5 and TRF4 (PharMingen). Results were expressed as pg/ml using murine recombinant IFN-γ and IL-5 (PharMingen) as the standards. The detection limit of the assay for the both cytokines was 40 pg/ml.

Statistical evaluation
Statistical differences between infected and control mice at each time point were evaluated using the nonparametric Mann-Whitney U test. A value of $P < 0.05$ was considered significant. The analysis was performed using the Statistica 6.O (Stat Soft, Tulsa, USA) statistical package.

Results

Parasite recovery (Tab. 1)
Number of *T. spiralis* adults in small intestine was decreasing from the beginning of the infection (mean value 304.5/mouse) up to day 30 p.i. (mean value 20.3/mouse). During muscle phase of the infection numbers of larvae continuously increased from value 15908 to 100139 larvae per mouse. The high numbers of *T. spiralis* larvae (over 10^5 in one mice) were already found from day 30 p.i.

Proliferative response of T lymphocytes to Con A (Fig. 1)
Healthy uninfected animals (control) did not show any relevant changes in any examined immune parameters throughout.
Stimulation of proliferative activity of splenic T cells was already found at first week p.i., reaching maximum on day 30 p.i. The T-cell response to Con A in infected mice was significantly ($P < 0.05$; $P < 0.01$) increased from day 20 till 45 p.i. In following days, the decrease in the proliferative activity was found and indices of stimulation were comparable to values in control group until the end of the experiment.

Proliferative response of B lymphocytes to LPS (Fig. 2)
In comparison to T cells, B-cell proliferative activity was increased 10 days later, i.e. from day 15 p.i. The proliferative response of B cells to LPS peaked on day 45 p.i. and a gradual decrease of stimulation indices was recorded from day 45 p.i. until the end of the experiment. The values of stimulation index in infected mice significantly ($P < 0.05$; $P < 0.01$) exceeded the values of control from day 30 to 75 p.i.

Table 1. Numbers of adults and larvae of *T. spiralis* from small intestine and muscles of mice infected with 400 larvae of *T. spiralis*

Day p.i.	Intestinal phase			Muscle phase
	Adults (mean value ± SD)			Larvae (mean value ± SD)
	♀	♂	Total ♀+♂	
0	infection with 400 larvae			
5	190.0 ± 22.1	116.5 ± 15.5	304.5 ± 40.5	
10	159.0 ± 31.4	91.3 ± 7.5	250.3 ± 38.9	
15	144.7 ± 21.4	97.0 ± 23.6	241.7 ± 45.0	
20	111.7 ± 16.7	78.7 ± 21.0	190.4 ± 30.6	15908.3 ± 2420.3
30	8.3 ± 0.6	12.0 ± 3.6	20.3 ± 3.6	114520.0 ± 15419.9
45				125700.0 ± 5565.1
60				130266.7 ± 2419.4
75				117900.0 ± 17936.8
90				104733.3 ± 2793.4
120				127808.3 ± 11542.9
150				111303.7 ± 10127.2
180				100139.3 ± 4026.6

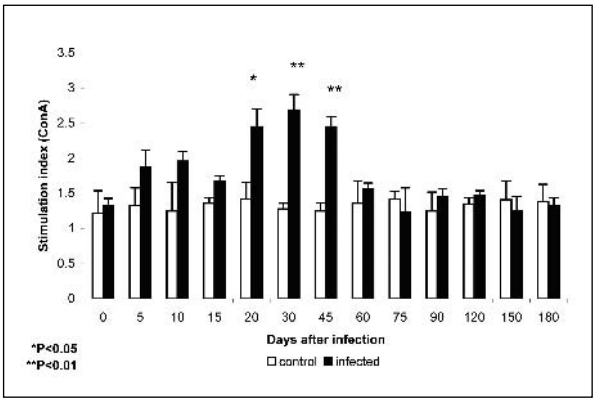

Fig. 1. The proliferative response of splenic T lymphocytes to Con A in mice infected with *T. spiralis*
infected n = 4, control n = 3; * significant differences between two groups (P < 0.05); ** significant differences between two groups (P < 0.01)

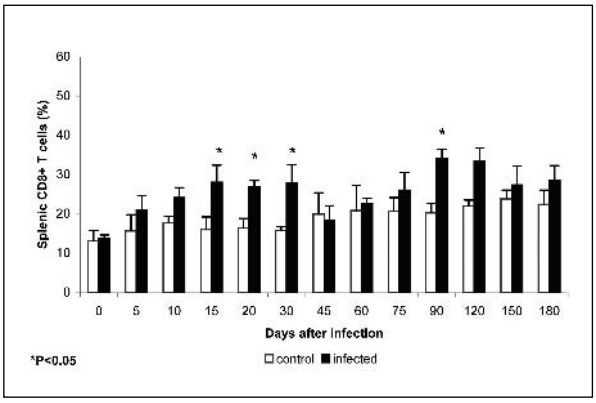

Fig. 4. The relative percentage of CD8+ T cells in spleen of mice infected with *T. spiralis*
infected n = 4, control n = 3; * significant differences between two groups (P < 0.05)

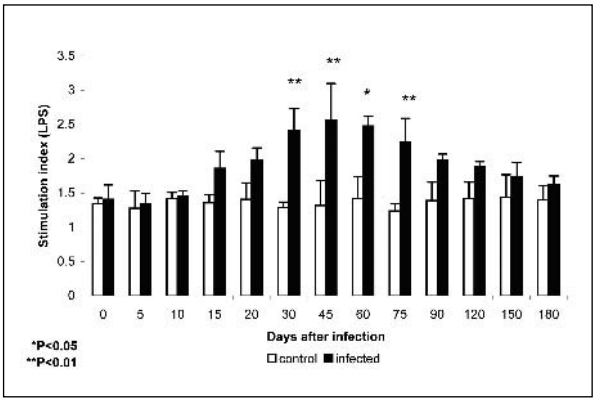

Fig. 2. The proliferative response of splenic B lymphocytes to LPS in mice infected with *T. spiralis*
infected n = 4, control n = 3; * significant differences between two groups (P < 0.05); ** significant differences between two groups (P < 0.01)

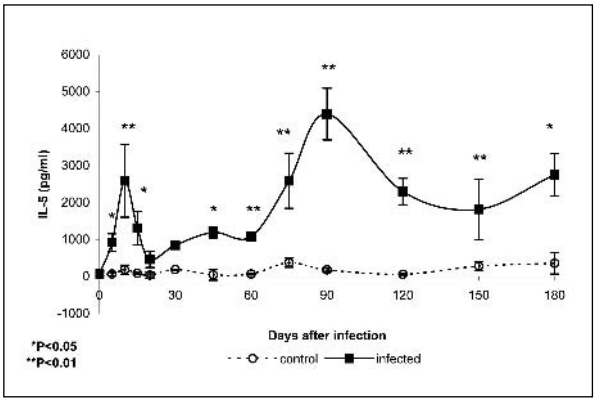

Fig. 5. Concentration of serum IL-5 in mice infected with *T. spiralis*
infected n = 4, control n = 3; * significant differences between two groups (P < 0.05); ** significant differences between two groups (P < 0.01)

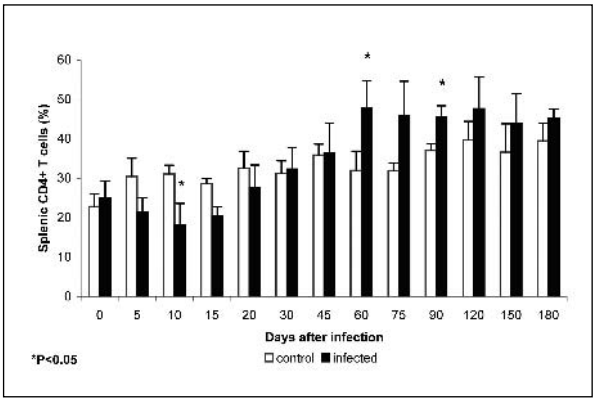

Fig. 3. The relative percentage of CD4+ T cells in spleen of mice infected with *T. spiralis*
infected n = 4, control n = 3; * significant differences between two groups (P < 0.05)

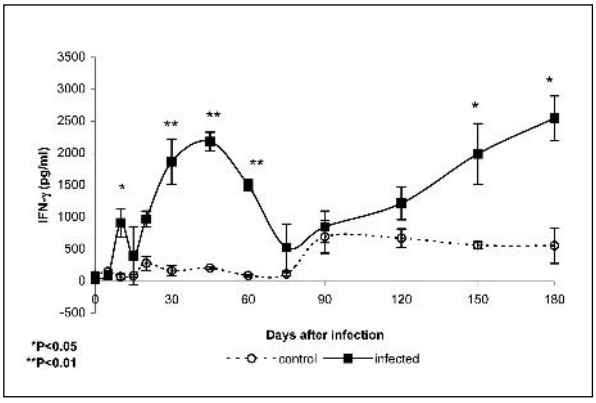

Fig. 6. Concentration of serum IFN-γ in mice infected with *T. spiralis*
infected n = 4, control n = 3; * significant differences between two groups (P < 0.05); ** significant differences between two groups (P < 0.01)

Distribution of CD4+ and CD8+ expressing T cells in spleen (Fig. 3 and Fig. 4)

After reduction of CD4+ cells in early phase of the infection (till day 20 p.i.) the permanent increase of this subpopulation was observed until day 60 p.i. The lowest ($P < 0.05$) presence of CD4+ T cells in spleen of infected mice was recorded on day 10 p.i. High values ($P < 0.05$) of this subpopulation were found from day 60 to 90 p.i. Presence of CD8+ cells in spleen of infected mice increased in two cycles, with maximal values ($P < 0.05$) on days 15 and 90 p.i.

Concentration of IL-5 and IFN-γin serum (Fig. 5 and Fig. 6)

Production of IL-5 was biphasic and peaked on days 10 and 90 p.i. After the first increase till day 15 p.i. ($P < 0.05$; $P < 0.01$) the level of serum IL-5 in infected mice declined and started to elevate after day 60 p.i. The second rise was stronger and values of IL-5 concentration in serum of infected mice remained high until the end of the experiment. Infected mice showed significantly ($P < 0.05$; $P < 0.01$) higher values of IL-5 cytokine almost any day post infection in comparison to control.

Concentration of IFN-γ had several cycles of higher and lower production. The first upgrade of IFN-γ was found on day 10 ($P < 0.05$), then on day 45 and other re-increase from day 120 p.i till the end of our observation. The significant rise in IFN-γ concentration of infected mice was recorded from days 30 to 60 p.i. ($P < 0.01$) and on days 150 and 180 p.i. ($P < 0.05$).

Discussion

Trichinella spiralis induces a chronic infection where a major role in host defense processes is played by cellular immunity (Mahida, 2003). The energy consumed by a host exclusively to mount an immune response is detrimental. Martinez *et al.* (2004) found that mass-controlled oxygen in *T. spiralis* infected rats had a clear tendency to increase during primary infection, especially in acute intestinal phase, and reported a positive correlation between oxygen consumption and number of larvae at 7 and 14 dpi. Expulsion of adults from the small intestine begins around day 6 p.i. In our study, after the oral infection of mice with 400 larvae of *T. spiralis*, adult parasites were present till 30 dpi with decrease of the number from the beginning of the experiment. The first findings from intestines were around 304.5 ± 40.5 adults per animal on day 5 p.i. After ingestion of larvae, a host develops an immunity-mediated inflammatory response in the intestine, and this is followed by rapid expulsion of some of the larvae from the intestine (Bell, 1998). Adult worms were decimated to 20.3 ± 3.6 exemplars in one mouse till day 30 p.i. Larvae in muscles of infected mice were detected from 20 dpi till the end of experiment, with the maximum number at 45 dpi. The growth of muscle larvae numbers was determinated by a fecundity of adults up to day 30 p.i., when the most of worms was eliminated from the gut.

The used mouse strain BALB/c appears to be resistant to *T. spiralis* infection with intensive immune responses. These mice are strong producer of IgE antibodies, which enhance parasite clearance and regulate mast cell responses during trichinellosis. BALB/c mice rejected the parasite burden in trichinella's infection with 450 larvae after 14 – 17 days p.i. (Gurish *et al.*, 2004). Pemberton *et al.* (2004) found the novel mouse intelectin-2 in goblet and Paneth cells of the jejunum in BALB/c mice, which expression during infection peaked at the time of worm expulsion. In contrast, the susceptible C57BL/10 mouse strain (lacking the gene for intelectin-2) showed delayed *T. spiralis* expulsion.

The absence of antigen on the cuticular surface of adult worms is in a sharp contrast to the findings described in muscle larvae, whose surface antigenicity is identical to that of stichocyte α-granules (Takahashi *et al.*, 1990, Appleton *et al.*, 1991). Therefore, no direct immune attack is likely to be exerted against adults. It has been suggested that the effects of serum antibodies on worms may be indirect and that worm expulsion is the result of inflammatory processes in the intestines evoked by the infection (Wakelin & Wilson, 1979).

Our results show that *T. spiralis* infection caused increased proliferation activity of spleen T lymphocytes even at the first week p.i. and B lymphocytes were activated from the second week p.i. Polyclonal lymphocyte activation of T-cells, but particularly B-cells, is responsible for the high levels of immunoglobulines IgG, IgM, and IgE observed in infected animals (Kazura, 1981) and in humans (Murrel & Bruschi, 1994). In our study, the decrease of stimulatory indices of splenocyte proliferative activity was recorded after day 45 p.i., when the majority of migratory larvae had been settled in muscle cells and had changed the host cell into nurse cell providing a suitable habitat for the larva (Despommier, 1993).

Expression of surface CD4+ and CD8+ antigens on T cells was monitored in order to examine impact of the infection on the balance between Th1 and Th2 – mediated cell immunity. Splenic CD4+ T subpopulation was reduced until day 20 p.i. Following days a permanent rise of the CD4+ cell numbers was found. During the host immune intestinal response, CD4+ and CD8+ lymphocytes infiltrate the jejunal muscle layers and they are capable to mediate tissue damage. CD4+ T cell mediated mucosal changes (Ishikawa *et al.*, 1997), which include intestinal goblet cell hyperplasia with high mucin secretion, play important role in the trapping and removal of intestinal worms from the gut (Miller, 1987). Persistent elevation of both CD4+ and CD8+ T cell subsets in pigs from 15 to 60 days after parasite exposure was observed by Ivanoska *et al.* (1990). Dillender and Lunney (1993) also confirmed an important role of T lymphocytes of the CD4+CD8+ phenotype in the regulation of the immune response to this parasite. In our experiment CD8+ cells were not decreased, but two peaks occurred during the experiment, on days 15 and 90 p.i. Similarly, Morales *et al.* (2002) analysed the phenotype of

the T cell subpopulations in human patients and found a trend toward an increase in the number of CD8+ cells and a decrease in the number of CD4+ cells. They also found that the CD4+ T subset was accompanied by a reduction in the number of naive cells and increase in the number of memory cells. Vallance *et al.* (1999) found in immunodeficient mice with trichinellosis that CD8+ T cells play no significant role in worm expulsion but that CD4+ T cells may make a significant contribution. Immune response involving CD4+ T cells and MHC II expression considerably accelerated worm loss, particularly between days 12 and 21 p.i.

Cytokines elaborated primarily by T helper cells play a dominant role in orchestrating both anti-parasite responses and pathology in many helminth infections including *T. spiralis* (Finkelman *et al.*, 1997). In order to examine a relation between Th1 versus Th2 cytokine profiles, serum levels of "marker" cytokines IFN-γ and IL-5 were monitored. In our work the production of serum IL-5 cytokine (Th2 type) of infected mice mounted firstly on day 10 p.i. and then lower level of its concentration was observed up to day 60 p.i. After this day a continual elevation of IL-5 was noticed until the second peak on day 90 p.i. IL-5 is a glycoprotein which is produced and secreted mainly from helper T cells following activation with antigen or mitogen. In present work, CD4+ T subset was reduced till day 20 p.i., what could caused lower IL-5 level in serum of infected mice. This proinflammatory cytokine is a key for production, differentiation and activation of eosinophils, for a B cell growth, and enhancing factor for IgA production (Beagley *et al.*, 1988; Kinashi *et al.*, 1986; Hom & Estridge, 1994). Eosinophils and IgA antibodies are potent factors in the defensive response generated against parasites (Coffman *et al.*, 1987). IL-5 can also delay the apoptosis of eosinophils (Simon & Blaser, 1995; Stern *et al.*, 1992) as well as induce their differentiation into a more active, hypodense state (Gleich *et al.*, 1993; Weller, 1994). Variation in the level of IL-5 indicates the severity of inflammation. IL-5 directly induced degranulation and superoxide production from eosinophils (Horic *et al.*, 1996; Dvorožňáková *et al.*, 2003) but did not elevate the activation of eosinophils (Kita *et al.*, 1992). Vallance *et al.* (2000) confirmed that IL-5 is not only essential for the onset of intestinal eosinophilia, but also makes a significant contribution to enteric host defence during challenge *T. spiralis* infections. IL-5 expression is only minimally protective during a primary *T. spiralis* infection but may protect against repeated exposure to gastrointestinal parasite. Developing protection against reinfection is important in determining the survival and viability of the host. Dent *et al.* (1997) suppose that eosinophilia and/or IL-5 may contribute to the survival of parasite *Schistosoma mansoni*. In our experiment antilarval response was insufficient in spite of high level of IL-5, numbers of muscle larvae were nearly constant from day 45 p.i. Doligalska (2000) also demonstrated an increase in the number of activated eosinophils and in the concentration of IL-5 was not associated with an increase in protective immune response.

According to our results, the IL-5 concentration in serum of mice with trichinellosis was enhanced at early phase of the infection and persisted increased during 6 months of the experiment. High level of IL-5 reflected a predominant Th2 immune response.

An expulsion of adult *T. spiralis* from the intestine is associated with increased production of Th2 cytokines and decreased production of Th1 cytokines (Finkelman *et al.*, 1997; Grencis *et al.*, 1991). The Th1 subset of T helper cells is known to orchestrate inflammation (Mosmann, 1991; Borošková *et al.*, 2003), a dominant feature of the enteric stage of infection with *Trichinella* (Khan & Collins, 2004). Pro-inflammatory cytokines, such as IL-18, IL-12, and IFN-γ are crucial regulators of Th2-mediated immunity to intestinal helminth infection (Helmby & Grencis, 2002; Helmby *et al.*, 2001).

On the contrary to huge Th2 type response in the present work, the representative cytokine of Th1 type - IFN-γ showed several cycles of higher and lower production in a course of the experiment. The first elevation of IFN-γ in serum was found on day 10 p.i., then on day 45 p.i. and reincrease from day 125 p.i. up to the end of the observation. It means that parallel expression of Th1 and Th2 responses were already recorded at the beginning of the infection. A shift in infected mice from Th2 to Th1 subset was noticed between days 15 and 60 p.i., and this reflects in a decreased level of IL-5 and a higher level of IFN-γ. The presence or absence of IFN-γ secretion determines, whether a T cell subset has protective activity against *T. spiralis* infection (Ramaswamy *et al.*, 1994). IFN-γ is crucially involved in protection against newborn larvae, but does not affect the expulsion of adult worms (Helmby & Grencis, 2003). In our experiment the second peak of IFN-γ was reached on day 45 p.i., and maximal values of the muscle larvae numbers were detected on days on 45 and 60 p.i. Mechanism of IFN-γ mediated immunity to newborn larvae may include enhanced cytotoxic killing by eosinophils, granulocytes and activated macrophages (Gansmuller *et al.*, 1987; Venturiello *et al.*, 1993, 1995). In spite of a rising IFN-γ concentration in serum of our experimental animals after day 125 p.i., only an insignificant reduction of muscle larvae was observed during the 2 last experimental months. While Machnicka and Dziemian (2001) recorded a remarkable reduction in muscle larval burden in rats on day 60 p.i. The Th1 cytokine IFN-γ enhances the macrophage's production of nitric oxide, which plays an important role in the cytotoxic activities of macrophages (Fultz *et al.*, 1993). The second rise in IFN-γ production from our experiment between days 15 and 45 p.i. correlates with higher nitric oxide production in *T. spiralis* infected mice at the same time after infection in study of Kolodziej-Sobocinska *et al.* (2005, in press).

IFN-γ has an antagonistic cytokine IL-10. IFN-γ inhibits macrophage secretion of IL-10 (Mosmann, 1994). The balance between IL-10 and IFN-γ determines the development of immunity against the life stages of the parasite (Helmby & Grencis, 2003). IL-10 deficiency results in in-

creased IFN-γ responses in vivo (Moore *et al.*, 2001). Beiting *et al.* (2004) revealed a role for IL-10 in limiting inflammatory responses during the early stages of muscle infection by *T. spiralis*, but the chronic inflammation is independent of IL-10 and is accompanied by a shift to a Th2 response following completion of parasite development in the muscle. In our work the IFN-γ level descended from day 45 to 75 p.i., what could be caused by IL-10 effect at the beginning of the muscular phase.

There is a little knowledge about a cytokine production during the muscular phase of trichinellosis. Morales *et al.* (2002) found in human study a maximal IL-5 production by peripheral blood mononuclear cells 2 months p.i. and IFN-γ production increased up to 9 months p.i. Similarly, our results document a rising IFN-γ level in a serum of infected mice from day 75 p.i.

Release of cytokines by mesenteric lymph node cells during early intestinal phase of trichinellosis was examined in rats by Stewart *et al.* (1999). They detected a significant elevation in IFN-γ production during the early stage (days 4, 6 and 9 p.i.) of enteral phase of infection. The highest level of IL-5 was seen at day 12 p.i. and its significant elevation to the end of the study, till day 20 p.i. In our work one of IFN-γ enhancement in mice was found on day 10 p.i. Th1 cells probably exert a significant influence on immunological events in the enteric environment out to day 10 p.i., as indicated by the highest levels achieved by IFN-γ until this day. Also other research works describe dominating Th1 type response in mice or rats within 2 – 9 days after *T. spiralis* infection and later it was replaced by the Th2 type response (Ishikawa *et al.*, 1998; Grencis *et al.*, 1987; Ramaswamy *et al.*, 1996). Some authors observed increase of IFN-γ later. Frydas *et al.* (1996) observed marked increases of IFN-γ, TNF-α and IL-4 levels in mice blood between days 14 and 20 p.i. Dzik *et al.* (2002) found a marked increase of IFN-γ-like activity in the serum of *T. spiralis* infected guinea-pigs between days 9 and 26 p.i.

Our results from the *T. spiralis* infection in mice show a significant stimulation of non-specific proliferative response of T and B splenocytes from day 20 p.i., when an intensive migration of newborn larvae was realised. Also splenic CD8+ cells had a high presence during early stage of muscular phase of the infection. Probably this CD8+ T subpopulation and its cytokine secretion have a great participation in an antiparasitic defence. A mixed production of Th1 and Th2 serum cytokines was detected during the first week p.i. and then high production of Th1 cytokine – IFN-γ occurred together with Th2 response (IL-5). Originally, it was believed that Th2 and Th1 responses would antagonize, but data of Helmby and Grencis (2003) and our study demonstrated that strong Th1 and Th2 responses can develop simultaneously *in vivo* and that the development of one type of the Th cell response does not necessary inhibit or prevent the development of the other. Our study suggests that the host develops strong Th1 response during newborn larvae migration until their encystation, what probably activate effector cells of immune response – macrophages to eliminate parasite.

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