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Trichinella spiralis reinfection: changes in cellular and humoral immune response in BALB/c mice

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Summary

Trichinella spiralis infection induces a host cell-mediated and humoral response. The role of T and B lymphocytes in the immune response of mice reinfected with 2 x 400 *T. spiralis* larvae was studied in relation to the parasite burden. BALB/c mice were infected on days 0 and 60 and immunological parameters were examined within a period of 180 days. In comparison with a single *T. spiralis* infection, T- and B-lymphocytes in reinfected mice responded by a significant increase in the proliferative activity during 10 days after reinfection. At the same time, the percentages of CD4+ T-cells of reinfected mice were also increased. In contrast, the CD8+ T-cell numbers were significantly reduced almost 30 days after reinfection. High concentration of serum IFN-γ lasted till the end of the experiment. The IL-5 level was increased only for 2 weeks after reinfection, followed by its decrease. Kinetics of specific anti-*Trichinella* immunoglobulins IgG_{2a} was not affected with reinfection, but specific antibodies IgG_1 significantly increased after reinfection and persisted elevated till the end of the experiment. Lower numbers of adults (69.2 % reduction) in the small intestine and 72.3 % reduction in muscle larvae were found after reinfection. Stimulation of the host immune response – the increased activity of CD4+ T lymphocytes and high levels of IFN-γ and specific IgG_1 after reinfection, contributed to the reduction of the parasite burden.

Keywords: *Trichinella* spiralis; T lymphocytes; cytokines IFN-γ and IL-5; immunoglobulines IgG_1 and IgG_{2a}

Introduction

Immunological aspects of the host-parasite relationship are widely discussed. Gastrointestinal nematodes provide good models for this discussion, because for many gastrointestinal nematodes, if the host-induced environmental changes are severe, the parasite is expelled and its reproductive life in the host is terminated. This may lead to strong resistance to reinfection (Wakelin, 1993). It is also known that there are differences in immunity during primary and challenge parasitic infections between host species or even among inbred mice strains which varied genetically (Behnke *et al*., 2006).

Trichinella spiralis is the intestinal nematode parasite with worldwide distribution and which causes trichinellosis – a serious zoonosis. The immunological hallmarks of trichinellosis – eosinophilia, mastocytosis, and hypergammaglobulinemia IgE, are induced by cytokines of Th2 subset (Finkelman *et al*., 1997). Repeated infection with helminths occurs often in both humans and animals. There are few publications focused on *Trichinella* reinfection (Grove *et al*. 1977; MacLean *et al*., 1989; Herndon & Kayes 1992; Santamarina *et al*., 1993; Soule *et al*., 1993; Kołodziej-Sobocińska *et al*., 2007). An early study on *T. spiralis* reinfection (Grove *et al*., 1977) demonstrated that a single, relatively light infection with 150 larvae, confers long-lasting resistance to reinfection at least in so far as the development of systemic infection and disease is concerned. The initial ability to expel adult worms from the intestine wanes but then returns in association with the development of humoral and cellular immunity. Immunological mechanisms activated after the secondary infection with *T. spiralis* are not described widely. Kołodziej-Sobocińska *et al*. (2007) examined the role of activated macrophages and free radicals after *T. spiralis* reinfection in mice. Studies on biological features of host-parasite relationship, which are based on the immune response of the host to reinfection are important for understanding the balance between parasite survival and its expulsion, for diagnostics or therapy of trichinellosis (Nöckler, 2003).

Most of studies about trichinellosis are concerned only with the early stages of infection, but trichinellosis is a chronic infection because living larvae persist in the muscles for a long time. 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. The mechanism of expulsion of worms is dependent on the Th2 type of response (involving IL-4, IL-13, and IL-9) which leads to the activation of mucosal mast cells (Urban *et al*., 2000; Khan *et al*., 2004). CD4+ T helper type 2 cells are critical in host protective immune and inflammatory responses during *T. spiralis* intestinal infection (Wakelin & Goyal, 1996). Muscle infection with *T. spiralis* elicited a chronic infection where a major role in host defense processes is played by cellular immunity (Mahida, 2003). Larvae 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; Dvorožňáková *et al*., 2010).
Our study was aimed at development of T-cell response and specific antibody response in mice after reinfection with larvae of *Trichinella spiralis* in relation to the parasite burden.

Materials and methods

The experiment was carried out on male BALB/c mice (n = 120) weighting 18 – 20 g. Mice were kept under a 12-h light/dark regime at room temperature (22 – 24 ºC) and 56 % humidity on a commercial diet and water. The experimental protocol was approved by the Parasitological Institute Animal Care Commitee. Animals were divided randomly into three groups as follows: Group 1 (n = 36) was uninfected and untreated (control), mice in Group 2 (n = 48) were infected *per os* with 400 *T. spiralis* larvae per mouse on day 0 of the experiment. Mice in Groups 3 (n = 36) were infected *per os* with 400 *T. spiralis* larvae per mouse on day 0 and reinfected with 400 *T. spiralis* larvae per mouse on day 60 of the experiment.
Samples of blood and spleen were obtained from three mice of each of groups on days: 0 (prior infection), 20, 45, 60, 65, 70, 75, 80, 90, 120, 150 and 180 post infection (p.i.).

The infective larvae Trichinella spiralis
The reference isolate of *Trichinella spiralis* (ISS 004) (obtained and assigned codes from the Trichinella Reference Centre in Rome), maintained by serial passage in ICR mice at the Institute of Parasitology SAS, was used for the infection. Larvae were released by artificial digestion (1 % pepsin, 1% HCl for 4 h at 37 ºC) 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. and post reinfection. The small intestine was cut into 5 – 10 cm long pieces, placed into a sieve and incubated in conical pilsner glasses in 37 ºC NaCl (0.9 % saline) overnight. After incubation, gut pieces were discarded and the sediment was counted under stereomicroscope at 60 x magnification.

Isolation of muscle larvae
The muscle phase of infection was examined on days 20, 30, 45, 60, 75, 90, 120, 150 and 180 p.i. and 5, 10, 15, 20, 30, 60, 90 and 120 post reinfection. Whole eviscerated carcasses were minced and artificially digested (1 % pepsin, 1 % HCl for 4 h at 37 ºC; both Sigma-Aldrich, Germany) according to Kapel & Gamble (2000). Samples were allowed to settle for 20 min before the supernatant was discarded 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 dished and counted using a stereomicroscope at 40 x magnification. Depending on the density of larvae either a subsample or the whole sample was counted.

T and B lymphocyte proliferation assay
The proliferative activity of splenic T and B was detected spectrophotometrically using an MTT assay (Dvorožňáková *et al*., 2011). Briefly, cells (5 x 10^6 cells /ml RPMI, Sigma-Aldrich, Germany) were incubated with 10 µg/ml of concanavalin A (Con A) (T cells) or lipopolysaccharide (LPS) (B cells) (Sigma-Aldrich, Germany) at 37 ºC in 5 % CO_2 and 85 % humidity for 72 hours. 20 µl aliquots of 3,4-dimethylthiazolyl 2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich, Germany) (0.1 % solution) were then added to the cell cultures, incubated for 4 h at 37 ºC and 5 % CO_2 followed by centrifugation at 800 x *g* for 5 min. The reaction was terminated with dimethylsulfoxide (Sigma-Aldrich, Germany) (200 µl/cell sample) and read at a 540 nm and 630 nm. The stimulation indices (SI) were calculated according to the formula: $SI = E_{540} - E_{630}$ (stimulated cells) / $E_{540} - E_{630}$ (unstimulated cells).

Percentage of CD4+ and CD8+ T cells
Lymphocytes from the spleens and depleted of erythrocytes were resuspended in PBS (pH 7.2) at a final concentration of 1x 10^6 cells /ml. The cellular subpopulations were detected by use of rat anti-mouse CD4+ fluorescein isothiocyanate-conjugated and rat anti-mouse CD8+ phycoerythrin-conjugated monoclonal antibodies (BD Biosciences PharMingen, Belgium) at the concentration of 0.4 µg/10^6 cells at 4 ºC for 30 min. Cells were then washed three times with PBS containing 0.1 % NaN_3 and analysed by the FACScan flow cytometer (Becton Dickinson Biosciences, Germany). All data files were analysed with CellQuest software. All data were expressed as a percentage of lymphocytes based on a gate set using forward and side scatter parameters.

Serum concentration of IFN-γ and IL-5
The capture ELISA was employed to determine the concentration of cytokines IFN-γ and IL-5 in the serum of mice from all the experimental groups after method of Šoltýs & Quinn (1999). IFN-γ and IL-5 were used as

Table1. Numbers of adults recovered from the small intestine of *T. spiralis* infected/reinfected mice

Single *Trichinella spiralis* infection (400 larvae)				***Trichinella spiralis* reinfection (2x400 larvae)**			
Day after infection	female	male	**All adults**	**Day after reinfection**	female	male	**All adults**
5	190.0 ± 22.1	116.5 ± 15.5	306.5 ± 40.5	**5**	95.3±35.5	48.3±12.9	**143.6±44.5
10	159.0 ± 31.4	91.3 ± 7.5	250.3 ± 38.9	**10**	25.8±9.3	11.7±2.9	**37.5±7.8
15	144.7 ± 21.4	97.0 ± 23.6	241.7 ± 45.0	**15**	3.7±3.2	1.6±1.1	**5.3±4.9
20	111.7 ± 16.7	78.7 ± 21.0	190.4 ± 30.6	**20**	0	0	**0
30	8.3 ± 0.6	12.0 ± 3.6	20.3 ± 3.6	**30**	0	0	*0

*P<0.05; **P<0.01 statistically significant from mice with a single infection

marker cytokines for the Th1 and Th2 responses, respectively. Two pairs of cytokine-specific monoclonal antibodies were used: R4-6A2 and XMG1.2 for IFN-γ and TRFK5 and TRF4 for IL-5 (BD Biosciences PharMingen, Belgium). Results were expressed as pg/ml using murine recombinant IFN-γ and IL-5 (BD Biosciences PharMingen, Belgium) as standards. The detection limit of the assay for the both cytokines was 40 pg/ml.

Detection of specific antibody production by iELISA

Specific *Trichinella spiralis* antibodies in serum were detected by indirect ELISA according to Reiterová *et al*. (1999). Excretory-secretory *T. spiralis* antigen diluted at 2 μg/ml carbonate buffer (pH 9.6) were bound to the microtitrate plates (Nunc, Denmark) overnight at 4 ºC. After triple washing of wells with phosphate buffered saline (PBS, pH = 7.2) with 0.5 % Tween 20 (PBS-T) non-specific bonds were blocked with by 0.5 % skimmed milk PBS after 1 hour incubation at room temperature. After triple washing with PBS-T the serum samples and conjugates were added step by step for 1 hour incubation at 37 ºC. Sera were diluted 1:100 in PBS-T. Anti-mouse horseradish peroxidase conjugates (all Sigma-Aldrich, Germany) were diluted: IgG_1 (1:2000), IgG_{2a} (1:500). The substrate ophenylene diamine (Sigma-Aldrich, Germany) at 0.05 mol/l in citrate buffer (pH 4.7) with 0.005 % H_2O_2 was used for a visual reaction. The reaction was stopped by 1M H_2SO_4 after 20 minutes incubation at room temperature in the dark. Plates were measured for the optical density at 490 nm (Revelation Quicklink, Opsys MR, Dynex Technologies, USA).

Statistical evaluation

Statistical differences were assessed using one-way ANOVA, followed by post hoc Tukey's test (a value of $P < 0.05$ was considered significant), which allowed comparison between each two groups at each time point. The analyses were performed using the Statistica 6.O (Stat Soft, Tulsa, USA) statistical package.

Table 2. Total larval burden in carcass of *T. spiralis* infected/reinfected mice

Single *Trichinella spiralis* infection (400 larvae)		***Trichinella spiralis* reinfection (2 x 400 larvae)**	
Day after infection	**Number of larvae**	**Day after reinfection**	**Number of larvae**
20	15908.3 ± 242.,3		
30	114520.0 ± 15419.9		
45	125700.0 ± 5565.1		
60	130266.7 ± 2419.4		reinfection
		5	85420.0 ± 821.5
		10	14900.0 ± 3915.4
75	117900.0 ± 17936.8	**15**	**9191.7 ± 6998.0
		20	12083.3 ± 2156.2
90	104733.3 ± 2793.4	**30**	**25517.7 ± 17692.3
120	127808.3 ± 11542.9	**60**	**22853.3 ± 9373.9
150	111303.7 ± 10127.2	**90**	**34130.0 ± 24079.0
180	100139.3 ± 4026.6	**120**	*57666.7 ± 15273.2

*P<0.05; **P<0.01 statistically significant from mice with a single infection

Results

Parasite burden – numbers of adults (Tab. 1)
The adults of *T. spiralis* were isolated from the small intestine of reinfected mice only till day 15 post reinfection and in reduced numbers in comparison with the single infection, where the adults were present even on day 30 p.i. The percentage of worm reduction after reinfection was 69.2 % (in range from 53.2 to 100).

Parasite burden – numbers of muscle larvae (Tab. 2)
Similarly, the strong reduction (72.3 %) of muscle larvae was recorded after reinfection. The smaller numbers of isolated larvae were found in reinfected mice in comparison to the single infection on days 15 post reinfection (reduction 92.2 %), 30 post reinfection (reduction 75.6 %), 60 post reinfection (reduction 82.1 %), 90 post reinfection (reduction 69.3 %) and 120 post reinfection (reduction 42.4 %).

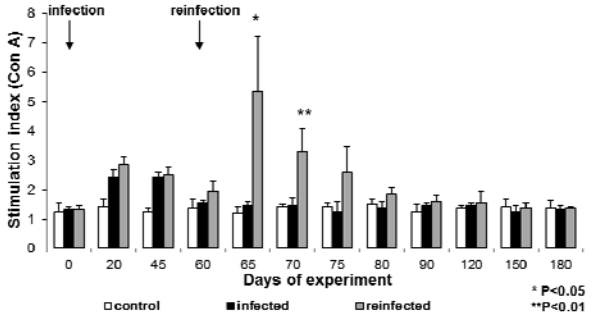

Fig. 1. Proliferative response of T lymphocytes to concanavalin A (Con A) in *T. spiralis* infected/reinfected mice
*P < 0.05; **P < 0.01 statistically significant from mice with a single infection

Proliferative response of T lymphocytes to Con A (Fig. 1)
The proliferative activity of T lymphocytes was significantly (P < 0.05; P < 0.01) stimulated during two weeks after the reinfection in comparison to the single infection. The maximum values of the stimulative indices in reinfected mice were found on day 65, i.e. 5th day after reinfection. In next days the proliferative activity of T cells in reinfected mice decreased to the level of other experimental groups.

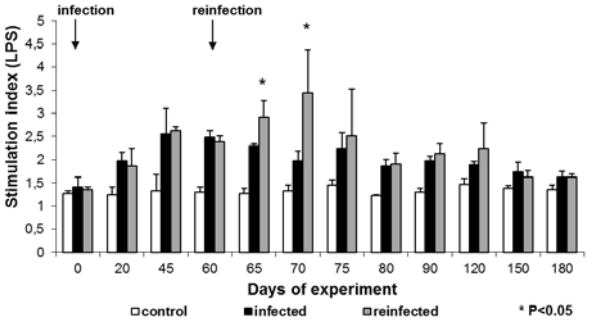

Fig. 2. Proliferative response of B lymphocytes to lipopolysaccharide (LPS) in *T. spiralis* infected/reinfected mice
*P < 0.05 statistically significant from mice with a single infection

Proliferative response of B lymphocytes to LPS (Fig. 2)
The B-cell proliferative activity induced by nonspecific mitogen lipopolysaccharide in reinfected mice showed a similar course, but contrary to the T cells, it peaked on day 70, i.e. on 10th day after reinfection (P < 0.05). Higher values of stimulative indices were recorded till day 120 of the experiment (4 or 3months after infection or reinfection) in both infected and reinfected group of mice.

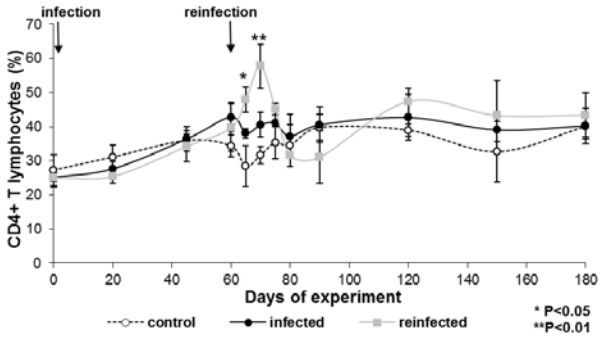

Fig. 3. Relative percentage of splenic CD4+ T cells in *T. spiralis* infected/reinfected mice
*P < 0.05; **P < 0.01 statistically significant from mice with a single infection

Percentage of splenic CD4+ T lymphocytes (Fig. 3)
The percentage proportion of helper CD4+ T cells in the spleen of reinfected mice significantly (P < 0.05; P < 0.01) increased after the repeated infection, with the maximum on day 70, i.e. on 10th day after reinfection. In the following days a continual reduction in this cellular subpopulation was observed till day 80.

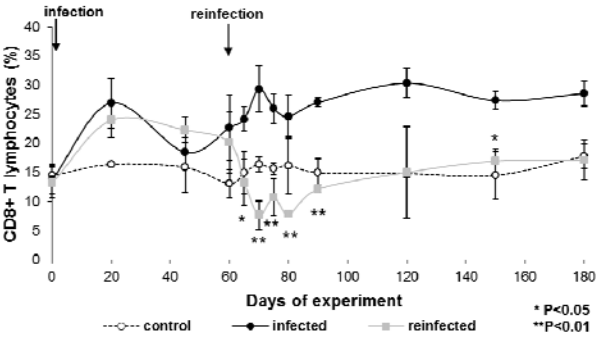

Fig. 4. Relative percentage of splenic CD8+ T cells in *T. spiralis* infected/reinfected mice
*P < 0.05; **P < 0.01 statistically significant from mice with a single infection

Percentage of splenic CD8+ T lymphocytes (Fig. 4)
In contrast to CD4+ T lymphocytes, the presence of CD8+ T cells in the spleen of reinfected mice was decreased (P < 0.05; P < 0.01) under the values found in mice with single infection and the lowest values were recorded on day 80 of the experiment (i.e.10th day after reinfection). The proportion of CD8+ T subpopulation achieved again the control values from day 90, i.e. 30 days after reinfection.

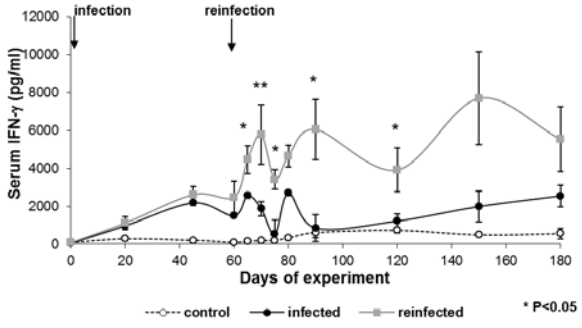

Fig. 5. Concentration of serum IFN-γ in *T. spiralis* infected/reinfected mice
*P < 0.05; **P < 0.01 statistically significant from mice with a single infection

Serum cytokine IFN-γ (Fig. 5)
In comparison to single infection changes in cytokine production were recorded. The level of serum IFN-γ rose (P < 0.05; P < 0.01) immediately after reinfection and the curve showed 3 peaks of its high concentration, on days 70, 90, and 150 of the experiment.

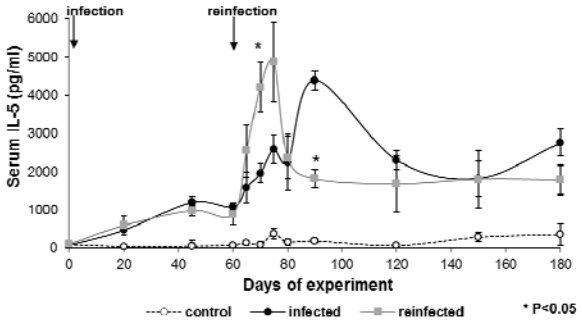

Fig. 6. Concentration of serum IL-5 in *T. spiralis* infected/reinfected mice
*P < 0.05 statistically significant from mice with a single infection

Serum cytokine IL-5 (Fig. 6)
The IL-5 concentration rose (P < 0.05) immediately after reinfection for a relatively short time, till day 75 (i.e. 15 days after reinfection). On the contrary, maximal values of IL-5 after the single infection were reached on day 90 p.i.

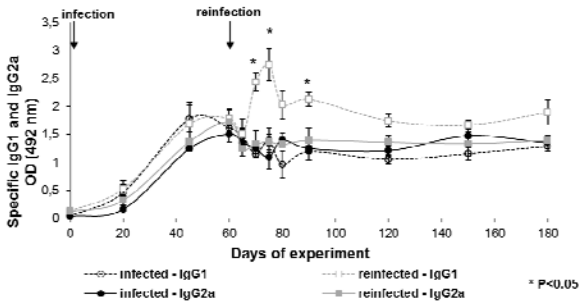

Fig. 7. Specific anti-*T. spiralis* immunoglobulines IgG_1 and IgG_{2a} in serum of *T. spiralis* infected/reinfected mice
*P < 0.05 statistically significant from mice with a single infection

Specific immunoglobulines IgG_1 and IgG_{2a} (Fig. 7)
The production of IgG_1 and IgG_{2a} was continually increased after the single infection until day 60 p.i. and then it was remained at higher values until the end of the experiment. The similar course of IgG_{2a} was recorded after reinfection, but IgG_1 significantly (P < 0.05) increased from days 70 to 90, i.e. 1 month post reinfection.

Discussion

Trichinella spp., the causative agent of trichinellosis, occupies two distinct intracellular niches within its host, the intestinal epithelium and the skeletal muscle (Despommier, 1998), where the interaction between the parasite and the host muscle is unusual for the biology of nematodes. Protective immunity against enteric helminths is often dependent on Th2 responses (Finkelman *et al*., 1997) and associated with high levels of serum IgE and IgG_1 together with an influx of eosinophils, basophils and mast cells to the infected site. The muscle infection with *T.spiralis* elicites 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). Antibody-dependent cell mediated cytotoxicity is directed against *T. spiralis* newborn larvae (Gurish *et al*., 2004). Protective immunity following secondary infection is directed against the larvae stages of the parasite and is dependent on CD4+ T cells and IL-4 production (Anthony *et al*., 2006), but does not require eosinophils (Urban *et al*., 2000).
It is known that immunity limits the duration of the intestinal phase of primary infection and drastically curtails the development of subsequent infections (Grove, 1977; Wakelin, 1993; Soule *et al*., 1993). Such protective responses can be initiated by very small infections (less than 10 worms) and act effectively against very large challenges (more than 600 larvae) (Wakelin, 1993). Our results confirmed rapid expulsion of adult worms after reinfection. After the single infection of mice with 400 larvae of *T. spiralis*, adult parasites were present until day 30 p.i. with decrease of the number from the beginning of the experiment. In comparison to a single infection, the worms after reinfection (2 x 400 larvae) were expelled out in a half shorter time (within 2 weeks) and their numbers were strongly reduced (almost 70 %). Similar results were recorded by Kołodziej-Sobocińska *et al*. (2007). Muscle larvae number after reinfection was not increased in comparison to single infection, on the contrary, a significant reduction (average 72.3 %) in their numbers was recorded from day 15 after reinfection to the end of the experiment. Kołodziej-Sobocińska *et al*. (2007) observed decreased number of muscle larvae after reinfection one month later, i.e. from day 60 after reinfection. Some authors also found muscle larvae reduction after primary infection in rats and mice (Machnicka & Dziemian 2001; Kołodziej-Sobocińska *et al*., 2006) and explained it by the destruction of larvae by inflammatory and immune mechanisms and calcification of larvae.

Our previous results (Dvorožňáková *et al*., 2005) showed that *T. spiralis* infection caused increased proliferation activity of spleen T lymphocytes as early as the first week p.i. and B lymphocytes were activated from the second week p.i. Results from this study showed strong stimulation of T- and B-cell proliferation after the reinfection during two weeks, in parallel with intestinal phase of the reinfection and migration of newborn larvae. Polyclonal lymphocyte activation of T-cells, but particularly B-cells, is responsible for the high levels of immunoglobulines IgG, IgM, and IgE (Murrel & Bruschi, 1994). In our study, the decrease of stimulatory indices of T lymphocyte proliferative activity was recorded after one month post reinfection, when the majority of migratory larvae have been settled in muscle cells and have changed the host cell into nurse cell providing a suitable habitat for the larva (Despommier, 1993), but B-cell proliferative activity remained increased until 2 months after reinfection, at the developed muscle phase.

T-cells play a central role in immune and inflammatory responses against *Trichinella* infection. During the host immune intestinal response, CD4 T cells play a key role in trapping and removal of intestinal worms from the gut, they mediate mucosal changes including intestinal goblet cell hyperplasia with high mucin secretion (Ishikawa *et al*., 1997; Urban *et al*., 2000). CD4 T cells are also related to a reduction of naive cells and an increased generation of memory cells (Morales *et al*., 2002). The memory CD4+ T cells are a heterogeneous population, consisting of both $CD62L^{high}$ central memory T cells (T_{CM}) and $CD62L^{low}$ effector memory T cells (T_{EM}) that are competent to produce the Th2 effector cytokine IL-4. Zaph *et al*. (2006) found that both subsets of memory CD4+ T cells develop after *Trichuris* infection (an intestinal helminth), persist in GALT, and mediate protective immunity to secondary infection. In our study expression of surface CD4+ and CD8+ antigens on T cells was monitored in order to examine impact of the reinfection on the balance between Th1 and Th2 – mediated cell immunity. Splenic CD4+ T subpopulation was significantly increased during 10 days after reinfection and on the contrary, CD8+ cell were decimated at this time. Such a huge presence of CD4+ T cells immediately after reinfection might be a result of memory immunity. Morales *et al*. (2002) analyzed the phenotype of the T cell subpopulations in human patients and 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. The immune response involving CD4+ T cells after reinfection in our work considerably accelerated worm loss, particularly between days 5 and 15 after reinfection.

Cytokines, elaborated primarily by T helper cells, play a dominant role in orchestrating both anti-parasite responses and pathology in *T. spiralis* infection. 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 mice with a single infection was continually elevated till day 60 p.i. and then markedly was increased until day 90 p.i. Similarly Morales *et al*. (2002) found in a human study a maximal IL-5 production by peripheral blood mononuclear cells 2 months p.i. *T. spiralis* reinfection in our study induced an immediate and sharp rise in IL-5 concentration during 20 days after reinfection, what correlated with CD4+ T subset stimulation. IL-5 is a glycoprotein which is secreted mainly from T helper cells following activation with antigen or mitogen. This cytokine IL-5 is an important factor for a B cell growth (Kinashi *et al*., 1986). An increased level of IL-5 after reinfection correlated with an increased B cell proliferation in our study. The levels of IL-5 increased continuously after the nurse cells formed. IL-5 induces eosinophil maturation and emigration from bone marrow and cytoprotection of the cells in peripheral tissues (Gurish *et al*., 2004). Kang *et al*. (2012) observed eosinophils around the nurse cells 4 weeks p.i. Eosinophils are not necessary for killing larvae and a recent study suggested that eosinophils may affect the immune response in a manner that would sustain chronic infection and ensure worm survival within the host (Fabre *et al*., 2009). *T. spiralis* muscle larvae death was correlated with enhanced IFN-γ and reduced IL-4 production (Kang *et al*., 2012). Vallance *et al*. (2000) and Doligalska (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, what correlated with our results during the intestinal phase after reinfection. IL-5 expression is only minimally protective during a primary *T. spiralis* infection but may be to protect against repeated exposure to gastrointestinal parasite. Developing protection against reinfection is important in determining the survival and viability of the host.

Despite the predominant role of Th2 response in helminthoses, the representative cytokine of Th1 type - IFN-γ showed a high concentration in serum of reinfected mice. Migration of *T. spiralis* newborn larvae after a single infection also increased IFN-γ production, confirming its participation in immune response at the muscle phase. 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, a sharp increase in IFN-γ level was recorded immediately after reinfection and high concentration of IFN-γ in serum persisted until the end of the experiment. Numbers of muscle larvae did not increase after reinfection but they started to significantly reduce from the second week after reinfection. The mechanism of IFN-γ mediated immunity to newborn larvae may include enhanced cytotoxic killing by eosinophils, granulocytes and activated macrophages (Venturiello *et al*., 1995). The muscular host immune response to *Trichinella* is partially regulated by the intestinal phase of the parasite which emphasizes the intensity of the following muscle inflammation compared with animals

infected by synchronized injections of newborn larvae. In eosinophil-ablated mice such as PHIL and GATA – animals it was observed that there was an increased NOS2 expression in macrophages, driven by higher IFN-γ release, thus responsible for muscle larva damage (Bruschi & Chiumiento, 2011). Kołodziej-Sobocińska *et al*. (2007) found an increased metabolic activity of peritoneal macrophages during the first months after reinfection. 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) showed that reduced muscle burdens result from IFN-γ-dependent immune response directed against newborn larvae. Beiting *et al*. (2004) concluded that IL-10 limits local and regional inflammation during the early stages of muscle infection but that chronic inflammation is controlled by an IL-10-independent mechanism that is coincident with Th2 response. The shift from IL-10-dependent to IL-10-independent control of inflammation was coincident with completion of parasite development in the nurse cell, together with the induction of a strong IgG_1 response to tyvelose-bearing glycoproteins that are synthesized only by mature first-stage larvae. The induction of an IgG_1 response suggests a role for Th2 cytokines in controlling inflammation during chronic infection. Beiting *et al*. (2004) results suggest that IL-10 exerts its anti-inflammatory effect in mice infected with *T. spiralis* by influencing the activities of large numbers of macrophages that surround infected muscle cells. *T. spiralis* promotes alternative activation of macrophages. Muscle-stage infection with *T. spiralis* may polarize infiltrating macrophages toward an alternative phenotype, suppressing their destructive properties.

A protective role for antibodies during helminth infection with positive correlation has being noted between antibodies specific for antigens from *T. spiralis* and reduced worm burdens (Gurish *et al*., 2004; Gu *et al*., 2008). Gurish *et al*. (2004) concluded that IgE promotes parasite expulsion from the gut following *T. spiralis* infection and participates in the response to larval stages of the parasite. Binding of IgE to the muscle larvae and evidence of increased cyst necrosis supported a role for IgE in immunity to primary infection with *T. spiralis*. Studies in rats (Gurish *et al*., 2004) suggested that IgE antibodies are important in the early response to cysts, mediating both the recruitment of eosinophils to encysted larvae in muscle and reducing the numbers of muscle cysts. In mice infected with *T. spiralis*, IgG_1 hypergammaglobulinemia was associated with high IgE levels (Bell, 1998). Polarization of the systemic response toward type 1 or type 2 profiles was not clear-cut. Moroever, at day 20 p.i., IgG_1 levels were higher than those of IgG_2, but were similar at day 60p.i. Our results showed a large elevation of specific IgG_1 antibodies from day 10 to 30 post reinfection and then IgG_1 antibodies remained at high levels. We have simultaneously observed high IgG_1 and IL-5 levels in serum of *T. spiralis* reinfected mice. At the same time a significant reduction in muscle larvae was found. These results emphasize the occurrence of an initial humoral orientated response, which then developed toward coexistent humoral and cellular immune responses (Picherot *et al*., 2007).

Results of Venturiello *et al*. (1996) suggested that during a chronic infection, resistance to reinfection may be modified. Early antibodies developing shortly after infection are cytotoxic, whereas blocking antibodies (IgG subclasses) predominated in the late population and were specific for newborn lavae and could not be adsorbed with muscle larvae. Muscle infection also elicited an antibody response, characterized initially by mixed isotypes directed at somatic larval antigens and changing to an IgG_1-dominated response directed at tyvelose-bearing excreted or secreted antigens. Immunoglobulines IgG_1 represent Th2-cell activation and IgG_2 antibodies reflect Th1 response (Else & Finkelman, 1998). A significant elevation of IgG_1 is often observed in trichinellosis (Li & Ko, 2001; Kołodziej-Sobocińska *et al*., 2006). The protective isotypes IgG_1 and IgG_2 are involved in the inflammatory response. An elevation of IgG_1 accompanies the muscle phase of infection (Doligalska, 2000) and newborn larvae are more sensitive *in vitro* to IgG_1 in antibody-dependent cellular cytotoxicity (Moskwa, 1999). *T. spiralis* specific serum IgG1 and IgG_{2a} levels in our study significantly increased after one month p.i. and followed *T. spiralis* reinfection increased significantly IgG_1, but the level IgG_{2a} did not change. These results correlate with study of Kang *et al*. (2012) those associated Th1 and Th2 cytokine production in the spleen with antibody production and concluded that Ig class switching depends on T-cell and their cytokines: IL-4 and IL-13 preferentially switch activated B-cells to the IgG_1 isotype (Th2 type). Conversely, IFN-γ and IL-12 enhance IgG_{2a} (Th1 type) (Rengarajan *et al*., 2000). Even though serum level of IFN-γ was increased after reinfection in our study, it was not sufficient to convert the immunity into predominant Th1 type. Therefore, Th2 cells activated by *T. spiralis* infections might generate IL-4 and IL-13 cytokines which enhance the switching of B cells to the IgG_1 type.

Our results suggest that observed components of cellular and humoral immunity contributed to increased protection of the host to repeated infection with *T. spiralis* and limited a parasite's invasion of the host. The increased functional activity of T and B lymphocytes after the reinfection supports a growth, maturation and differentiation of these cells. The rise of helper CD4+ T cells with the maximal numbers during newborn larval migration could activate B lymphocytes to secrete high level of IgG_1 and consequently induced antibody dependent cell cytotoxicity, which is directed against newborn larvae. The reinfection induced the changes in Th1 and Th2 polarization of immune response. The increased IFN-γ production could advance inflammatory response and help to rapid expulsion of worms from the intestine, it protected the host against a migration of newborn larvae and destroyed muscle larvae. We suppose that the amplification of the immune response after the reinfection was result of the memory immunity and it participated in reduction of parasite exemplars in comparison to the single infection.

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