Chapter 9

Immunotherapy Can Enhance Anthelmintic Efficacy in Alveolar Echinococcosis

Emília Dvorožňáková

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/59732

Abstract

The immune response of the intermediate host with alveolar echinococcosis was investigated on mice intraperitoneally infected with *Echinococcus multilocularis* protoscoleces. The study was focused on cell-mediated immune response (dependent on interactions of T lymphocytes and macrophages), which is considered protective in alveolar echinococcosis. The immune response to *E. multilocularis* is regulated by Th1/Th2 cytokines produced by the CD4+ T lymphocyte subpopulation. Metacestode has been known for its ability to modify immune functions and suppress effective specific cell response to ensure its survival in host organism. The influence of immunomodulatory substances – muramyltripeptide (L-MTP-PE), glucan (GI), glucan with zinc (GIZn), and transfer factor (TF) – applied alone or combined with anthelmintic albendazole (ABZ) on regulative and effector components of immunity were tested and at the same time, antiparasitic efficacy of immunomodulators was evaluated.

Keywords: *Echinococcus multilocularis*, therapy, muramyltripeptide, glucan, transfer factor

1. Introduction

The larval stage of *Echinococcus multilocularis* causes alveolar echinococcosis, the serious helminthozoonosis with a high mortality in patients with late treatment [1]. The disease is characterized by an infiltrative, tumor-like growth of the *E. multilocularis* larval cysts, affecting

the liver of intermediate hosts such small mammals or man [2]. The therapy of this disease represents an important problem. The treatment with available anthelmintics (benzimidazoles, praziquantel a.o.) is long term and relatively little effective. A surgical extirpation of cysts is not always successful owing to their ability to form metastatic foci in secondarily infected organs [3, 4]. The available treatment has only parasitostatic effect and is therefore performed over many years, often through the patient's life [5]. This brings the risk of adverse reactions of drugs, among which gastrointestinal disturbances, reversible alopecia, hepatitis, proteinuria, neurological symptoms are the most frequent [5, 6], apart from a strong teratogenic effect [7]. The disadvantage of benzimidazoles consists also in irresponsiveness to treatment in some patients and recurrence of the disease after intermission of the therapy [8]. There is still no consensus regarding the effective dosage and duration of treatment [9, 10]. Moreover, the immunological status of the host, host susceptibility, and actual stage of infection influence the results of treatment [11]. The generalized immunosuppression induced by *E. multilocularis* may complicate therapy efficacy in alveolar echinococcosis. *E. multilocularis* evades the host immune response by mechanisms that protect the parasite or modify the host immunity to ensure its long-lasting survival in the host organism [12, 13]. Application of immunomodulatory substances could improve host immune status during *E. multilocularis* infection and limit the growth of the parasite. Results of alternative therapeutic strategies with use of immunomodulatory substances seem to be promissing and might contribute to higher efficacy of the anti-*Echinococcus* treatment.

E. multilocularis induces parasite-specific cellular and humoral immune response in intermediate host [14]. The infection induces strong cellular immune response and production of all antibody isotypes, which are not effective in killing and elimination of parasites. Cellular immune response, leading to granulomatous infiltration of peri-parasite tissue, plays the dominant role in the fight with echinococcus [15]. Cell-mediated immune response depending on interaction of macrophages and T lymphocytes is regarded as protective against *E. multilocularis* infection [16]. On the contrary, *E. multilocularis* is able to evade host immune response or modify it to ensure its long-lasting survival in the organism of the intermediate host [12, 13]. Different activation of T cells subsets (CD4+, CD8+) and the combined Th1 and Th2 cytokine profile appear crucial for prolonged metacestode growth and survival. Regressive, as well as progressive course of the disease correlates with stadium-specific granuloma cell composition and antigen-specific T cell response [17]. An activation of Th1 CD4 T lymphocytes is connected with the control of the infection [18] and a reversion to Th2 response may contribute to long-lasting manifestation of *E. multilocularis* infection in humans [12, 19]. The *E. multilocularis* metacestode can specifically manipulate the balance between Th1 and Th2 response leading to lowered effectiveness of immune response [20]. Macrophages on the periphery of the periparasitic granuloma can produce proinflammatory cytokines continually serving as mediators of acute phase of protein secretion and fibrogenesis [14]. Granuloma generation and fibrosis can restrict larval growth, but also reduces anthelmintic drug transport to the lesion and thus may be the reason for partial or total ineffectiveness of treatment [20]. Application of immunomodulatory substances could improve the host immune status during *E. multilocularis* infection and limit the growth of the parasite. New therapeutic approaches to alveolar echinococcosis use immunomodulators to overcome patient's immunosuppression (caused by *E. multilocularis* metacestode) that complicates benzimidazole therapy. The application of cytokine IFN-γ or IL-12 together with benzimidazoles stopped the progression

http://dx.doi.org/10.5772/59732

of disease or limited the metacestode growth [17, 21], suggesting its usefulness in therapy. However, the use of recombinant cytokines in therapy is technically and financially demanding. Therefore, new ways to support a secretion of endogenous cytokines in patients are sought.

In our study, we focused on the activity of three immunomodulators (liposomized muramyltripeptide, glucan with zinc, and transfer factor) in enhancing of the host antiparasite defence and the efficacy of anthelmintic albendazole treatment in alveolar echinococcosis.

2. Materials and methods

Experiments were performed on pathogen-free BALB/c mice, males, weighing 20–25 g. Mice were kept under a 12-h light/dark regime at room temperature (21±3°C) and 50–60% relative humidity on a commercial diet and water. The experimental protocols complied with the current Slovak ethics law.

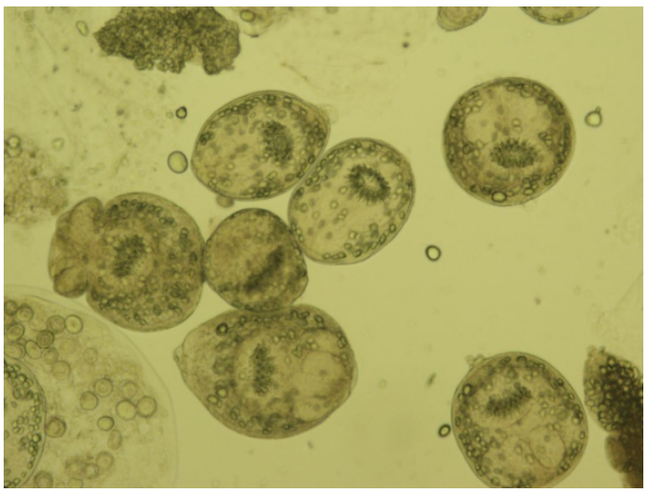

Figure 1. Protoscoleces of *Echinococcus multilocularis*.

2.1. Infection

E. multilocularis metacestode (strain provided by Department of Medical Parasitology, Clinical Institute of Hygiene and Medical Microbiology, Medical University of Vienna, Austria) was passaged in our laboratory by intraperitoneal injection of Mongolian jirds *Meriones unguiculatus*.

Parasite cysts were isolated 4 months post infection (p.i.) and cut into pieces in sterile RPMI 1640 medium (Sigma-Aldrich, Germany) supplemented with antibiotics, 100 U/ml penicillin and 100 μg/ml streptomycin (Sigma-Aldrich, Germany), and passed through a Cell Dissociation Sieve Tissue Grinder Kit using apertures ranging from 380 to 45,7 μm (Sigma-Aldrich, Germany).

Protoscoleces obtained after the last filtration were maintained in RPMI and counted for an infective dose.

2.2. Efficacy of treatment

The antiparasitic efficacy of immunotherapy was evaluated by the cyst development in infected mice. *E. multilocularis* cysts were isolated from sacrificed mice and parasite cysts were weighed and subsequently mean value ± S.D. was determined (n=3 or n=4).

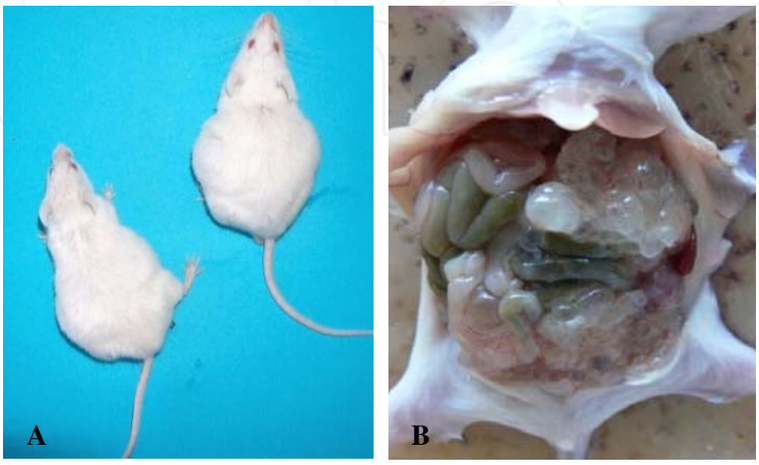

Figure 2. A – Mice infected with *E. multilocularis*. B – Cysts of *E. multilocularis* in the peritoneal cavity of an infected mouse (4th month after the infection).

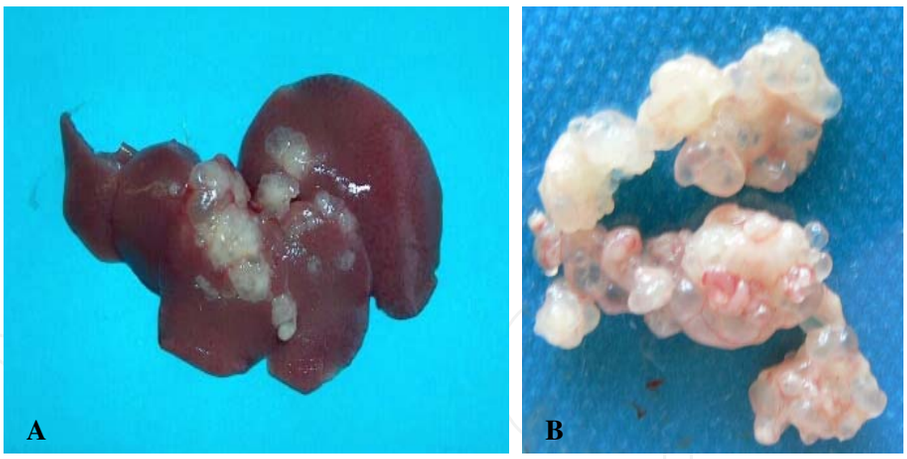

Figure 3. A – The affected liver with *E. multilocularis* cysts. B – Cysts of *E. multilocularis* isolated from the peritoneal cavity of an infected mouse (4th month after the infection).

2.3. T and B lymphocyte proliferation assay

The spleen was aseptically homogenized in phosphate-buffered saline (PBS) (pH 7.2) to obtain cells. Cell suspension was washed twice with PBS and finally with RPMI 1640 medium (Sigma-Aldrich, Germany). Erythrocytes were removed by lysis in hypotonic solution (0.85 % NH_4Cl) and lymphocytes were resuspended to a final concentration of 5 x 10^6 cells /ml in RPMI 1640

medium. The assay was performed in 96 wells plates (Nunc, Denmark) and cells were incubated in RPMI 1640 medium (100 μl) containing 10 % bovine fetal serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. Mitogens Concanavalin A (Con A) (T cells) and lipopolysaccharide (LPS) (B cells) (Sigma-Aldrich, Germany) were added in a dose 100 μl (concentration 10 μg/ml) to the cell suspensions and incubated at 37 °C in 5 % CO_2 and 85 % humidity for 72 h. Then 20 μl of 3,4-dimethylthiazolyl 2,5-diphenyltetrazolium bromide (Sigma-Aldrich, Germany) (0.1 % solution) was added to the cell suspensions and incubated at 37 °C and 5 % CO_2 for 4 h followed by centrifugation at 800 x *g* for 5 min. Reaction was terminated with dimethylsulfoxide (Sigma-Aldrich, Germany) (100 μl/cell sample) and read on ELISA reader (Multiskan Plus, Labsystem, Finland) at 540 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)

Proliferative responses were measured separately for lymphocytes isolated from each mouse per group.

2.4. Number 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. Monoclonal antibodies rat anti-mouse CD4 fluorescein isothiocyanate-conjugated and rat anti-mouse CD8 phycoerythrin-conjugated monoclonal antibodies (BD Biosciences PharMingen, Belgium) were used at the concentration of 0.4 μg/10^6 cells at 4°C for 30 min. After washing in PBS three times, cells were analyzed by the FACScan flow cytometer (Becton Dickinson Biosciences, Germany) and CellQuest software. Cells from each mouse per group were analyzed individually. The final numbers of both cell populations were calculated as proportion from the total isolated lymphocytes per spleen/mouse.

2.5. Concentration of IFN-γ and IL-5 in serum

The capture ELISA was employed to determine the concentration of cytokines IFN-γ and IL-5 in serum according to the method [22]. IFN-γ and IL-5 were used as marker cytokines for the Th1 and Th2 responses, respectively. Cytokine-specific monoclonal antibodies were used, for IFN-γ detection: pure anti-mouse IFN-γ (R4-6A2) and biotin anti-mouse IFN-γ (XMG1.2); for IL-5 detection: pure anti-mouse IL-5 (TRFK5) and biotin anti-mouse IL-5 (TRF4) (all BD Biosciences PharMingen, Belgium). Results were expressed at 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.

2.6. Superoxide anion assay

Production of superoxide anion (O_2^-) by peritoneal macrophages was detected as superoxide dismutase (SOD) – reduction of ferricytochrome C with and without stimulation with phorbol myristate acetate (PMA) [23]. Cells were obtained by peritoneal lavage and after washing in PBS were diluted at concentration of 1 x 10^6 cells/ml in RPMI 1640 (Sigma-Aldrich, Germany).

Cell suspension (1 ml/well) was added to 24-well plate (Falcon, France) and incubated at 37 °C in 5 % CO_2 and 85 % humidity for 2 h. Nonadherent cells were removed by washing with ice-cold Earls Balanced Salt Solution (EBSS) (pH 7.2). The reaction was carried out in 0.3 ml/well of 160 μM ferricytochrome C (Sigma-Aldrich, Germany) in EBSS. In control, the reaction was immediately blocked by 300 μg SOD/10 μl in EBSS. The stimulation of cells was induced by 10 μl of PMA in ethanol. Cells were incubated at 37 °C in 5 % CO_2 and 85 % humidity for 2 h. Supernatant from wells was centrifuged at 170 x *g* for 3 min at 4 °C. The optical density (OD) of supernatant was measured at 550 nm in a 96-well plate reader (Multiscan Plus, Labsystems, Finland). Bradford protein microassay was used to determine cell-protein concentration in each well with standard reagent and bovine serum albumin as the protein standard (BioRad, UK). The OD was read at 595 nm. The resulting value was used to calculate nmol of O_2^- produced according to the formula: nmol O_2^- = ($OD_{blocked\ by\ SOD}$ - $OD_{without\ SOD}$ /6.3) x 100 and determinated for 1 mg of cell proteins.

2.7. Statistical evaluation

Statistical differences were assessed using Kruskal-Wallis ANOVA and post hoc Tukey's HSD test (a value of $p<0.05$ was considered significant) in the program Statistica 6.0 (Stat Soft, Tulsa, USA) statistical package.

3. Muramyltripeptide

Muramylpeptides – components of bacterial cellular wall are classified as biological immunomodulators. Muramylpeptides primarily activate macrophages to a high production of oxygen radicals and a secretion of inflammatory cytokines, which activate neutrophils, T and B lymphocytes [24]. Muramyldipeptide (MDP) is the smallest bacterial structure with immunopotent activity. The positive effect of MDP on the host immune response was also observed in several parasitic infections [25–27]. Muramyltripeptide phosphatidylethanolamine (MTP-PE) is a lipofilic derivate of MDP. Liposome-encapsulated MTP-PE has an enhanced effect on macrophages to secrete pro-inflammatory cytokines, which results in increasing of cytotoxicity of these cells [28–30].

Affinity of liposomized forms of MTP-PE to the reticuloendotelial system, in particular to macrophages located in the liver and the spleen [30, 31], could be useful in therapy of alveolar echinococcosis, by which the parasite cysts primarily develop in the liver. The biological effect of MTP-PE takes place in Kuppfer cells in the liver (macrophage's equivalent) [32]. Macrophages activated by liposomized MTP-PE stimulate Th1 subpopulation of lymphocytes and proinflammatory mediators via cytokine secretion [28, 29, 33, 34]. T lymphocytes therefore constitute an active component of immune reactions after immunomodulation with muramylpeptides.

The effect of muramyltripeptide phosphatidylethanolamine incorporated into multilamellar liposomes (L-MTP-PE) on immune response of intermediate host infected with *E. multilocularis* was examined.

http://dx.doi.org/10.5772/59732

3.1. Experimental design

Experiments were carried out on male BALB/c mice (n=150) weighing 20–25 g. Mice were randomly divided into five groups as follows:

Group 1 – uninfected and untreated (control)

Group 2 – infected intraperitoneally with 5000 *E. multilocularis* protoscoleces/mouse on Day 0 and no treatment

Group 3 – *E. multilocularis* infected (as Group 2) and treated with liposomized muramyltripeptide phosphatidylethanolamine (L-MTP-PE) (Ciba-Geigy, Switzerland) intravenously (i.v.) twice a week at the dose of 1 mg/kg of body weight (b.w.) starting at weeks 5 and 6 post infection (p.i.)

Group 4 – *E. multilocularis* infected (as Group 2) and treated with albendazole (ABZ) (Sigma, Germany) per os (p.o.) twice a week at the dose of 10 mg/kg of b.w. starting at week 5 up to week 10 p.i.

Group 5 – *E. multilocularis* infected (as Group 2) and treated with combination of L-MTP-PE and ABZ as above.

Samples of blood, spleen, and peritoneal macrophages were obtained at the following weeks: 0 (prior infection), 2, 4, 8, 10, 12, 14, 18, 22, and 26 p.i. from all groups (3 mice per experimental day).

4. Results and discussion

In our experiment, the *E. multilocularis* infection induced an inhibition of the proliferative activity of T and B lymphocytes almost in the course of the whole experiment (Figures 4, 5).

However, the application of L-MTP-PE to infected mice had a positive stimulatory effect on T and B lymphocytes, especially in combination of L-MTP-PE+ABZ, where proliferative activity of lymphocytes was increased for a long time, from week 8 p.i. (2 weeks after the end of the therapy) till week 14 p.i. (lasted almost 2 months). Muramylpeptides belongs to the polyclonal activators of B lymphocytes, inducing a high proliferation and production of polyclonal antibodies. Muramyldipeptide activates B cells particularly during a late phase of their differentiation because the specific receptors for MDP are expressed on B cell surface at a definitive stage of their maturation [35].

T lymphocytes play a major role in the control of immune response in intermediate host organism infected with *E. multilocularis* [36]. In alveolar echinococcosis CD4+ T cells represent the main T subpopulation, being abundant in the first phase of the periparasitic granuloma formation and later these cells are replaced by CD8+ T cells [17]. A long-term stimulation of CD4+ T cell subpopulation after the immunotherapy could participate in antiparasite defence (to form the periparasitic granuloma) and increase its efficacy. Both L-MTP-PE alone and

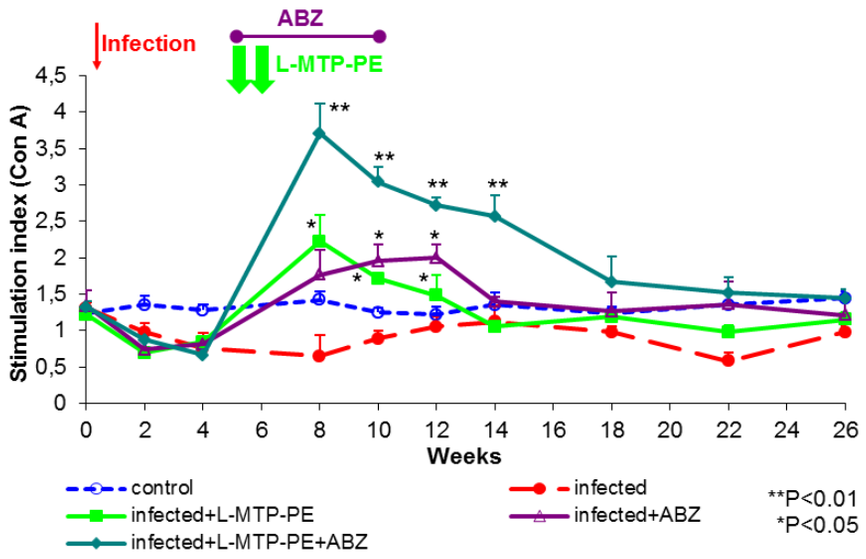

Figure 4. Proliferative activity of T lymphocytes in *E. multilocularis* infected mice after therapy L-MTP-PE+ABZ. *(p<0.05); **(p<0.01) statistically significant from infected mice.

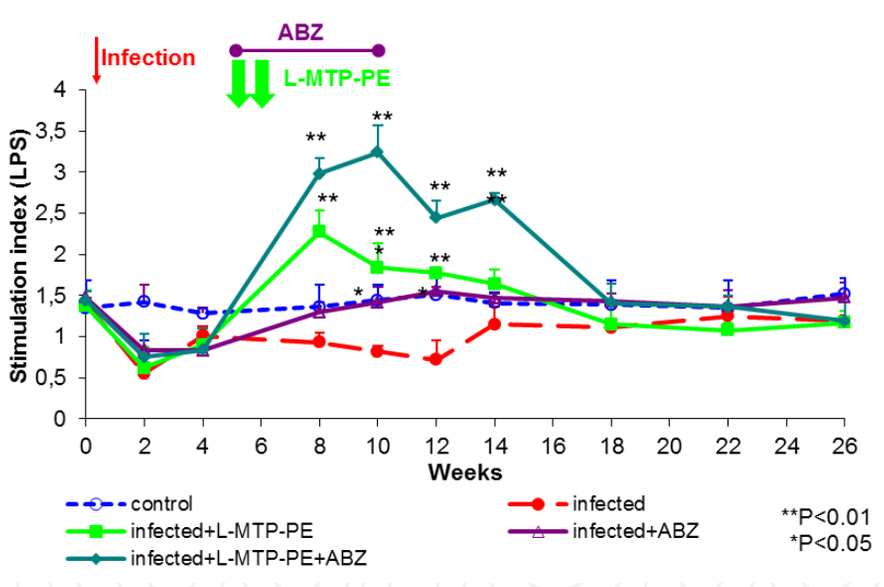

Figure 5. Proliferative activity of B lymphocytes in *E. multilocularis* infected mice after therapy L-MTP-PE+ABZ. *(p<0.05); **(p<0.01) statistically significant from infected mice.

combination of L-MTP-PE+ABZ increased the presence of CD4+ T cells in the spleen of infected mice within 6 weeks after the immunotherapy (Figure 6).

Previous experimental studies have manifested that *E. multilocularis* infection can lead to an expansion of CD8+ T cell clones [18, 37, 38]. In our study (Figure 7), the CD8+ T cells occurrence was higher for a short time in mice after therapy with L-MTP-PE than in infected and non-treated mice. Combined therapy L-MTP-PE+ABZ induced high values of CD8+ T cells, which remained increased till the end of the experiment. Splenic suppressive CD8+ T cells in alveolar

http://dx.doi.org/10.5772/59732

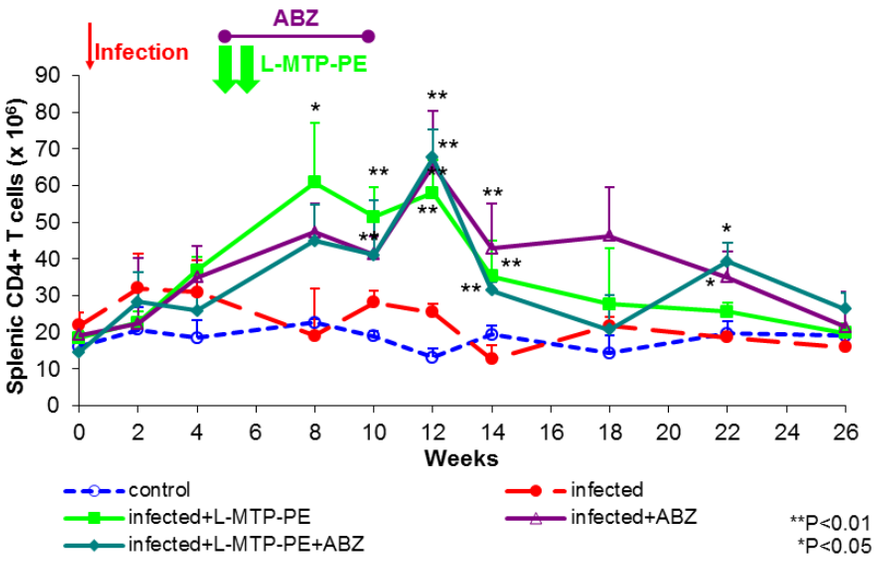

Figure 6. Number of splenic CD4 T cells in *E. multilocularis* infected mice after therapy L-MTP-PE+ABZ. *(p<0.05); **(p<0.01) statistically significant from infected mice.

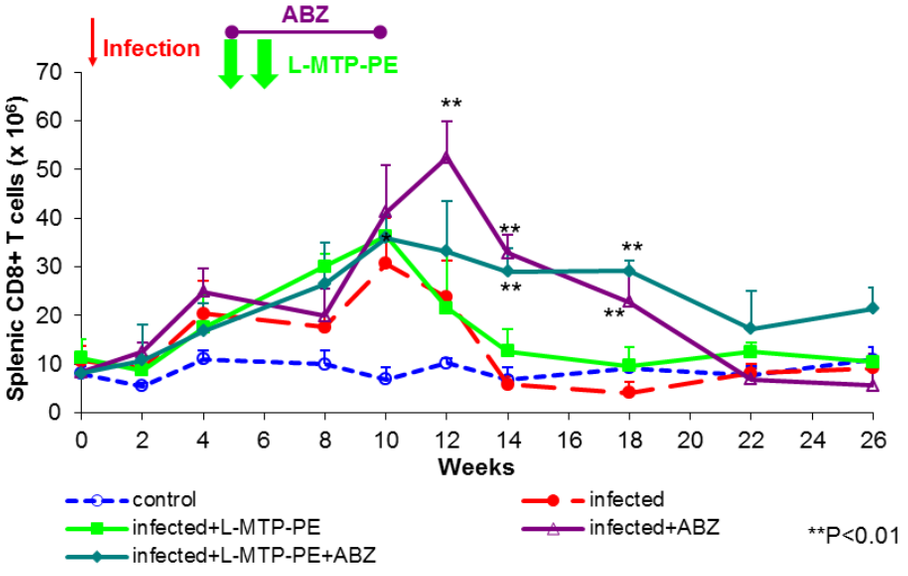

Figure 7. Number of splenic CD8 T cells in *E. multilocularis* infected mice after therapy L-MTP-PE+ABZ. **(p<0.01) statistically significant from infected mice.

echinococcosis have a key role in modulation of immunosuppression and ensure the parasite survival in a host [20, 38]. Results in our experiment do not differentiate between cytotoxic and suppressor subtypes of CD8+ T cells and which one of them dominates. In alveolar echinococcosis, the CD8+ T splenocytes consist mostly of T suppressors with a low density of the CD8 antigen [37]. In our experiment, the immunotherapy suppressed the proliferation of *E. multilocularis* cysts and therefore we suppose L-MTP-PE could increase the occurrence of cytotoxic CD8+ T cells. Immunomodulator L-MTP-PE has also stopped a decrease of the CD4/

CD8 ratio and the CD4/CD8 index was stabilized over 2.00. This reflects that CD4+ T lymphocytes were in the majority despite the increased values of CD8+ T lymphocytes. CD4+ T cells together with macrophages and IFN-γ actively participate in parasite's destruction [39].

In many parasitic infections, the clinical outcome of the disease is associated with Th1 or Th2 cell activation. *E. multilocularis* metacestode is able to direct a host immune response to a less efficient, "tolerant" Th2 profile [20, 40]. In [34, 41] it was found that liposomized immunomodulators induce a development of splenic Th1 lymphocyte subpopulation. The dominance of Th1 response is important in defensive reactions of a host infected with *E. multilocularis*. IFN-γ is an important activator of macrophages and affects their energetic system [21]. In our study (Figure 8), the level of IFN-γ peaked after L-MTP-PE+ABZ therapy from weeks 8 to 18 p.i. (i.e., almost 3 months), which outlines an important credit of common administration of immunomodulators and anthelmintic drugs for therapy of alveolar echinococcosis. The protective effect of combined therapy L-MTP-PE+ABZ was the most evident during the time with IFN-γ stimulation (lasting 3 months after the therapy), manifesting in breaking of parasite's development (Figure 11).

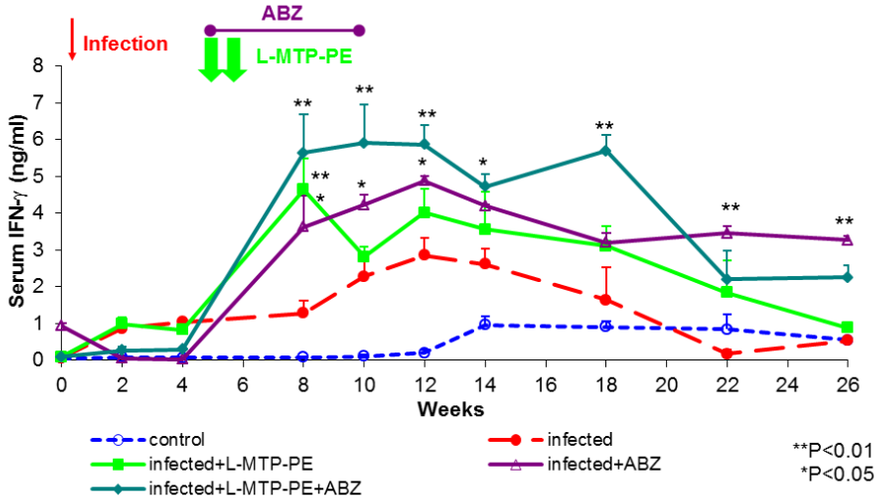

Figure 8. Serum IFN-γ in *E. multilocularis*-infected mice after therapy L-MTP-PE+ABZ. *(p<0.05); **(p<0.01) statistically significant from infected mice.

Liposomized MTP-PE suppressed the Th2 response, associated with a progressive development of larval cysts in *E. multilocularis* infection. In our experiment, the Th2 response was suppressed in mice treated with L-MTP-PE; we recorded almost twofold reduction of IL-5 production (Figure 9) during the whole experiment. We observed a total IL-5 cytokine suppression in mice treated with combined therapy L-MTP-PE+ABZ. The cytokine IL-5 is related to eosinophilia, IgE production, and may induce allergic reactions of the disease [19, 42, 43]. The therapeutic regime of L-MTP-PE in our experiment was timed on the first stage of the *E. multilocularis* infection, which is characterized by secretion of Th1 cytokines IL-2 and IFN-γ [20]. In this early phase of the infection, Th2 response has not been activated in substantial extent. Th2 response decreases a synthesis of proinflammatory Th1 cytokine IFN-γ by

http://dx.doi.org/10.5772/59732

antagonistic IL-10 secretion and by inhibited IL-12 production in macrophages [44, 45]. Therefore, there is a higher possibility of an activation of protective Th1 response by immunomodulator before the stage when an expansive metacestode growth takes place (approx. from week 8 p.i.).

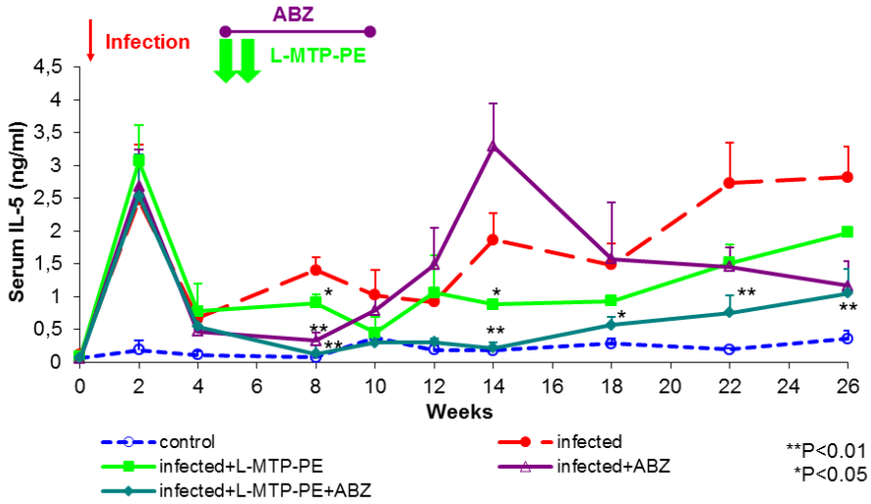

Figure 9. Serum IL-5 in *E. multilocularis* infected mice after therapy L-MTP-PE+ABZ. *(p<0.05); **(p<0.01) statistically significant from infected mice.

Macrophages participate in the destruction of the parasite through synthesis of O_2^- and other free radicals and, together with T cells, control the parasite development [39]. In our experiment (Figure 10), therapy L-MTP-PE stimulated O_2^- generation in infected mice from weeks 8 to 12 p.i. (i.e. for 1 month). The combination of L-MTP-PE+ABZ significantly increased the superoxide production from weeks 8 to 18 p.i. (almost for 3 months).

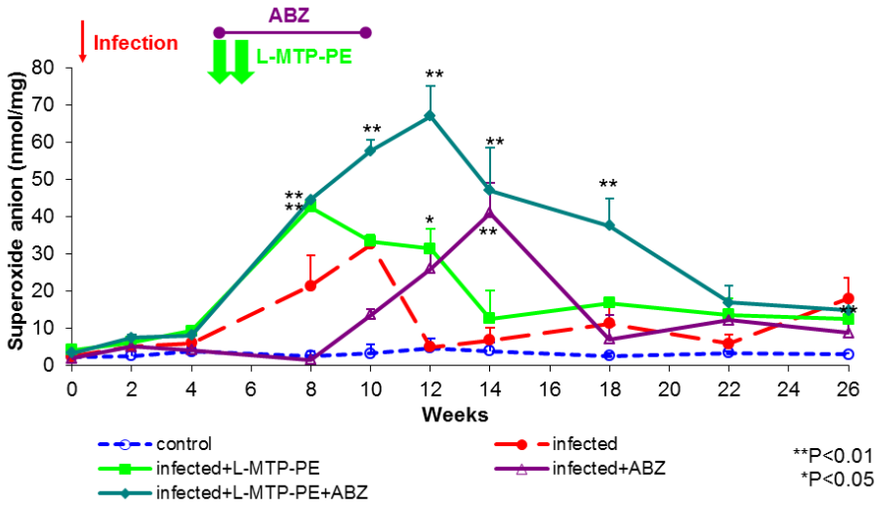

Figure 10. Macrophage's activity in *E. multilocularis* infected mice after therapy L-MTP-PE+ABZ. *(p<0.05); **(p<0.01) statistically significant from infected mice.

It documents a long-term and strong activation of macrophage's metabolism accompanied with a huge release of other biologically effective substances. In parallel, the proliferative activity of B cells was also increased, which could be related to macrophages' stimulation induced by B cell secrets [46]. Positive effect of L-MTP-PE on macrophages had been confirmed in immunosuppressed mice [25], in which the immunomodulator induced a macrophages restoration. An induction of cytotoxicity of liver macrophages against tumor cells after stimulation with L-MTP-PE was observed [28]. In addition, an increased tumoricidal activity of Kupffer cells in mice after application of L-MTP-PE was recorded [47]. Their results were later corroborated in the work [48], which described an activation of Kupffer cells with an increased production of superoxide anion and subsequent reduction of micrometastases in rats after MTP-PE therapy.

Macrophages' stimulation *in situ* is selectively influenced by a way of application of liposomized immunomodulator, and also by other biological substances. In [49], L-MTP-PE administered alone stimulated peritoneal macrophages with less effect than after coincubation of macrophages with L-MTP-PE and IFN-γ together, which doubled their cytotoxic activity. The synergetic and potentiating effect of IFN-γ in the immunomodulatory acting was also verified on macrophages infected with *Listeria monocytogenes* [50], in mice infected with *Klebsiella pneumoniae* [34]. These data confirmed an important role of T cells in immune activation of the host organism after the immunomodulator administration.

Potentiation of effector components of the immunity after the immunomodulation and anthelmintic therapy of *E. multilocularis* infected mice in our experiment resulted in a restriction of the parasite development and reduced larval cysts in a host organism (Figure 11). The immunomodulator L-MTP-PE administered alone slowed down *E. multilocularis* proliferation till week 14 p.i. and combination of L-MTP-PE+ABZ reduced the parasite development till week 22 p.i. (i.e., 3 months after the end of the experiment). A similar synergism of liposomized MDP and antiparasitic drug Glucantime was observed in visceral leishmaniasis [51].

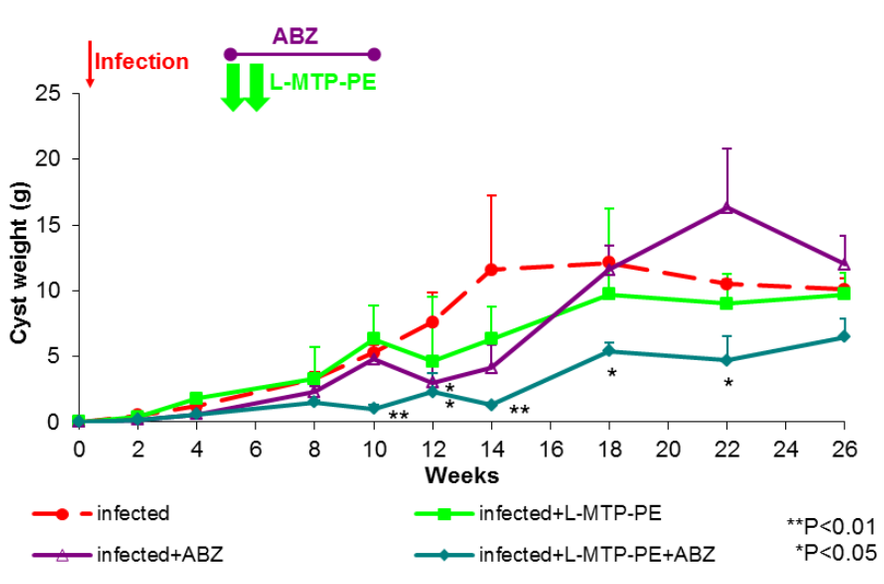

Figure 11. Cyst's weight in *E. multilocularis* infected mice after therapy L-MTP-PE+ABZ. *(p<0.05); **(p<0.01) statistically significant from infected mice.

http://dx.doi.org/10.5772/59732

In conclusion, our results show the possibility to increase antiparasitic efficacy of ABZ in *E. multilocularis* infection with L-MTP-PE, which properly stimulates the host immune response. Combined therapy L-MTP-PE+ABZ positively increased CD4+ T cells numbers and cytokine production that regulate the host immune response. The immunotherapy suppressed the Th2 response and *vice versa* activated the Th1 response with a restrictive impact on the parasite development. The immunomodulator L-MTP-PE stimulated macrophages' metabolism to produce reactive oxygen substances and together with IFN-γ increased an antiparasitic efficacy of albendazole.

5. Glucan and zinc

Glucans are β-(1,3)-D polymers of glucose occuring naturally as the basic component of the cell walls of bacteria, fungi, and yeast [52–54]. Glucans activate mainly nonspecific stimulation of the immune system [22], particularly proliferation and functional activity of phagocytic cells (macrophages, NK cells) as the part of innate immunity [55, 56], but also have an influence on specific immunity – increase of the T-lymphocyte activity and production of circulating antibodies [57–59]. The immunostimulative effect of glucan could be increased by its combination with the other components – immunoglobulin G, zinc, or vitamin C [60, 61]. Zinc interacts with cytokines and proteases and indirectly influences the immune system [62]. Its immunostimulative effect on T lymphocytes, macrophages has been known [63–65] and so zinc could potentiate the immunostimulative effect of the glucan during therapy of alveolar echinococcosis. Also living *Echinococcus* needs zinc for growth and its reproduction [66]. An obvious association between serum zinc concentration and disease severity in patients with alveolar echinococcosis was confirmed in [67]. Decreased zinc concentration in progressive cases may be a consequence of enhanced immune activation and also consumption of zinc by the growing parasite. Zinc deficiency in the host may thus contribute to the observed immunosupression in alveolar echinococcosis.

The role of glucan immunomodulator (GI, soluble β-(1,3)-D glucan) and/or glucan immunomodulator supplemented with zinc (GIZn) in stimulation of a host defence mechanism against *E. multilocularis* infection and the immunomodulator's effect on albendazole antiparasitic efficacy was observed.

5.1. Experimental design

Experiments were carried out on male BALB/c mice (n=220) weighing 20–25 g. Mice were divided randomly into five groups as folows:

Group 1 – uninfected and untreated (control)

Group 2 – infected intraperitoneally with 5000 *E. multilocularis* protoscoleces/mouse on Day 0 and no treatment

Group 3 – *E. multilocularis* infected (as Group 2) and treated with albendazole (ABZ) (Sigma, Germany) per os (p.o.) twice a week at the dose of 10 mg/kg of b.w. starting at week 5 up to week 10 p.i.

Group 4 – *E. multilocularis* infected (as Group 2) and treated with ABZ (as above) and glucan immunomodulator (GI) (Dimenzia, Slovak Republic) twice a week at the dose of 5 mg/kg b.w. at weeks 5 and 6 p.i.

Group 5 – *E. multilocularis* infected (as Group 2) and treated with ABZ (as above) with glucan supplied with zinc (GIZn) (Mevac, Slovak Republic) twice a week at the dose of 5 mg/kg b.w. at weeks 5 and 6 p.i.

Samples of blood, spleen, and peritoneal macrophages were obtained at the following weeks: 0 (prior infection), 2, 4, 6, 8, 10, 12, 14, 18, 22, and 26 p.i. from all groups (four mice per experimental day).

6. Results and discussion

The *Echinococcus multilocularis* infection in our experiment inhibited the proliferative activity of T and B lymphocytes within 26 weeks of the experiment (Figures 12, 13), with the minimum at weeks 2 and 4 p.i., that is, in the early stage of infection, during the time important for the parasite establishment. These data correlate to results of other authors [17, 68] and it could be caused by immunosuppressive factors released by protoscoleces of *E. multilocularis*. The T cell proliferation (Figure 12) was stimulated in infected mice with GI+ABZ therapy from week 6 to 10 p.i., at the beginning of the progressive larval growth in infected mice without treatment. The GI stimulatory activity was prolonged for 4 weeks by zinc supplementation in GIZn immunomodulator. This confirms an important role for zinc in immune response, specifically lymphocyte proliferation induced by mitogens. T-cell division, maturation, and differentiation require an adequate zinc content [65, 69]. Even after ABZ therapy, the proliferative activity of T lymphocytes was increased during drug's administration, from week 6 to 12 p.i., similarly to another works [68, 70]. The modulatory effect of albendazole could be derived from the fact that a drug induces direct morphological and structural changes of parasite cysts walls and protoscoleces [71], which lead to the revealing of normally unexposed structural antigens, which are presented to T lymphocytes. It results in inducing a Th2 response (chronic antigen stimulation) or a Th1 response (small parasitic lesions or reduced antigen output/recognition) [72]. The positive synergistic effect of anthelmintic ABZ and immunomodultor GIZn resulted in a prolonged increased T cell proliferation for 8 weeks after the end of drug's administration.

Immunomodulators GI and GIZn did not induce such stimulative effect on B cell proliferation (Figure 13); they restored a suppressed proliferative activity of B cells only for a short time. The similar restoring effect of glucan was observed in other parasitic infections with *Ascaris suum* or *Toxocara canis* [60, 73–75]. The weaker influence of GIZn on B cells could be explained by the fact that severe zinc deficiency impairment of B cell function is rarely seen [76, 77] and also the humoral response of patients with progressive alveolar echinococcosis was not affected by zinc deficiency [67]. Both combined therapies GI+ABZ and GIZn+ABZ reinforced

http://dx.doi.org/10.5772/59732

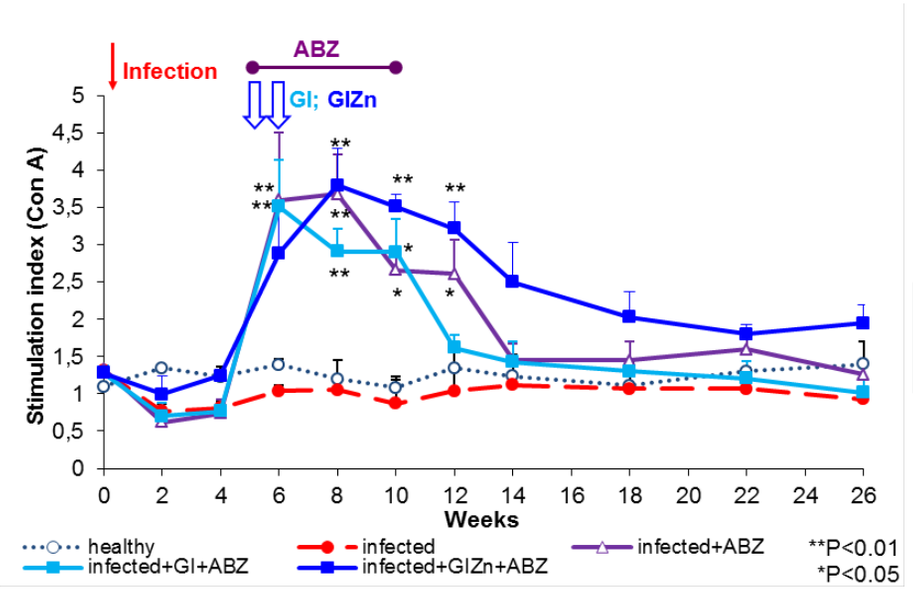

Figure 12. Proliferative activity of T lymphocytes in *E. multilocularis* infected mice after immunotherapy with GI or GIZn. *(p<0.05); **(p<0.01) statistically significant from infected mice.

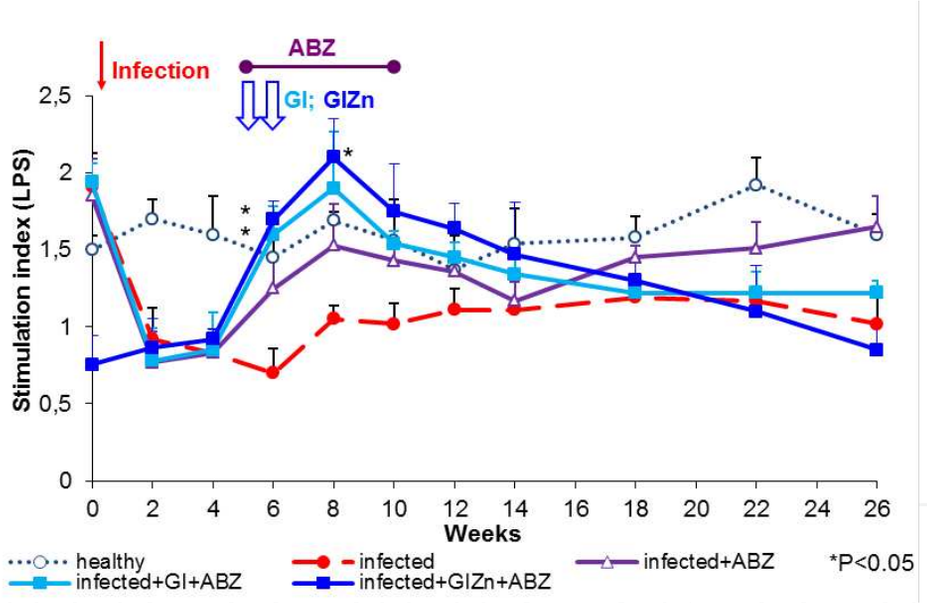

Figure 13. Proliferative activity of B lymphocytes in *E. multilocularis* infected mice after immunotherapy with GI or GIZn. *(p<0.05) statistically significant from infected mice.

a stimulatory activity of ABZ on B cell subpopulation from week 6 to 14 p.i., during the intensive cyst's growth in infected mice without treatment. The functional activity of T lymphocytes in cellular response to *E. multilocularis* infection is associated with two subpopulations of CD4+ and CD8+ T cells. A time-dependent supression of T cell proliferative response was observed in [78], and authors also recorded *in vitro* CD4 T cells' decrease and *vice versa* CD8 T cells' increase in splenocytes after activation with *E. multilocularis* protoscoleces.

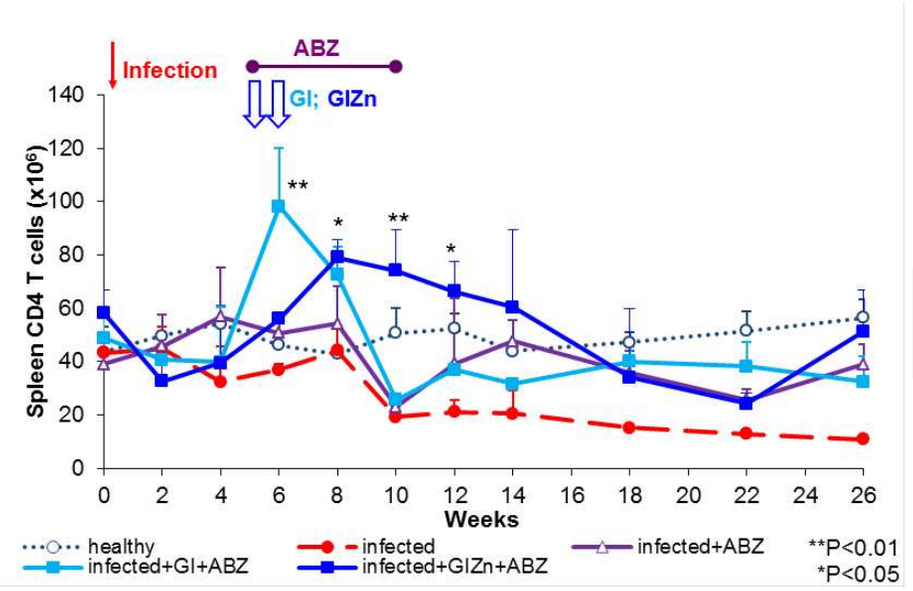

Figure 14. Number of splenic CD4 T cells in *E. multilocularis* infected mice after immunotherapy with GI or GIZn. *(p<0.05); **(p<0.01) statistically significant from infected mice.

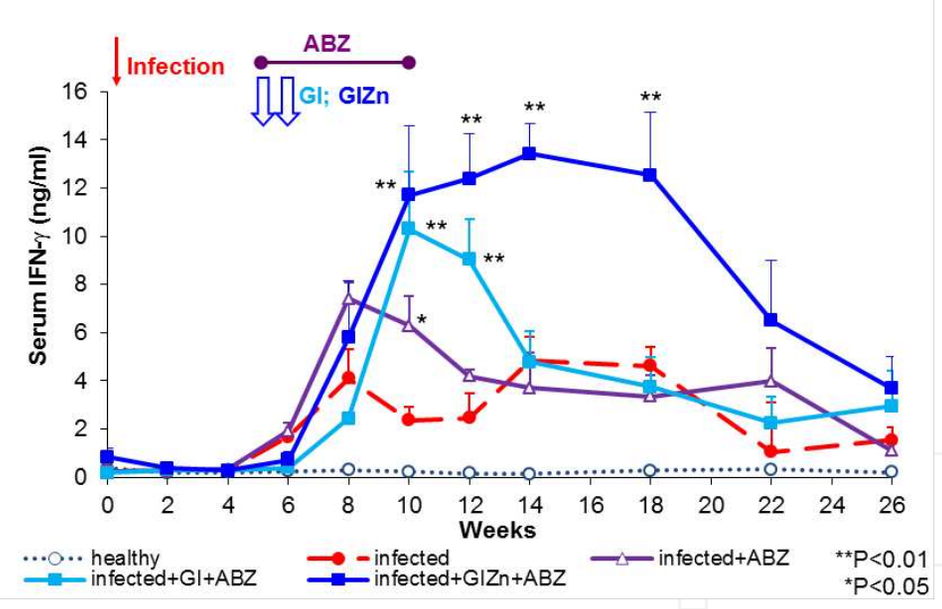

Figure 15. Serum IFN-γ in *E. multilocularis* infected mice after immunotherapy with GI or GIZn. *(p<0.05); **(p<0.01) statistically significant from infected mice.

CD4 T cells represent the main T subpopulation in the first phase of alveolar echinococcosis in granuloma formation around the parasite [17, 79]. In our experiment (Figure 14), the presence of CD4 T cells was stimulated from weeks 6 to 8 p.i. in the spleen of mice infected and treated with GI+ABZ. Supplementation of glucan with zinc could contributed to prolong an increase in the CD4 T cell subpopulation in mice treated with GIZn+ABZ from week 6 to 14 p.i. It has been known that zinc deficiency reduces the production of IL-4 cytokine acting

http://dx.doi.org/10.5772/59732

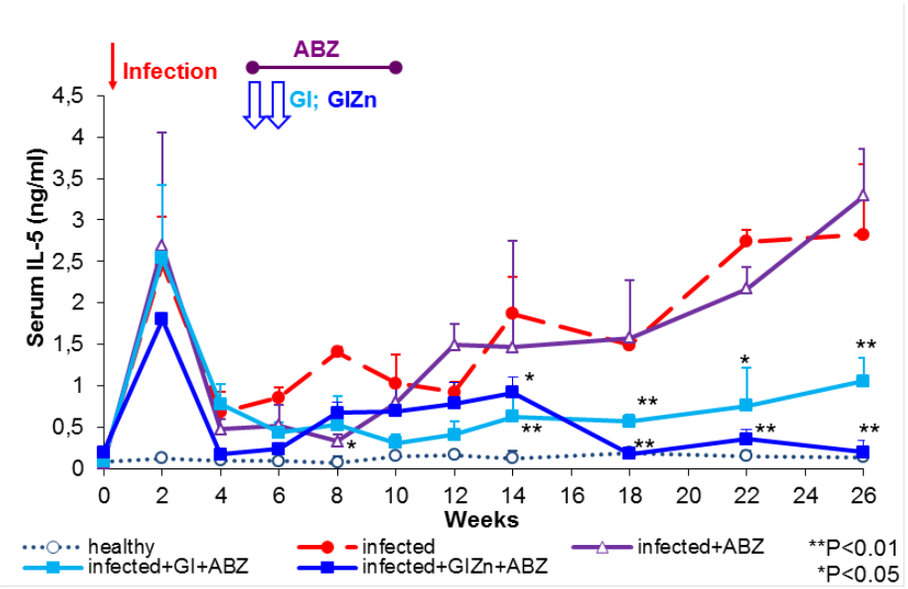

Figure 16. Serum IL-5 in *E. multilocularis* infected mice after immunotherapy with GI or GIZn. *($p<0.05$); **($p<0.01$) statistically significant from infected mice.

as a growth factor for helper CD4 T cells [80]. The long-term stimulation of CD4 T cells after GIZn+ABZ therapy could increase antiparasite defence. Periparasitic granuloma in regressive alveolar echinococcosis is composed of great numbers of CD4 T lymphocytes, macrophages, and myofibroblasts [81]. In patients with progressive disease, the CD8 T lymphocytes are dominant [82]. In our experiment, *E. multilocularis* infection did not induce serious changes in T cells. The CD8 T lymphocytes were not influenced by immunotherapy, except for a light growth in the CD8 T cell numbers at weeks 12 and 14 p.i. in mice infected and treated with GIZn+ABZ, which could be helpful in the development of immunosuppression of the host.

The Th1 and Th2 cytokine balance regulates the immune response to *E. multilocularis* [19]. The possible effect of the immunotherapy on the Th1/Th2 balance was evaluated according to serum levels of cytokines IFN-γ and IL-5. GIZn+ABZ therapy stimulated the level of serum IFN-γ with the strongest and longest effect in mice with *E. multilocularis* infection from week 8 to 22 p.i. (Figure 15). This result documents the positive effect of GIZn immunomodulator on preferential Th1 stimulation. Immunomodulators GI and GIZn decreased the serum level of IL-5 from week 6 p.i. (Figure 16). The strong Th2 downregulative effect of both glucan immunomodultors stopped the rise of IL-5 (recorded in mice infected and without the treatment) from week 14 p.i till the end of the experiment.

IFN-γ is widely recognized as a major priming signal for macrophages' activation, which have a key role in effector phase of immune response to *E. multilocularis.* Macrophages provide for the protoscoleces destruction by reactive oxygen metabolites production (superoxide anion, hydrogenperoxide) [20]. The activation of peritoneal macrophages in the course of therapy was evaluated by generation and release of superoxide anion (O_2^-). Although the most increase in IFN-γ serum level in our experiment was found after GIZn therapy, GI had the strongest impact on O_2^- release (Figure 17). Both GI+ABZ and GIZn+ABZ therapies resulted in the huge generation of O_2^- from week 12 to 18 p.i., but with lower values in mice treated with GIZn+ABZ.

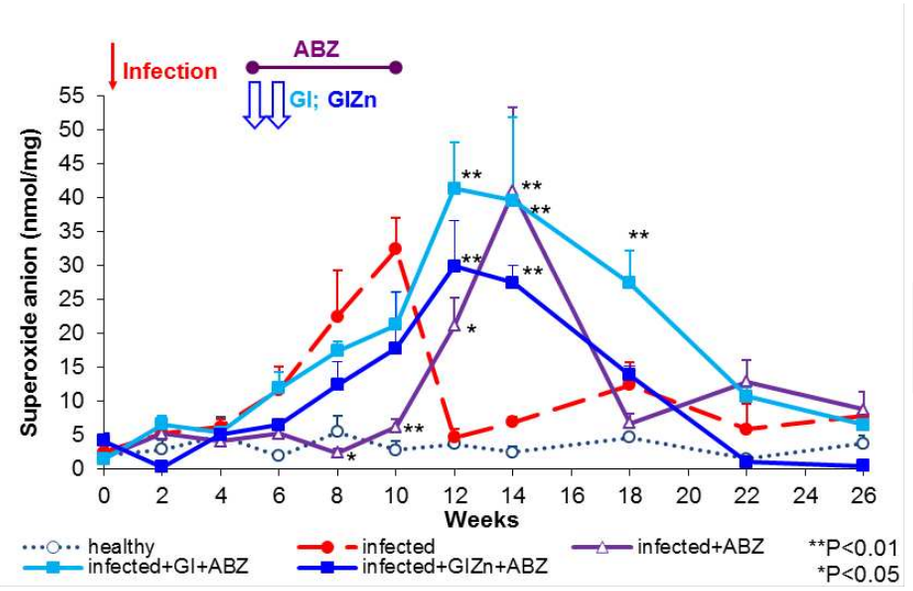

Figure 17. Macrophages' activity in *E. multilocularis* infected mice after immunotherapy with GI or GIZn. *(p<0.05); **(p<0.01) statistically significant from infected mice.

A notable finding in the treatment efficacy was that GI's presence in GI+ABZ therapy partly inhibited the parasitostatic effect of ABZ (Figure 18).

It could be explained by GI's stimulatory effect on fibrosis in periparasitic granuloma, which could inhibit anthelmintic drug from penetrating to parasite vesicle. The glucan's profibrotic activity in metacestode infection *Mesocestoides corti* was found in [83], where glucan therapy stimulated mononuclear-phagocytic cells in the liver followed by increased fibrotic process around parasite's larvae. In our experiment, GIZn+ABZ therapy was evaluated as the most effective therapeutic approach to alveolar echinococcosis, because it stopped larval cyst's growth in the host till week 14 p.i. This coincides with the highest levels of IFN-γ in our experiment – the cytokine with an antifibrotic property [21]. Cytokine IFN-γ plays a key role in the regulation of collagen metabolism and might ameliorate liver fibrogenesis. Also in [84] it was documented that IFN-γ downregulated lysyl oxidase gene expression, an essential catalyst for the cross-linking of extracellular collagen and elastin.

In our experiment, the stimulation of IFN-γ production induced by GIZn could reduce the irreversible fibrosis in periparasitic granuloma and thus allow penetration of anthelmintics to larvocyst. IFN-γ has not only an antifibrotic property, but it is also engaged in Th1 immune reactions, which are protective against *E. multilocularis* and include fibrogenesis. A correlation of T cell immune response and hepatic fibrosis was found in [85]; serum IFN-γ levels were negatively correlated with serological markers of fibrosis. Another immunological component connected with fibrogenesis appear to be reactive oxygen metabolites produced by macrophages. Superoxide anion, hydrogenperoxide, and hydroxyl radical are important stimuli to colagen gene induction of hepatic stellate cells (HSC) [86]. Collagen synthesis and subsequent cross-linking of collagen by pyridinoline, leading to irreversible fibrosis in periparasitic granuloma, is a substantial component of the host immune reaction in forming a resistance against parasite growth [87]. Therefore, the stimulation of fibrotic process could be beneficial

http://dx.doi.org/10.5772/59732

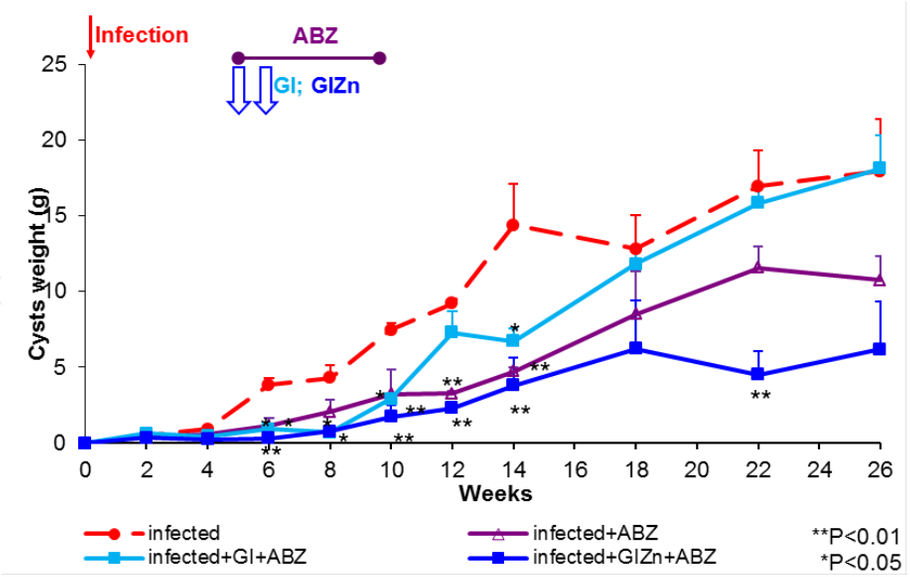

Figure 18. Cyst's weight in *E. multilocularis* infected mice after immunotherapy with GI or GIZn. *(p<0.05); **(p<0.01) statistically significant from infected mice.

for the host. On the contrary, overproduction of fibrous tissue may be harmful for host, as fibrosis results in many complications of alveolar echinococcosis in patients: vasculary and biliary obstructions in the liver [88]. In our experiment, the greatest inducer of O_2^- generation in peritoneal macrophages (connected with hepatic fibrosis) was GI. On the other hand, GI supplemented with zinc induced a short-time and a weaker production of this metabolite.

Zinc plays an important role as cofactor of enzymes involved in collagen synthesis [89] and zinc supplemention has favorable inhibitive effects on hepatic fibrosis [90, 91]. This fact can explain better the antiparasitic efficacy of treatment with GIZn and nearly twofold higher efficacy of GIZn+ABZ therapy in comparison with ABZ treatment (Figure 18).

According to host's immunosuppression and zinc deficiency in patients with alveolar echinococcosis [67], the combination of immunomodulator GIZn and anthelmintic drug ABZ achieved the most effective control of the parasite infection. The parasitostatic effect of therapy GIZn+ABZ lasted for the longest time; also after the end of the therapy it reached the greatest reduction of *E. multilocularis* cysts in the host. Zinc supplemention of glucan immunomodulator improved its immunostimulatory effects on cellular immunity, which activated Th1 response and significantly stimulated IFN-γ cytokine synthesis. We suppose zinc positively downregulated the liver fibrosis induced by glucan, because GIZn immunomodulator suppressed the generation of superoxide – an activator of hepatic stella cells involved in the fibrotic process.

7. Transfer factor

The dialysable leucocyte extract, a product of the immune system, is known as transfer factor (TF). Transfer factors are low molecular weight dialysable products extracted from immune

cells which transmit the ability to express delayed-type hypersensitivity and cell-mediated immunity from sensitized donors to nonimmune recipients [92]. TF increases macrophage activation and IL-1, IL-2, and IFN-γ production *in vitro* and enhances leucocyte chemotaxis and natural killer function [93, 94]. The TF preparates activated the proliferation of splenocytes from animals with hypothyroidism [95] and stimulated hemopoiesis [96]. Transfer factor stimulated antigen-specific cell-mediated response in patients with various infections, and so administration of transfer factor has been recommended for patients with selective deficits in cell-mediated immunity such as refractory neoplasms and chronic infections [94]. Administration of dialysable leucocyte extract has been shown to be free of hypersensitivity, long-lasting side effects or complications, except for transitory hyperpyrexia.

Nonspecific transfer factor (TF) from the blood leukocytes of immunized swine was studied as an appropriate candidate for immunotherapy, supplementing conventional ABZ treatment of alveolar echinococcosis.

7.1. Experimental design

Experiments were carried out on male BALB/c mice (n=165) weighing 20–25 g. Mice were randomly divided into five groups as follows:

Group 1 – uninfected and untreated (control)

Group 2 – infected intraperitoneally with 5000 *E. multilocularis* protoscoleces/mouse on Day 0 and no treatment

Group 3 – *E. multilocularis* infected (as Group 2) and treated with transfer factor (TF) (Imunor, ImunomedicA, Czech Republic) per os (p.o.) twice a week at the dose of 200 μg/kg of body weight (b.w.) starting at week 5 up to week 10 p.i.

Group 4 – *E. multilocularis* infected (as Group 2) and treated with albendazole (ABZ) (Sigma, Germany) per os (p.o.) twice a week at the dose of 10 mg/kg of b.w. starting at week 5 up to week 10 p.i.

Group 5 – *E. multilocularis* infected (as Group 2) and treated with combination of TF and ABZ as above

Samples of blood, spleen, and peritoneal macrophages were obtained at the following weeks: 0 (prior infection), 2, 4, 6, 8, 10, 12, 14, 18, 22, and 26 p.i. from all groups (3 mice per experimental day).

8. Results and discussion

Transfer factors (TFs) are proteins that transfer specific cellular immunity from an immune donor to a nonimmune recipient and possess a number of nonspecific activities, such as the ability to increase the numbers of immunocompetent cells, to stimulate phagocytosis, to induce the production of interferons and interleukins, to stimulate hemopoiesis, etc. TF includes a lot

of molecules, acting as antigens (MW cca 5 kDa), or acting as immunomodulators (less than 3.5 kDa) [97]. Transfer factors are very efficient in diseases in which cellular immunity plays an important role in protection and control of the disease, for example, viral, bacterial, and parasite infections, as well as immunodeficiencies and some types of cancer [98].

The host cellular immunity plays a principal role in the control of the *E. multilocularis* proliferation, which was documented by experiments, in which mouse strains not developing cellular immune response do not control metacestode growth, while mice deficient in humoral immunity control parasite growth up to a certain level [99]. Furthermore, T-cell immunity significantly contributes to the control of alveolar echinococcosis in human patients, demonstrated by the rapid fatal outcome of the infection in an immunodeficient patient coinfected with human immunodeficiency virus (HIV) [100]. Upon restoration of CD4-immunocompetence in another AIDS-patient coinfected with *E. multilocularis*, the course of disease was positively influenced again [101].

In our work, both the therapy with TF or combination of TF+ABZ abolished a suppressory impact of *E. multilocularis* infection on T- and B-cell proliferation (Figures 19, 20) and the therapeutic stimulatory effect was recorded up to week 14 p.i., that is, one month after the end of the therapy. Immunomodulating activity of TF on lymphocyte functional status was also recorded by [95], who detected an increased proliferation of splenocytes from rats with hypothyroidism induced by TF. A significant stimulative effect of TF on T- and B-cell populations was also confirmed in experimental ascariasis in pigs [63].

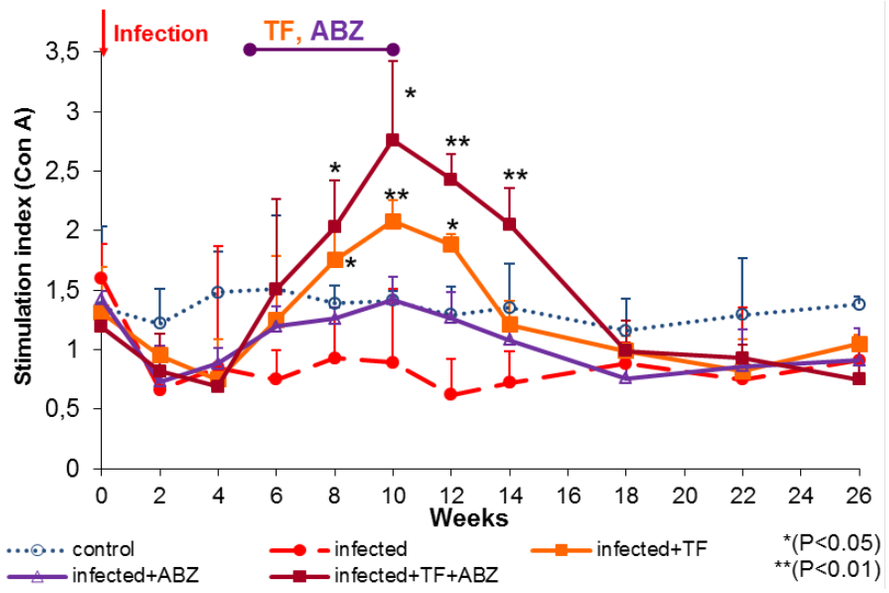

Figure 19. Proliferative activity of T lymphocytes in *E. multilocularis* infected mice after therapy TF+ABZ. *(p<0.05); **(p<0.01) statistically significant from infected mice.

T lymphocytes play a key role in the control of immune response in alveolar echinococcosis [18, 36]. In our study, the administration of TF alone or in combination of TF+ABZ caused the increase in numbers of CD4+ T cells in the spleen of infected mice up to week 14 p.i. (Figure 21). Patients with active alveolar echinococcosis showed increased numbers of CD8+ T lymphocytes [20] and also our results documented an increase in this subpopulation after *E.*

multilocularis infection from weeks 4 to 8 p.i., that is, during the massive proliferation of larval cysts. The CD8+ T cells were not markedly influenced with TF immunomodulator, because all therapeutic approaches inhibited the CD8+ T cell numbers during drug administration, from weeks 6 to 10 p.i., but with no effect after the end of therapies. The splenic CD8+ T subpopulation in alveolar echinococcosis are mostly T suppressor cells [78]. Therefore, we deduce that TF therapy stopped the development of host immunosuppression only for a short time, during the drug administration. The increase in CD4+ T lymphocytes induced by TF was also observed in viral infection (herpes zoster) and CD8+ T lymphocytes showed less decrease [102], similar to our results.

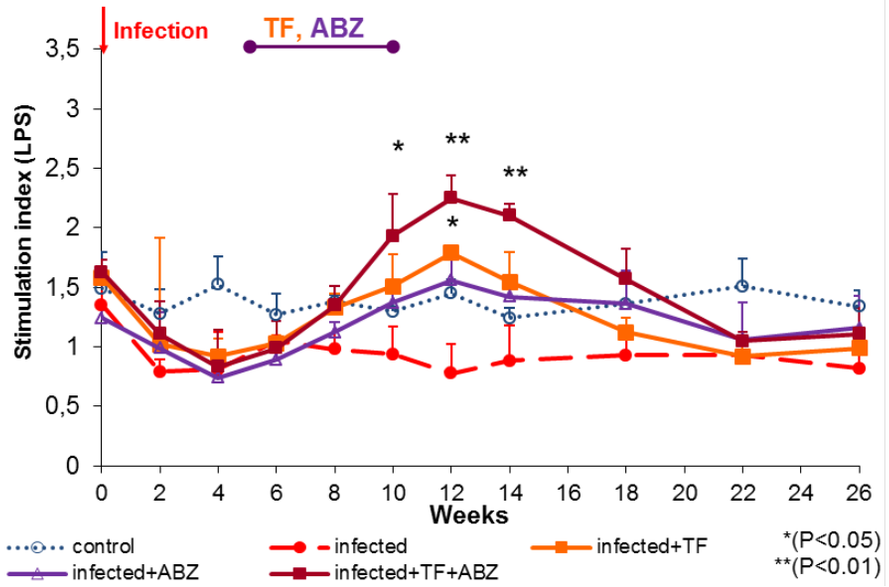

Figure 20. Proliferative activity of B lymphocytes in *E. multilocularis* infected mice after therapy TF+ABZ. *(p<0.05); **(p<0.01) statistically significant from infected mice.

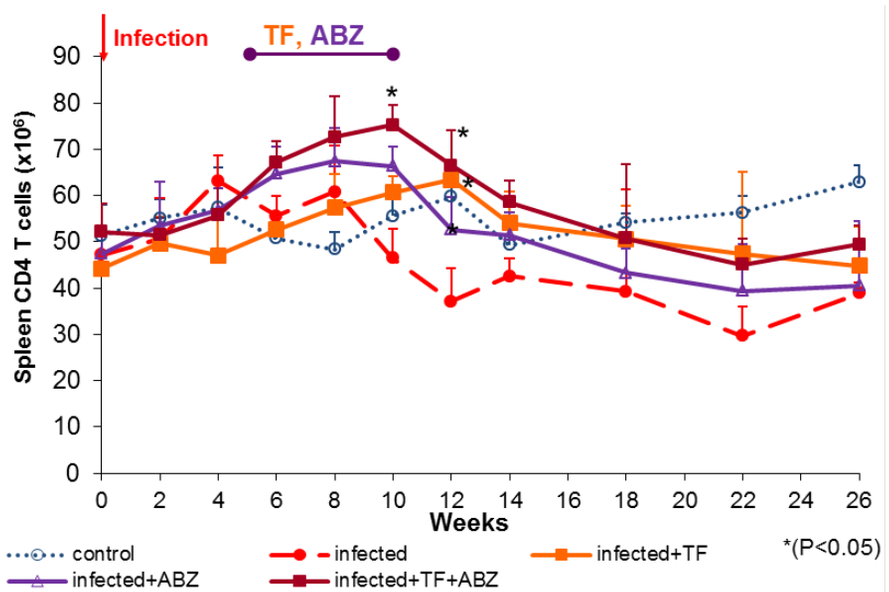

Figure 21. Number of splenic CD4 T cells in *E. multilocularis* infected mice after therapy TF+ABZ. *(p<0.05) statistically significant from infected mice.

http://dx.doi.org/10.5772/59732

Active alveolar echinococcosis is related to Th2 response – dominance of IL-5 and IL-10 and week production of IFN-γ [20]. The superiority of Th1 response plays a key role in the host defence reactions against *E. multilocularis*. TF therapy selectively affects cytokine production in response to antigen stimulation, particularly by secreting IFN-γ [93]. In our experiment, the results of cytokine proteins detected by capture ELISA may not correlate with the levels of bioactive cytokine protein. ELISA may utilize anticytokine antibodies that cannot discriminate between the precursor (inactive) and mature (bioactive) forms of a cytokine protein. Moreover, an ELISA may detect partially degraded cytokine proteins, which have retained their immunoreactive properties but may have lost their bioactivity [103, 104]. But capture ELISA is a helpful indicator of cytokine presence, although it does not reflect the biological potency of the detected cytokines.

The concentration of serum IFN-γ in mice infected with *E. multilocularis* and treated with TF or both TF+ABZ (Figure 22) was increased during therapy and also for one month after therapy (from weeks 6 to 14 p.i.). The stimulative effect of TF on Th1 response, particularly together with drug TF+ABZ, was observed in IFN-γ production *in vitro* [105], where high release of this cytokine from splenocytes was found till the end of the experiment. Similar results were achieved in mice with tuberculosis and treated with TF and antibiotics [98]. Th1-inducing effect of TF was also confirmed in antitumour immunotherapy [92]. The concentration of IL-5 in serum in mice infected with *E. multilocularis* and treated with TF or TF+ABZ in our work was suppressed till the end of the experiment. This is consistent with an inhibited production of IL-5 *in vitro* after immunotherapy with TF and TF+ABZ [105], which was significantly decreased two weeks after the end of the therapy. The intensive Th2 downregulative effect of TF was found by [93], who detected low splenocytes secretion of IL-10 and no IL-4. This cytokine IL-5 modulates eosinophilia, IgE production, and can enhance allergic reactions of the disease [20].

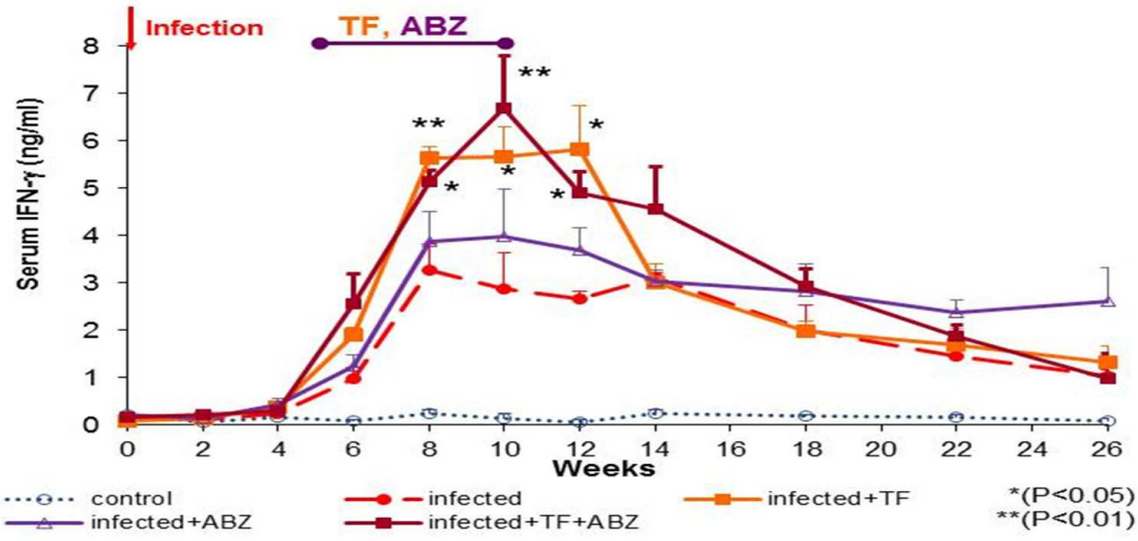

Figure 22. Serum IFN-γ in *E. multilocularis* infected mice after therapy TF+ABZ. *(p<0.05); **(p<0.01) statistically significant from infected mice.

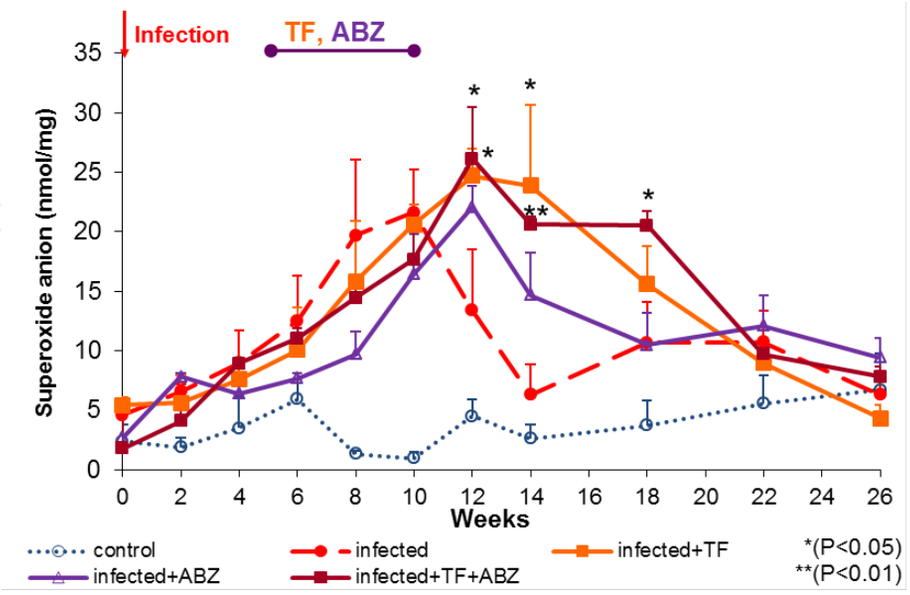

Figure 23. Macrophages' activity in *E. multilocularis* infected mice after therapy TF+ABZ. *(p<0.05); **(p<0.01) statistically significant from infected mice.

IFN-γ (that was stimulated after TF therapy) is an important activator of macrophages and affects their energetic system [21]. Immunotherapy with TF or TF+ABZ activated the respiratory burst in macrophages with high generation of O_2^- for a long time, from weeks 8 to 18 p.i. (Figure 23). This is documented by a long-term stimulation of macrophages' metabolic activity accompanied with a high production of also other biologically effective substances. At the same time, the proliferation of B cells and the IFN-γ level were increased. B cells release stimulation factors for macrophages after activation with antigens from destroyed *Echinococcus* protoscoleces [46] and cytokine IFN-γ markedly affects the macrophage energetic system. In our study, high concentrations of IFN-γ time-matched with intensive O_2^- production and slow parasite proliferation in infected mice treated with TF or TF+ABZ. Stimulative effect of TF on macrophages' metabolism had also been recorded by [106], who found the TF dialysis fraction (less than 3500 MW) activating the capacity of peritoneal macrophages to produce superoxide anion.

Activation of effector components of immune response after the immunotherapy of *E. multilocularis* infected mice in our experiment reduced the parasite growth and limited the development of larval cysts in the host (Figure 24). The immunomodulatory effect of TF suppressed the parasite proliferation till week 14 p.i. with the similar activity to anthelmintic drug albendazole and prolonged the parasite-reducing effect for one month, that is, till week 18 p.i. The TF-inhibiting effect on the parasite development was associated with the time of TF administration and a short time after it. The addition of TF to albendazole therapy resulted in a high antiparasitic effect with a significant cyst's reduction up to week 18 p.i. (2 months after the end of the therapy) in comparison to untreated group. Thereafter, the protective effect of anthelmintic therapy was lost.

http://dx.doi.org/10.5772/59732

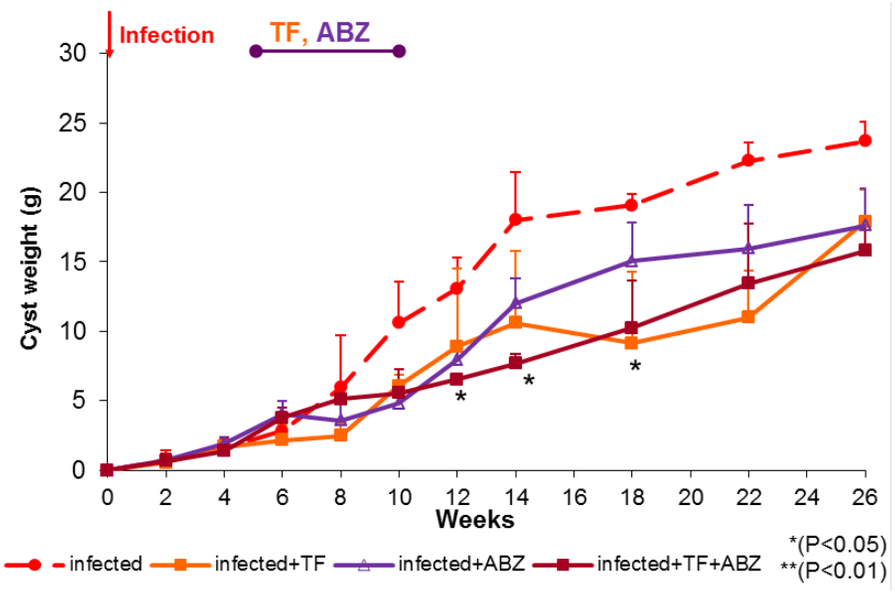

Figure 24. Cyst's weight in *E. multilocularis* infected mice after therapy TF+ABZ. *(p<0.05); **(p<0.01) statistically significant from infected mice.

In conclusion, the results of our study suggest that TF can have a positive effect on the host cellular immune response against the development of *E. multilocularis*. Nowadays, it is known that TF consists of a high quantity of low molecular weight proteins (> 200), and the exact chemical nature and molecular mechanisms of its action have not been defined yet. Low synthesis of IFN-γ induced by the *E. multilocularis* infection was restored with TF and exceeded the control concentration. The rise recorded in IFN-γ cytokine could be explained as a result of immunorestoration of CD4+ Th1 lymphocytes or due to the proliferation of another CD4 subpopulation. We think that regulation of immune homeostasis with TF therapy, which occurred between the CD4 cells and IFN-γ, played an important role in the immune response to *E. multilocularis* infection. Regulatory and effector mechanisms (CD4+ T cells, IFN-γ, and macrophages) which actively participate in the parasite destruction were stimulated with TF and supported antiparasitic effect of albendazole.

9. Conclusion

Our results suggest the possible way to increase ABZ antiparasitic efficacy in *E. multilocularis* infection by stimulation of the host immune response.

L-MTP-PE was recognized as a strong macrophage activator in mice infected with *E. multilocularis*. This immunomodulator stimulated the superoxide anion production for 3 months. The activity of macrophages was supported by dominant Th1 protective response – considered the highest serum IFN-γ level in that time. The antiparasitic effect of combined L-MTP-PE +ABZ therapy was manifested by marked reduction of cysts growth for 3 months after the end of treatment.

GIZn+ABZ has been shown to be the most effective immunomodulatory and antiparasitic treatment, the greatest reduction of metacestode growth was observed as early as 2 weeks from the beginning of this therapy, lasting till the end of experiment (for 4 months). The addition of zinc to immunomodulatory glucan substance has greatly contributed to the reduction of intensive fibrosis after glucan treatment, which attenuated the antiparasitic effect of ABZ alone.

TF could correct the immune balance disturbance after the *E. multilocularis* infection, which is responsible for lymphocyte suppressor activity. Inhibited IFN-γ production after the *E. multilocularis* infection was restored and increased with TF, which could result from a restoration of CD4+ Th1 lymphocytes. TF administration prolonged the parasitostatic effect of ABZ for 2 months. Restoration of suppressed host immune mechanisms that are important for restriction of parasite expansive growth seems to be a promissing approach to *E. multilocularis* infection.

Immunomodulatory agents can be beneficial in the treatment of alveolar echinococcosis, where the developed Th1 immune response supports the improvement of the anthelmintic action of benzimidazoles. The type and development of the immune response are generally managed by the lymphocyte network and its immunoregulatory mechanism. Immunomodulators could regulate the immune disbalance after the *E. multilocularis* infection, which suppresses lymphocytes' and macrophages' activities. Restoring of this immunosuppresion may be one promising way to overcome this situation and to control chronic alveolar echinococcosis.

Acknowledgements

This study was supported by the Slovak VEGA agency, grants No. 2/0172/13 and No. 2/0081/15.

Author details

Emília Dvorožňáková*

Address all correspondence to: dvoroz@saske.sk

Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovak Republic

References

[1] Wilson JF, Rausch RL, Wilson FR. Alveolar hydatid disease. Review of the surgical experience in 42 cases of active disease among Alaskan Eskimos. *Ann Surg* 1995;221(3) 315-323.

[2] Zhang W, McManus DP. Recent advances in the immunology and diagnosis of echinococcosis. *FEMS Immunol Med Microbiol* 2006;47(1) 24-41.

[3] Kern P. Medical treatment of echinococcosis under the guidance of Good Clinical Practice (GCP/ICH). *Parasitol Int* 2006;55(Suppl) S273-S282.

[4] Kinčeková J, Hrčková G, Bober J, Vrzgula A, Szabadošová V, Bohuš P, Zachar M. A rare case of alveolar echinococcosis in a 14-year-old child. *Helminthologia* 2008;45(1) 28-31.

[5] Reuter S, Jensen B, Buttenschoen K, Kratzer W, Kern P. Benzimidazoles in the treatment of alveolar echinococcosis: a comparative study and review of the literature. *J Antimicrob Chemo* 2000;46(3) 451-456.

[6] Venkatesan P. Albendazole. *J Antimicrob Chemo* 1998;41(2) 145-147.

[7] Capece BPS, Navarro M, Arcalis T, Castells G, Toribio L, Perez F, Carretero A, Ruberte J, Arboix M, Cristofol C. Albendazole sulphoxide enantiomers in pregnant rats embryo concentrations and developmental toxicity. *Vet J* 2003;165(3) 266-275.

[8] Ammann RW, Hirsbrunner R, Cotting J, Steiger U, Jacquier P, Eckert J. Recurrence rate after discontinuation of long-term mebendazole therapy in alveolar echinococcosis (preliminary results). *Am J Trop Med Hyg* 1990;43(5) 506-515.

[9] McManus DP, Zhang WB, Bartley PB. Echinoccosis. Lancet 2003; 362(9392) 1295-1304.

[10] Altintas Nuray, Orenay S, Reyhan E, Turk M, Asci M, Turel S, Yolasigmaz A, Altintas Nazmiye. Genotoxic effects of albendazole in patients medicated for cystic echinococcosis. *Helminthologia* 2007;44(2) 57-61.

[11] El-On J. Benzimidazole treatment of cystic echinococcosis. *Acta Tropica* 2003;85(2) 243-252.

[12] Gottstein B, Haag K, Walker M, Matsumoto J, Mejri A, Hemphill A. Molecular survival strategies of *Echinococcus multilocularis* in the murine host. *Parasitol Int* 2006;55(Suppl) S45-S49.

[13] [13]Vuitton DA, Zhang SL, Yang Y, Godot V, Beurton I, Mantion G, Bresson-Hadni S. Survival strategy of *Echinococcus multilocularis* in the human host. *Parasitol Int* 2006;55(Suppl) S51-S55.

[14] Gottstein B, Hemphill A. Immunopathology of echinococcosis. In: Freedman DO. (ed.) *Immunopathogenetic Aspects of Disease Induced by Helminth Parasites*. Book Series: *Chem Immunol*, 1997,66:177-208.

[15] Dixon JB. Echinococcosis. *Comp Immunol Microbiol Infect Dis* 1997;20(1) 87-94.

[16] Reimann J, Kaufmann SHE. Alternative antigen processing pathways in anti-effective immunity. *Curr Opin Immunol* 1997;9(4) 462-469.

[17] Emery I, Liance M, Deriaud E, Vuitton DA, Houin R, Leclerc C. Characterization of T-cell immune responses of *Echinococcus multilocularis*-infected C57BL/6J mice. *Paras Immunol* 1996;18(9) 463-472.

[18] Manfras BJ, Reuter S, Wendland T, Boehm BO, Kern P. Impeded Th1 CD4 memory T cell generation in chronic-persisting liver infection with *Echinococcus multilocularis*. *Int Immunol* 2004;16(1) 43-50.

[19] Sturm D, Menzel J, Gottstein B, Kern P. Interleukin-5 is the predominant cytokine produced by peripheral blood mononuclear cells in alveolar echinococcosis. *Infect Immun* 1995;63(5) 1688-1697.

[20] Vuitton DA. The ambiguous role of immunity in echinococcosis: protection of the host or of the parasite? *Acta Tropica* 2003;85(2) 119-132.

[21] Liance M, Ricard-Blum S, Emery I, Houin R, Vuitton DA. *Echinococcus multilocularis* infection in mice: in vivo treatment with a low dose of IFN-gamma decreases metacestode growth and liver fibrogenesis. *Parasite* 1998;5(3) 231-237.

[22] Šoltýs J, Quinn MT. Modulation of endotoxin- and enterotoxin-induced cytokine release by in vivo treatment with beta-(1,6)-branched beta-(1,3)-glucan. *Infect Immun* 1999;67(1) 244-252.

[23] Hrčková G, Velebný S. Effect of praziquantel and liposome-incorporated praziquantel on peritoneal macrophage activation in mice infected with *Mesocestoides corti* tetrathyridia (Cestoda). *Parasitology* 1997;114(5) 475-482.

[24] Pabst MJ, Beranova-Giorgianni S, Krueger JM. Effects of muramyl peptides on macrophages, monokines, and sleep. *Neuroimmunomodulation* 1999;6(4) 261-283.

[25] Adam A, Lederer E. Muramylpeptides as immunomodulators. *Atl Sci - Immunol* 1988;1(3-4) 205-214.

[26] Hui GS, Tam LQ, Chang SP, Case SE, Hashiro C, Siddiqui WA, Shiba T, Kusumoto S, Kotani S. Synthetic low-toxicity muramyl dipeptide and monophosphoryl lipid A replace Freund complete adjuvant in inducing growth-inhibitory antibodies to the *Plasmodium falciparum* major merozoite surface protein, gp195. *Infect Immun* 1991;59(5) 1585-1591.

[27] Dvorožňáková E, Borošková Z, Dubinský P, Tomašovičová O, Hříbalová V, Machnicka B. Immunomodulative effect of muramyldipeptide in mice with larval toxocarosis. *Parasitol Res* 1999;85(12) 1034-1040.

[28] Dieter P, Ambs P, Fityke E, Schwende H. Lipopolysaccharide and liposome-encapsulated MTP-PE-induced cytotoxicity and release of eicosanoids, tumor necrosis factor-alpha and nitric oxide in liver macrophages. In: Honn KV, Marnett LJ, Nigam S, et al. (eds.) *Eicosanoids And Other Bioactive Lipids in Cancer, Inflammation, and Radiation Injury 3* Book Series: *Adv Exper Med Biol* 1997;Vol407 485-490.

[29] Asano T, McWatters A, An T, Matsushima K, Kleinerman ES. Liposomal muramyl tripeptide up-regulates interleukin-1 alpha, interleukin-1 beta, tumor necrosis factor-alpha, interleukin-6 and interleukin-8 gene expression in human monocytes. *J Pharmacol Exper Therap* 1994;268(2) 1032-1039.

[30] Melissen PM, van Vianen W, Leenen PJ, Bakker-Woundenberg IA. Tissue distribution and cellular distribution of liposomes encapsulating muramyltripeptide phosphatidyl ethanolamide. Tissue and cellular distribution of LE-MTPPE. *Biotherapy* 1993;7(1) 71-78.

[31] Gay B, Cardot JM, Schnell C, van Hoogevest P, Gygax D. Comparative pharmacokinetics of free muramyl tripeptide phosphatidyl ethanolamine (MTP-PE) and liposomal MTP-PE. *J Pharmaceut Sci* 1993;82(10) 997-1001.

[32] Dieter P, Hempel U, Malessa B, Fitzke E, Tran-Thi TA, MacLouf J, Creminon C, Kanaoka Y, Urade Y. Lipopolysaccharide- and liposome-encapsulated MTP-PE-induced formation of eicosanoids, nitric oxide and tumor necrosis factor-alpha in macrophages. In: Honn KV, Marnett LJ, Nigam S, et al. (eds.) *Eicosanoids And Other Bioactive Lipids in Cancer, Inflammation, and Radiation Injury 4* Book Series: *Ad Exper Med Biol* 1999;Vol469 443-448.

[33] Hsieh CS, Macatonia SE, Tripp CS, Wolf SF, O'Garra A, Murphy KM. Development of TH1 CD4+ T cells through IL-12 produced by *Listeria*-induced macrophages. *Science* 1993;260(5107) 547-549.

[34] ten Hagen TL, van Vianen W, Savelkoul HF, Heremans H, Buurman WA, Bakker-Woundenberg IA. Involvement of T cells in enhanced resistance to *Klebsiella pneumoniae* septicemia in mice treated with liposome-encapsulated muramyl tripeptide phosphatidylethanolamine or gamma interferon. *Infect Immun* 1998;66(5) 1962-1967.

[35] Souvannavong V, Brown S, Adam A. The synthetic immunomodulator muramyl dipeptide (MDP) can stimulate activated B cells. *Mole Immunol* 1988;25(4) 385-391.

[36] Graichen DAS, Gottstein B, Matsumoto J, Müller N, Zanotto PMA, Ayala FJ, Haag KL. 2007) Expression and diversity of *Echinococcus multilocularis* AgB genes in secondarily infected mice: evaluating the influence of T-cell immune selection on antigenic variation. *Gene* 2007;392(1-2) 98-105.

[37] Kizaki T, Kobayashi S, Ogasawara K, Day NK, Good RA, Onoe K. Immune suppression induced by protoscoleces of *Echinococcus multilocularis* in mice. Evidence for the presence of CD8dull suppressor cells in spleens of mice intraperitoneally infected with *E. multilocularis*. *J Immunol* 1991;147(5) 1659-1666.

[38] Gottstein B, Wunderlin E, Tanner I. *Echinococcus multilocularis*: parasite-specific humoral and cellular immune response subsets in mouse strains susceptible (AKR, C57B1/6J) or 'resistant' C57B1/10) to secondary alveolar echinococcosis. *Clin Exper Immunol* 1994;96(2) 245-252.

[39] Harraga S, Godot V, Bresson-Hadni S, Mantion G, Vuitton DA. Profile of cytokine production within the periparasitic granuloma in human alveolar echinococcosis. *Acta Tropica* 2003;85(2) 231-236.

[40] Hübner MP, Manfras BJ, Margos MC, Eiffler D, Hoffmann WH, Schulz-Key H, Kern P, Soboslay PT. *Echinococcus multilocularis* metacestodes modulate cellular cytokine and chemokine release by peripheral blood mononuclear cells in alveolar echinococcosis patients. *Clin Exper Immunol* 2006;145(2) 243-251.

[41] Sehra S, Chugh L, Gangal SV. Role of liposomes in selective proliferation of splenic lymphocytes. *Mole Cell Biochem* 1998;183(1-2) 133-139.

[42] Jenne L, Kilwinski J, Scheffold W, Kern P. IL-5 expressed by CD4+ lymphocytes from *Echinococcus multilocularis*-infected patients. *Clin Exper Immunol* 1997,109(1):90-97.

[43] Vuitton DA. Echinococcosis and allergy. *Clin Rev Aller Immunol* 2004;26(2) 93-104.

[44] Sher A, Fiorentino D, Caspar P, Pearce E, Mosmann T. Production of IL-10 by CD4+ T lymphocytes correlates with down-regulation of Th1 cytokine synthesis in helminth infection. *J Immunol* 1991;147(8) 2713-2716.

[45] Fiorentino D, Zlotnik A, Vieira P, Mosmann TR, Howard M, Moore KW, O'Garra A. IL-10 acts on the antigen-presenting cell to inhibit cytokine production by Th1 cells. *J Immunol* 1991;146(10) 3444-3451.

[46] Šoltýs J, Turčeková L, de Rycke PH. The effect *Echinococcus* hydatid cyst fluid and protoscoleces on mouse peritoneal macrophages and spleen lymphocytes. *Helminthologia* 1999;36(1) 25-30.

[47] Xu Z, Fidler IJ. The *in situ* activation of cytotoxic properties in murine Kupffer cells by the systemic administration of whole *Mycobacterium bovis* organisms or muramyl tripeptide. *Can Immunol Immunother* 1984;18(2) 118-122.

[48] Karpoff HM, Jarnagin W, Delman K, Fong Y. Regional muramyl tripeptide phosphatidylethanolamine administration enhances hepatic immune function and tumor surveillance. *Surgery* 2000;128(2) 213-218.

[49] Tanguay S, Bucana CD, Wilson MR, Fidler IJ, von Eschenbach AC, Killion JJ. *In vivo* modulation of macrophage tumoricidal activity by oral administration of the liposome-encapsulated macrophage activator CGP 19835A. *Canc Res* 1994;54(22) 5882-5888.

[50] Melissen PM, van Vianen W, Bidjai O, van Marion M, Bakker-Woundenberg IA. Free versus liposome-encapsulated muramyl tripeptide phosphatidylethanolamide (MTPPE) and interferon-γ (IFN-γ) in experimental infection with *Listeria* monocytogenes. *Biotherapy* 1993;6(2) 113-124.

[51] Adinolfi LE, Bonventre PF, Vanderpas M, Eppstein DA. Synergistic effect of glucantime and a liposome-encapsulated muramyl dipeptide analog in therapy of experimental visceral *Leishmania*sis. *Infect Immun* 1985;48(2) 409-416.

[52] Morikawa K, Takeda R, Yamazaki M, Mizuno D. Induction of tumoricidal activity of polymorphonuclear leukocytes by a linear beta-1,3-D-glucan and other immunomodulators in murine cells. *Can Res* 1985;45(4) 1496-1501.

[53] Sakurai T, Suzuki I, Kinoshita A, Oikawa S, Masuda A, Ohsawa M, Yadomae T. Effect of intraperitoneally administered beta-1,3-glucan, SSG, obtained from *Sclerotinia sclerotiorum* IFO 9395 on the functions of murine alveolar macrophages. *Chem Pharmaceut Bull* (Tokyo) 1991;39(1) 214-217.

[54] Williams DL, Pretus HA, Mcnamee RB, Jones EL, Ensley HE, Browder IW, Di Luzio NR. Development, physicochemical characterization and preclinical efficacy evaluation of a water soluble glucan sulfate derived from *Saccharomyces cerevisiae*. *Immunopharmacology* 1991;22(3) 139-155.

[55] Větvička V, Thornton BP, Ross GD. Soluble beta-glucan polysaccharide binding to the lectin site of neutrophil or natural killer cell complement receptor type 3 (CD11b/CD18) generates a primed state of the receptor capable of mediating cytotoxicity of iC3b-opsonized target cells. *J Clin Invest* 1996;98(1) 50-61.

[56] Sakurai T; Kaise T; Yadomae T; Matsubara C. Different role of serum components and cytokines on alveolar macrophage activation by soluble fungal (1-->3)-beta-D-glucan. Eur J Pharmacol 1997;334(2-3) 255-263.

[57] Kidd PM. The use of mushroom glucans and proteoglycans in cancer treatment. Alt Med Rev 2000;5(1) 4-27.

[58] Gallin EK, Green SW, Patchen ML. Comparative effects of particulate and soluble glucan on macrophages of C3H/HeN and C3H/HeJ mice. *Int J Immunopharmacol* 1992;14(2) 173-183.

[59] Rasmussen LT, Seljelid R. Novel immunomodulators with pronounced *in vivo* effects caused by stimulation of cytokine release. *J Cell Biochem* 1991;46(1) 60-68.

[60] Šoltýs J, Borošková Z, Dubinský P, Tomašovičová O, Auer H, Aspock H. Effect of glucan immunomodulator on the immune response and larval burdens in mice with experimental toxocarosis. *Appl Parasitol* 1996;37(3) 161-167.

[61] Ditteová G, Velebný S, Hrčková G. Modulation of liver fibrosis and pathophysiological changes in mice infected with *Mesocestoides corti* (*M. vogae*) after administration of glucan and liposomized glucan in combination with vitamin C. *J Helminthol* 2003;77(3) 219-226.

[62] James K. Interactions between cytokines and alpha 2-macroglobulin. *Immunol Today* 1990;11(12) 163-166.

[63] Benková M, Borošková Z, Šoltýs J. Immunostimulatory effects of certain substances in experimental ascaridiasis in pigs. *Vet Med* (Prague) 1991;36(12) 717-724.

[64] Wellinghausen N, Kirchner H, Rink L. The immunobiology of zinc. *Immunol Today* 1997;18(11) 519-521.

[65] Rink L, Kirchner H. Zinc-altered immune function and cytokine production. *J Nutr* 2000;130(Suppl) S1407-S1411.

[66] Chowdhury N, Singh R. Distribution of zinc in parasitic helminths. *J Helminthol* 1989; 63(2) 149-152.

[67] Wellinghausen N, Jochle W, Reuter S, Flegel WA, Grunert A, Kern P. Zinc status in patients with alveolar echinococcosis is related to disease progression. *Paras Immunol* 1999;21(5) 237-241.

[68] Borošková Z, Dvorožňáková E, Ševčíková Z. Cellular immune reactions of mice with alveolar echinococcosis after albendazole therapy. *Helminthologia* 2003;40(4) 187-194.

[69] Baum MK, Shor-Posner G, Campa A. Zinc status in human immunodeficiency virus infection. *J Nutr* 2000;130(Suppl) S1421-S1423.

[70] Dvorožňáková E, Hrčková G, Borošková Z, Velebný S, Dubinský P. Effect of treatment with free and liposomized albendazole on selected immunological parameters and cyst growth in mice infected with *Echinococcus multilocularis*. *Parasitol Int* 2004;53(4) 315-325.

[71] Pérez-Serrano J, Denegri G, Casado N, Rodríguez-Caabeiro F. *In vivo* effect of oral albendazole and albendazole sulphoxide on development of secondary echinococcosis in mice. *Int J Parasitol* 1997;27(11) 1341-1345.

[72] Abbas AK, Murphy KM, Sher A. Functional diversity of helper T lymphocytes. *Nature* 1996;383, 787-793.

[73] Šoltýs J, Benková M, Borošková Z. Immunorestorative effect of glucan immunomodulator on guinea pigs with experimental ascariosis. *Vet Immunol Immunopathol* 1994;42(3-4) 379-388.

[74] Borošková Z, Dvorožňáková E, Dubinský P, Velebný S, Tomašovičová O, Machnická B. Effect of free and liposomised albendazole on the immune responses in healthy and *Toxocara canis* infected mice. *Vet Med* (Prague) 1998;43(10) 293-300.

[75] Dvorožňáková E, Akao A. 2000: Th1 and Th2 response after glucan immunomodulation in murine larval toxocarosis. Reports of Czech Parasitological Society, Prague, Czech Republic, 2000. S8:53.

[76] Bach JF. The multi-faceted zinc dependency of the immune system. *Immunol Today* 1981;2(11) 225-227.

[77] Crea A, Guérin V, Ortega F, Hartemann P. Zinc and the immune system. *Annales de Médecine Interne* 1990;141(5) 447-451.

[78] Kizaki T, Ishige M, Kobayashi S, Bingyan W, Kumagai M, Day NK, Good RA, Onoe K. Suppression of T-cell proliferation by CD8+ T cells induced in the presence of protoscoleces of *Echinococcus multilocularis in vitro*. *Infect Immun* 1993;61(2) 525-533.

[79] Bresson-Hadni S, Liance M, Meyer JP, Houin R, Bresson JL, Vuitton DA. Cellular immunity in experimental *Echinococcus multilocularis* infection. II. Sequential and comparative phenotypic study of the periparasitic mononuclear cells in resistant and sensitive mice. *Clin Exper Immunol* 1990;82(2) 378-383.

[80] Dowd PS, Kelleher J, Guillou PJ. T-lymphocyte subsets and interleukin-2 production in zinc-deficient rats. *Brit J Nutr* 1986;55(1) 59-69.

[81] Vuitton DA. New trends in the treatment of echinococcosis. *Helminthologia* 1999;36(3) 167-170.

[82] Gottstein B. Molecular and immunological diagnosis of echinococcosis. *Clin Microbiol Rev* 1992;5(3) 248-261.

[83] Hrčková G, Velebný S, Daxnerová Z, Solár P. Praziquantel and liposomized glucan-treatment modulated liver fibrogenesis and mastocytosis in mice infected with *Mesocestoides vogae* (*M. corti*, Cestoda) tetrathyridia. *Parasitology* 2006;132(4) 581-594.

[84] Song YL, Ford JW, Gordon D, Shanley CJ. Regulation of lysyl oxidase by interferon-gamma in rat aortic smooth muscle cells. *Arterioscler, Thromb Vasc Biol* 2000;20(4) 982-988.

[85] Tang JT, Fang JY, Gu WQ, Li EL. T cell immune response is correlated with fibrosis and inflammatory activity in hepatitis B cirrhotics. *World J Gastroenterol* 2006;12(19) 3015-3019.

[86] Casini A, Ceni E, Salzano R, Biondi P, Parola M, Galli A, Foschi M, Caligiuri A, Pinzani M, Surrenti C. Neutrophil-derived superoxide anion induces lipid peroxidation and stimulates collagen synthesis in human hepatic stellate cells: Role of nitric oxide. *Hepatology* 1997;25(2) 361-367.

[87] Guerret S, Vuitton DA, Liance M, Pater C, Carbillet JP. *Echinococcus multilocularis*: relationship between susceptibility/resistance and liver fibrogenesis in experimental mice. *Parasitol Res* 1998;84(8) 657-667.

[88] Miguet JP, Bresson-Hadni S, Vuitton DA. Echinococcosis of the liver. In: Mcintyre N, Benhamou JP, Bicher J, Rizetto M, Rodes J. (eds.) *Oxford Textbook of Clinical Hepatology*. Oxford University, Oxford, 1991; 721-730.

[89] Anttinen H, Ryhanen L, Puistola U, Arranto A, Oikarinen A. Decrease in liver collagen accumulation in carbon tetrachloride-injured and normal growing rats upon administration of zinc. *Gastroenterology* 1984;86(3) 532-539.

[90] Kojima-Yuasa A, Ohkita T, Yukami K, Ichikawa H, Takami N, Nakatani T, Opare Kennedy D, Nishiguchi S, Matsui-Yuasa I. Involvement of intracellular glutathione in zinc deficiency-induced activation of hepatic stellate cells. *Chemico-Biologic Interact* 2003; 146(1) 89-99.

[91] Kojima-Yuasa A, Umeda K, Ohkita T, Opare Kennedy D, Nishiguchi S, Matsui-Yuasa I. Role of reactive oxygen species in zinc deficiency-induced hepatic stellate cell activation. *Free Rad Biol Med* 2005;39(5) 631-640.

[92] Pineda B, Estrada-Parra S, Pedraza-Medina B, Rodriguez-Ropon A, Pérez R., Arrieta O. Interstitial transfer factor as adjuvant immunotherapy for experimental glioma. *J Exp Clin Can Res* 2005;24(4) 575-583.

[93] Alvarez-Thull L, Kirkpatrick Ch. Profiles of cytokine production in recipients of transfer factors. *Biotherapy* 1996;9(1-3) 55-59.

[94] Foschi FG, Marsigli L, Bernardi M, Salvi F, Mascalchi M, Gasbarrini G, Stefanini G. Acute multifocal cerebral white matter lesions during transfer factor therapy. *J Neurol Neurosurg Psychiat* 2000;68(1) 114-115.

[95] Holeva OH, Paster IP, Liubchenko TA, Paster IeU, Kholodna LS, Zamotaierva HA, Hrodzinskyi DM. The immune reactivity transfer factor as a modulator of lymphocyte functional activity in rats. *Fiziolohichnyĭ zhurnal* 2000;46(4) 58-65.

[96] Vacek A, Hofer M, Barnet K, Čech K, Pekárek J, Schneiderová H. Positive effects of dialyzable leukocyte extract (DLE) on recovery of mouse haemopoiesis suppressed by ionizing radiation and on proliferation of haemopoietic progenitor cells in vitro. *Int J Immunopharmacol* 2000;22(8) 623-634.

[97] Foschi FG, Marsigli L, Bernardi M, Salvi F, Mascalchi M, Gasbarrini G, Stefanini G. Acute multifocal cerebral white matter lesions during transfer factor therapy. *J Neurol Neurosurg Psychiat* 2000;68(1) 114-115.

[98] Kirkpatrick CH. Activities and characteristics of transfer factors. *Biotherapy* 1996;9(1-3) 13-16.

[99] Fabre RA, Pérez TM, Aguilar LD, Rangel MJ, Estrada-Garcia I, Hernández-Pando R, Estrada Parra S. Transfer factors as immunotherapy and supplement of chemotherapy in experimental pulmonary tuberculosis. *Clin Exp Immunol* 2004;136(2) 215-223.

[100] Dai WJ, Waldvogel A, Siles-Lucas M, Gottstein B. *Echinococcus multilocularis* proliferation in mice and respective parasite 14-3-3 gene expression is mainly controlled by an alpha beta(+) CD4(+) T-cell-mediated immune response. *Immunology* 2004;112(3) 481-488.

[101] Sailer M, Soelder B, Allerberger F, Zaknun D, Feichtinger H, Gottstein B. Alveolar echinococcosis of the liver in a six-year-old girl with acquired immunodeficiency syndrome. *J Pediat* 1997;130(2) 320-323.

[102] Zingg W, Renner-Schneiter EC, Pauli-Magnus C, Renner EL, vanOverbeck J, Schlapfer E, Weber M, Weber R, Opravil M, Gottstein B, Speck RF. Swiss HIV Cohort Study. Alveolar echinococcosis of the liver in an adult with human immunodeficiency virus type-1 infection. *Infection* 2004;32(5) 299-302.

[103] Estrada-Parra S, Nagaya A, Serrano E, Rodriguez O, Santamaria V, Ondarza R, Chavez R, Correa B. Comparative study of transfer factor and acyclovir in the treatment of herpes zoster. *Int J Immunopharmacol* 1998;20(10) 521-535.

[104] Mosmann TR, Fong TAT. Specific assays for cytokine production by T cells. *J Immunol Meth* 1989;116(2) 151-158.

[105] Carter LL, Swain SL. Single cell analyses of cytokine production. *Curr Opin Immunol* 1997;9(2) 177-182.

[106] Dvorožňáková E, Porubcová J, Ševčíková Z. Immune response of mice with alveolar echinococcosis to therapy with transfer factor, alone and in combination with albendazole. Parasitol Res 2009; 105(4):1067-1076.

[107] Karhumaki E, Marnela KM, Krohn K. Chromatographic and enzymatic effects on transfer factor-like activity from human leukocytes and porcine spleen dialysate. Int J Biochem 1988;20(10) 1067-1072.

IntechOpen