

# The influence of immunosuppression on apoptosis and necrosis during experimental trichinellosis in mice

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## Abstract

A study was carried out to determine the influence of immunosuppressive drug on apoptosis and necrosis lymphocytes in the spleen and mesenteric lymph nodes of mice experimentally infected with *Trichinella spiralis*. CFW mice were infected with 200 larvae of *Trichinella spiralis*. Dexametason was injected intra-peritoneally, at a dose of 25 mg/kg body weight, ten times at 24 hour intervals (between 1-5 and 18-22 day of infection). The lymphocytes obtained from the spleen and mesenteric lymph nodes on 7, 14, 21, 28, 35, 42 and 60 day after infection were incubated using Annexin-V-Fluos Staining Kit. Results of the study demonstrated that dexametason increased the percentage of apoptotic lymphocytes in the spleen and mesenteric lymph nodes from 14 day after infection in mice infected with *T. spiralis* and decreased the percentage of the necrotic lymphocytes in both examined lymphatic organs.

**Key words:** *T. spiralis*, apoptosis, necrosis, immunosuppression, dexametason, mice.

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## Introduction

Trichinellosis is a zoonotic infection caused by the parasite nematodes belonging to the genus *Trichinella*. Pathological changes during infection are closely connected with the development of two generations of *Trichinella spiralis* in the intestine and in the muscle tissue. In the intestinal phase of trichinellosis a protective response is induced in the mucosal immunological system. Inflammatory infiltrations developing in the intestine after infection with *T. spiralis* have features of allergic reactions [1]. T-helper (Th) 2-type lymphocytes have a significant participation in modulating immunological reaction. Both Th1 cells as well as Th2 cell type response is in lymphatic organs but in the spleen Th1 lymphocytes are more active. However, the Th2 are more active in mesenteric lymph nodes [2, 3]. Although they do not directly participate in the elimination of the parasite, they determine the type, the intensity and the speed of the immune response via secreted cytokines [4, 5].

Expulsion of *T. spiralis* from intestine is associated with synthesis of specific IgE, mastocytosis prolonged under IL-4 and eosinophilia induced by IL-5. The intestinal muscle hypercontractility and goblet cells hyperplasia appear as a result of infection-induced immune-mediated changes in intestinal physiology which is associated with the worm expulsion [6]. Parasites developed a lot paths in order to avoid the immune response of the host and one of them is arousing the apoptosis of effector cells [7]. It was demonstrated that excretory/secretory antigens of helminths trigger the apoptosis in subpopulation of lymphocytes in lymphatic organs and can contribute to existence the immunosuppression and finally to the longer survival in the body of the host. It is also not possible to exclude their participation in triggering necrosis which leads to rapid disintegration of a cell with all consequences of this process. The apoptosis is regarded as the natural death of cells which ensures the homeostasis, so this kind of death is more

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favourable. *T. spiralis* invasion induces apoptosis of some inflammatory infiltration cells in the lamina propria of intestinal mucosa and also in muscle tissue [8]. In mice infected with *T. spiralis* and subjected to actions of immunostimulators, an increase of percentage of apoptotic lymphocytes and decrease of percentage of necrotic lymphocytes in spleen and mesenteric lymph node lymphocytes was observed. However, no significant impact of these processes was demonstrated on the number of parasites in intestines and muscles [9]. The aim of the current study was to detect apoptosis and necrosis among lymphocytes of spleen and mesenteric lymph nodes in mice infected with *T. spiralis* under condition of immunosuppression.

Glucocorticoids are used in the treatment of early stage of trichinellosis for the reason that they suppress the symptoms of immediate-type hypersensitivity. They show the anti-inflammatory and immunosuppressive activity through inhibition the activity of immune cells (including lymphocytes) and through inducing the changes in population together with decreasing cytokine synthesis [10, 11].

## Material and methods

The study was carried out on 126 CFW inbred mice. The mice were about three months old, and weighed about 20 grams. 84 mice were orally infected with 200 *T. spiralis* larvae, T1 (ISS1820 Poland). The larvae used in the infection were recovered from the muscle tissue of mice infected 2-3 months earlier by digestion with 1% pepsin/HCl solution for 4 h at 37°C.

Dexametason (Dexametason Jelfa, series 011005) was administered intra-peritoneally, at a dose of 25 mg/kg body weight, ten times at 24 hour intervals. The first set of five doses of dexametason (DEX) was administered starting on the 1<sup>st</sup> day of infection, and the second set of five doses was administered starting on the 18<sup>th</sup> day of infection.

The mice were divided into three experimental groups:

- Control: mice infected with *T. spiralis*,
- DEX<sup>+</sup>: mice infected with *T. spiralis* and treated with DEX,
- DEX: uninfected mice treated with DEX.

All mice were maintained under identical conditions for the duration of the experiment.

The study protocol was approved by the Local Ethics Committee in Wroclaw, Poland (No 29/2008).

## Isolation of lymphocytes

Six mice from each group were sacrificed using halothane (Narcotan, Leciva) on days 7, 14, 21, 28, 35, 42 and 60 after infection. The spleen and mesenteric lymph nodes were removed aseptically, transferred to sterile phospho-buffered saline (PBS) at 4°C and pressed through a nylon cell strainer to produce a single cell suspension. Lymphocytes were isolated by Ficoll 400/Uropolinum 75% density – gradient in centrifugation for 15 min at 4°C [12].

Cells collected from the interphase were washed twice with PBS with 1% bovine serum albumin (BSA; Sigma) at 4°C. For spleen samples, this fraction was treated with 0.84% NH<sub>4</sub>Cl in order to disrupt erythrocytes. After a second wash, the cells were adjusted to 1 × 10<sup>6</sup> cell/ml. Cell viability was determined by staining with trypan blue. The proportion of viable lymphocytes was greater than 96%.

## Measurement of apoptosis and necrosis

Annexin V-Fluos Staining Kit (Roche-Diagnostics) was used to detect apoptotic and necrotic cells by flow cytometry. The method depends on the selective binding of Annexin-V to phosphatidylserine residues on the cell surface of apoptotic cells, and the staining by propidium iodide of cells that had lost membrane integrity. The staining procedure was performed in accordance with the procedure recommended by the manufacturer. Cells were analyzed using a FACS Calibur flow cytometer (Becton-Dickinson Biosciences). Data were analyzed using the CellQuest 3.1f software package.

## Statistical analysis

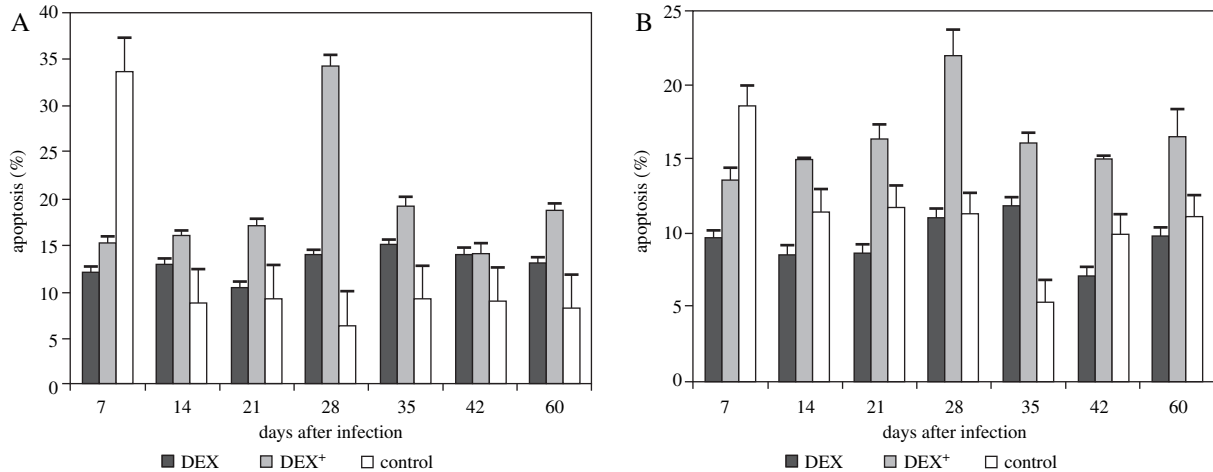
The results were analyzed by two-way analysis of variance (ANOVA) with a Tukey test to determine the significance for multiple comparison. *P*-value < 0.05 were considered statistically significant. Numerical calculations were carried out using the STATISTICA ver. 9.0 software package.

## Results

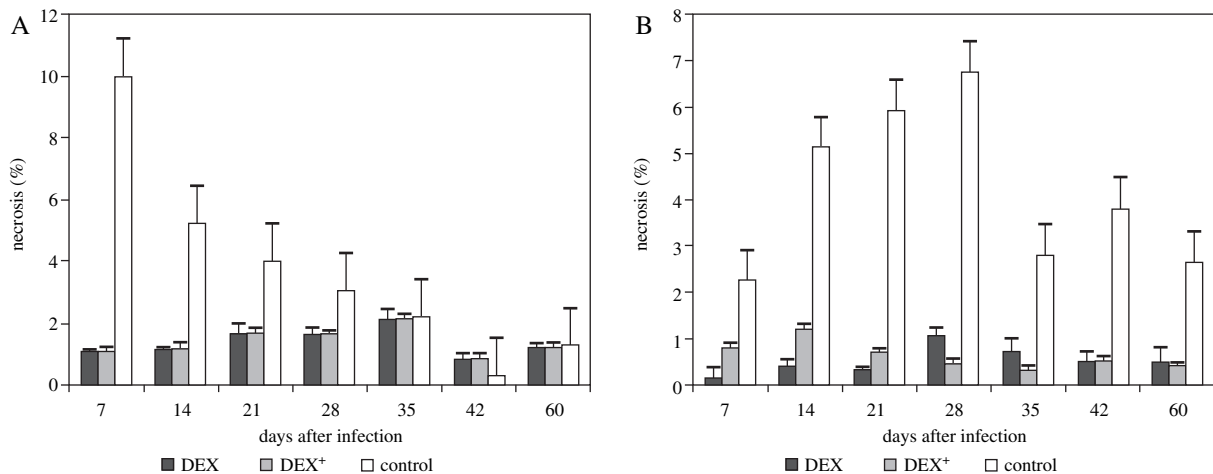
### Apoptosis

The percentage of apoptotic lymphocytes in the group of mice infected with *T. spiralis* (Control group) was significantly higher at 7 day after infection (*dai*) both in the spleen (37.5%) and in mesenteric lymph nodes (32.8%). The percentage of apoptotic lymphocytes in the spleen in group DEX<sup>+</sup>, was significantly higher than in the Control group from 14 *dai* (16%, *p* < 0.05) with a maximum of 34.3% at 28 *dai* (*p* < 0.001). For all of the other sampling times except 42 *dai*, the percentage of apoptotic lymphocytes was significantly higher with an average of about 18%. In the spleen of mice uninfected and treated with dexametason (group DEX) the dynamics of the population of apoptotic lymphocytes was similar to the dynamics of the lymphocyte population observed in group DEX<sup>+</sup>, but recorded values was lower (Fig. 1A).

The percentage of apoptotic lymphocytes in mesenteric lymph nodes in the group DEX<sup>+</sup> was significantly higher than in the Control group from 14 *dai* with a maximum of 22% at 28 *dai* (*p* < 0.001). In the mesenteric lymph nodes in group DEX they were less apoptotic cells than in Control group and group DEX<sup>+</sup> with exception of 35 *dai* (Fig. 1B).



**Fig. 1.** Percentage of apoptotic lymphocytes: A – in the spleen, B – in mesenteric lymph nodes



**Fig. 2.** Percentage of necrotic lymphocytes: A – in the spleen, B – in mesenteric lymph nodes

**Necrosis**

The highest percentage of necrotic lymphocytes was observed in the spleen in Control group at 7 dai (36.1%) and at 28 dai in mesenteric lymph nodes (6.8%) (Fig. 2A, B). The percentage of necrotic lymphocytes in the spleen in group DEX+ was significantly lower than in the Control group from 7 to 28 dai. The percentage of necrotic lymphocytes in the spleen in group DEX was approximately the same as in group DEX+ (Fig. 2A).

The percentage of necrotic lymphocytes in mesenteric lymph nodes in group DEX+ was for all sampling times significantly lower than in the Control group. In the mesenteric lymph nodes there were no significant differences in the necrotic lymphocyte between group DEX+ and group DEX (Fig. 2B).

**Discussion**

In the present study, the lymphocyte apoptosis and necrosis in lymphatic organs of mice infected with *T. spiralis* and treated with dexametasone was evaluated in order to asses lymphocyte response during immunosuppression.

In contrast to necrosis, which is a passive and genetically uncontrolled process followed by an inflammatory reaction of surrounding tissue, apoptosis is an active process, which balances the cell proliferation [13]. The level of parasite infection depends on intensity of inflammatory reaction, therefore nematodes inhibits innate and adaptive pro-inflammatory immune response. Cytokines regulate and mediate the inflammatory response, therefore parasites try to modify the level and composition of pro- and anti-inflammatory cytokines [14].

The studies conducted over last few years demonstrated that parasites can induce and regulate apoptosis of T cells contribute to the suppression of the immune response [15]. Parasites may trigger apoptosis directly by secretion of active mediators or indirectly by producing an inflammatory milieu that promotes death of subpopulations of T lymphocytes [16-18].

Dexametason is a glucocorticoid and acts as an anti-inflammatory agent and immunosuppressant. It, like other drugs in that class, reduces the permeability of capillaries and inhibits migration of cellular elements to surrounding tissues preventing exudations and extravasations [10, 19]. It was demonstrated that single, small doses of dexametason administered to mice before infection with *T. spiralis* significantly extend the duration of the intestinal phase as well as increases infestation in muscles. Moreover, it reduces elimination of the parasites from the intestine and inhibits the response of TCD4<sup>+</sup> lymphocytes in the intestine [20]. Dexametason suppressed T CD4<sup>+</sup>, T CD8<sup>+</sup> and B lymphocytes in the lamina propria of the intestinal mucosa, and T CD4<sup>+</sup> lymphocytes in inflammatory infiltrates in the muscle tissue [21]. In the previous studies conducted in mice infected with *T. spiralis* we have found that treatment with dexametason increased the proportion of apoptotic lymphocytes in muscle tissue only at 28 days after infection, and significantly increased the proportion of necrotic lymphocytes in muscle at 21 days after infection. Moreover, the number of larvae in the muscle tissue was slightly higher in mice treated with dexametason than in the control group [22]. In the present study in the spleen and in mesenteric lymph nodes in mice infected with *T. spiralis* and treated with dexametason an increase in number of apoptotic cells was observed (Fig. 1A, B). The percentage of necrotic lymphocytes in both of examined lymphatic organs was weaker and less clear (Fig. 2A, B).

In earlier experiments, the stimulator of T cells, thymus factor X (TFX – Jelfa) was used to modulate the immune response of mice during the experimental trichinellosis [9]. Although the percentage of apoptotic lymphocytes in the spleen, mesenteric lymph nodes and muscle tissue was higher in the treated mice, this had no effect on parasite load in intestine and on the survival of parasite larvae in muscle tissue. Moreover, in mice treated with TFX, inflammatory infiltrates in muscle tissue were more extensive, and the number of inflammatory cells inside the cysts containing the larvae was higher.

Current results confirm the observation of a previous study that the extent of inflammatory exudates may be more responsible for parasite survival than percentage of lymphocytes undergoing apoptosis. Dexametason, as it know, inhibits the activity of the cells of the immune system, including lymphocytes, by changing the composition of the cell subpopulation and by affecting the process of apoptosis.

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