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ORIGINAL RESEARCH ARTICLE

Plasmagenesis dynamics in experimental *Trichinella* infection

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Abstract. The aim of this work was to evaluate the plasmacytic reaction dynamics during *Trichinella* infection in rats. Twenty outbred white rats 5-6 months old weighting 230-250 g were infected *per os* with 10 *Trichinella spiralis* muscle larvae (L_1) per g of weight. Five uninfected rats with the same age and weight constituted the control group. After infection, rats were euthanized at days 4, 8, 26 and 38 p.i. The spleens were collected and stamps on microscope slides were fixed in methanol and stained with Pappenheim. Plasma lineage cells were counted in 50 optical microscope fields and plasmacytic reaction was evaluated. Three types of plasma cells were observed: plasmablasts, immature plasma cells and mature plasma cells. Mitotic activity (the percentage of proliferating cells from the total cells count) and chromosome aberrations were evaluated in spleen and bone marrow cell populations after carmine acetate staining. Plasmacytic reaction directly depended on the *T. spiralis* development stage. High levels of blasts, mitotic and plasma cells were observed in cell populations at early intestinal (4-5 days p.i.) and muscle stages (30 days p.i.). On the contrary, during the migration stage of the parasites a decrease of plasmacytic reaction, lower than that of the control group, along with a decrease of proliferation and mitotic activities were observed.

At 38-40 days p.i., a normalization of plasmacytic reaction and mitotic activity happened. At all stages of the parasite development, the numbers of cells with chromosome aberrations remained similar. The degree of immune maturation of spleen and bone marrow cells directly depended on *T. spiralis* development stage. Stimulating effect of the parasites was pronounced at early intestinal and early muscle stage, whereas suppressive effect of this parasite was evident at migrating stage.

Keywords: *Trichinella*; Plasma cells; Experimental infection; Plasmagenesis.

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Dinamica plasmagenezei în infecţia experimentală cu *Trichinella*

Rezumat. Scopul acestei lucrări a fost evaluarea dinamicii reacţiei plasmocitare în timpul infecţiei cu *Trichinella* la şobolani. Douăzeci de şobolani albi non-consangvini, în vârstă de 5-6 luni, cu o greutate de 230-250 g, au fost infectaţi *per os* cu 10 larve musculare (L_1) de *Trichinella spiralis* per g de greutate. Cinci şobolani neinfectaţi cu aceeaşi vârstă şi greutate au constituit grupul de control. După infecţie, şobolanii au fost eutanasiaţi în zilele 4, 8, 26 şi 38 p.i. A fost colectată splina şi amprentele pe lamelele microscopului au fost fixate în metanol şi colorate cu Pappenheim. Celulele de linie plasmatică au fost numărate în 50 de câmpuri de microscop optic şi a fost evaluată reacţia plasmocitară. Au fost observate trei tipuri de celule plasmatice: plasmablaste, celule plasmatice imature şi celule plasmatice mature. Activitatea mitotică (procentul de celule proliferante din numărul total de celule) şi aberaţiile cromozomiale au fost evaluate în populaţiile de celule de splină şi măduvă osoasă, după colorarea cu acetat de carmin. Reacţia plasmocitară a depins direct de stadiul de dezvoltare al *T. spiralis.* La populaţiile de celule au fost observate niveluri ridicate de blaşti, celule mitotice şi plasmatice, la stadiile intestinale timpurii (4-5 zile p.i.) şi musculare (30 de zile p.i.). Dimpotrivă, în etapa de migraţie a paraziţilor s-a observat o scădere a reacţiei plasmocitare, mai mică decât în cazul grupului de control, împreună cu o scădere a proliferării şi a activităţilor mitotice.
La 38-40 zile p.i., a avut loc o normalizare a reacţiei plasmocitare şi a activităţii mitotice. În toate etapele dezvoltării parazitului, numărul de celule cu aberaţii cromozomiale a rămas similar. Gradul de maturare imună a splinei şi celulelor măduvei osoase a depins direct de stadiul de dezvoltare al *T. spiralis.* Efectul stimulant al paraziţilor a fost pronunţat la stadiul muscular precoce şi intestinal, în timp ce efectul supresor al acestui parazit a fost evident în stadiul de migrare.

Cuvinte cheie: *Trichinella;* Celule plasmatice; Infecţie experimentală; Plasmageneză.

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Introduction

Trichinellosis is a human zoonotic parasitic disease caused by nematodes belonging to *Trichinella* genus. Humans and animals acquire the infection through the consumption of raw or undercooked infected meat from domestic or wild animals. The infection is widely distributed and is a serious danger for humans and animals, causing a great damage to economy and to society as whole (Murrell and Pozio, 2011; Pozio et al., 2009). Most of the human infections are caused by *Trichinella spiralis* (Murrell and Pozio, 2011; Gottstein et al., 2009; Pozio and Murrell, 2006). Acute and chronical helminthiasis lead to an appropriate development of immune reactions in the host organism, and these play a leading part in formation of a pathological process (Gottstein et al., 2009; Givanyan et al., 2011; Dvorožňáková et al., 2013). Many experimental studies allowed to conclude that a morphological substrate for immune processes is a lymph tissue, richly represented by spleen and bone marrow (Dvorožňáková et al., 2012; Wernersson and Pejler, 2014). A spleen's role is to provide immune competent cells which are then transformed into antibody producing cells (Wernersson and Pejler, 2014; Shapiro-Shelef and Calame, 2005). Various immune reactions are based on processes of cell transformations in these tissue systems (Shapiro-Shelef and Calame,

2005). Generally, literature data show the important part that immunity plays in the parasite-host relationships and this is particularly evident during *Trichinella* infection (Maizels et al., 2004; Oltean et al., 2012; Boyett and Hsieh, 2014). It's well known that macrophages, lymph cells and plasma cells play immediate part in immune processes in the host. Macrophages capture and digest the antigen, transfer information to lymphocytes and stimulate them. As result, lymphocytes undergo differentiation and proliferation processes. Starting mitosis provides for the development of immune active cells – plasma cells which secrete circulating antibodies. Immune competent cells here are small lymphocytes which are able, after antigen stimulation, to be transformed into immune effector cells synthesizing antibodies. *Trichinella* larvae migrating inside the host are able to interrupt processes of cell proliferation inside him (Maizels et al., 2004). Literature contains some data on studies of plasma cell reaction in immune competent spleen cells under helminthiasis effects (Movsesyan et al., 2009), as well as on changes in mitotic activity in bone marrow and spleen cells in cases of several parasitic diseases (Petrosyan et al., 2012; Odoevskaya et al., 2008a).

Recently our team of Russian, Armenian and Bulgarian parasitologists had performed series of studies dedicated to important aspects of trichinellosis as: characteristics of *Trichinella* adaptation to their hosts; parasite-host relationships; electrophoretic studies of six *Trichinella* species *(Trichinella spiralis, T. pseudospiralis, T. nativa, T. britovi, T. nelsoni, T. murrelli),* all of them aimed at discovering specific features and defining eco-biological peculiarities within *Trichinella* genus (Petrosyan et al., 2012; Odoevskaya et al., 2008a; Odoevskaya et al., 2008b; Odoevskaya et al., 2010; Dilcheva et al., 2013; Odoyevskaya et al., 2014).

Important aspect of *Trichinella* infection is triggering the host immunity. Therefore, the aim of this work was to evaluate the plasmacytic reaction dynamics in white rats experimentally infected with *Trichinella spiralis.*

Material and methods

Experimental infection

Twenty outbred white rats 5-6 months old weighting 230-250 g were infected *per os* with 10 *T. spiralis* muscle larvae per g of weight. Larvae viability was determined on the basis their motility. Five uninfected rats with the same age and weight constituted the control group.

Plasmacytic reaction studies

Plasmacytic reaction was studied in the spleen using the stamp method proposed by Gurvich and Schumakova (1957). To this end, rats were euthanized at 4th, 8th, 26th and 38th days after infection. The spleens were collected and their stamps on a microscope slide were made. After methanol fixing (10 min), stamps were stained with Pappenheim and plasma lineage cells counted in 50 microscope vision fields. In counting, nomenclature of immune competent cells proposed in 1960 at the Prague Symposium and furtherly redefined (Pokrovskaya et al., 1965; Movsesyan et al., 2009) was used.

Three cell types among plasma lineage elements, depending on the differentiation stage, were observed (figure 1). It is important to take into account numbers of plasmablasts, immature and mature plasma cells to differentiate cells with various functional activity and immunological importance. Plasmablasts are the least mature form; their cytoplasm is highly basophilic due to intense protein synthesis, they play an important role in immunogenesis processes. Immature plasma cells are significantly smaller than plasmablasts. They undergo active mitosis and propagation. They present a clear basophilia of their cytoplasm as result of the active synthesis of specific antibodies. Mature plasma cells have smaller size and nucleus-cytoplasm ratio. Their cytoplasm is strongly basophilic and bright blue in color. According to some authors (Shapiro-Shelef and Calame, 2005; Petrosyan et al., 2012; Ford and Hammerton, 1956; Calame, 2001) mature plasma cells are less active as

antibody producers than plasmablasts and immature plasma cells; they do not propagate mitotically and are degrading elements. The number of each cell type was expressed as the mean ± SD of each group of rat. Each group was compared with the control non infected group, a level of $P < 0.01$ was considered as statistically significant by Student *t*-test.

Mitotic activities studies

Mitotic activity and chromosome aberrations were determined in cell populations from the rats' spleen and bone marrow cells. Cells were fixed in acetate alcohol (1:3) then stained with acetate carmine. Further, mitotic activity and numbers of cells with chromosome aberrations were evaluated using the anaphase-telophase method (Ford and Hammerton, 1956).

Mitotic activity was defined as percent ratio of proliferating cells to total number of cell counted. Therefore, among 100-150 cells found to be at late anaphase and early telophase, cells with chromosome aberrations (bridges, acentric fragments, bridges with fragments) were counted (figure 2).

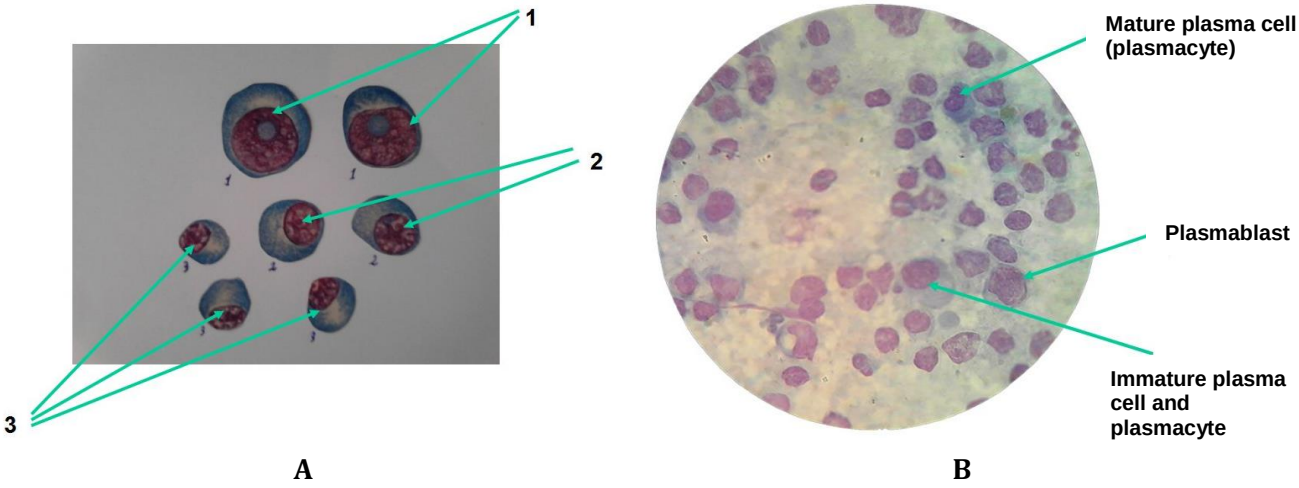

Figure 1. A, Cells of plasma lineage: 1 – plasmablasts; 2 – immature plasma cells; 3 – mature plasma cells. B, Plasma lineage cells found at early intestinal stage of infection at magnification ×630.

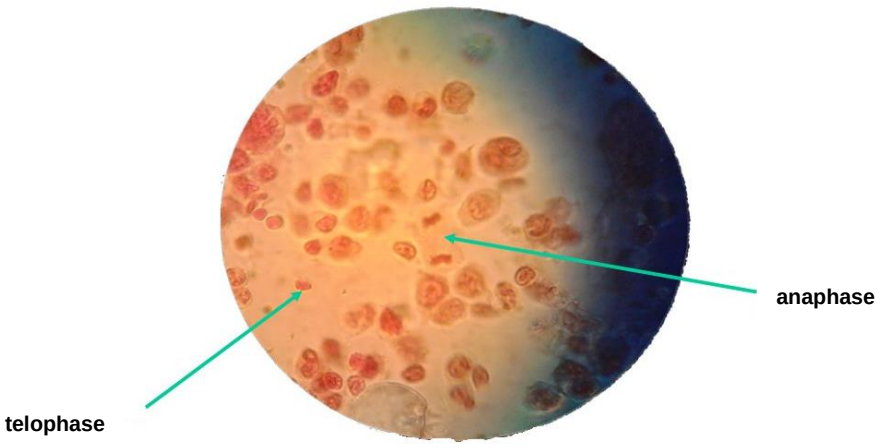

Figure 2. Mitosis in marrow cells (metaphase, late anaphase, at magnification ×630).

Results and discussion

Changes in composition of plasma lineage cells show that during the period of the parasite's early intestinal stage (4-5 days p.i.) a pronounced growth of numbers of plasma cells in spleen occurs, mainly through immature plasma cells (figure 1), reaching 40.0 ± 0.8 cells whereas 17 ± 0,9 cells were counted in the control group ($P < 0.05$; figure 3). The number of mature plasma cells was the same in the experimental and control groups. Regarding proliferation activity, spleen and marrow cells grows synchronous with dynamics of plasmacytic reaction, i.e. the mitotic activity was 1.3 ± 0.12% in spleen (0.7 ± 1.2% in control) and 2.1 ± 0.04% in marrow (1.8±0.07% in control) (figures 2 and 4).

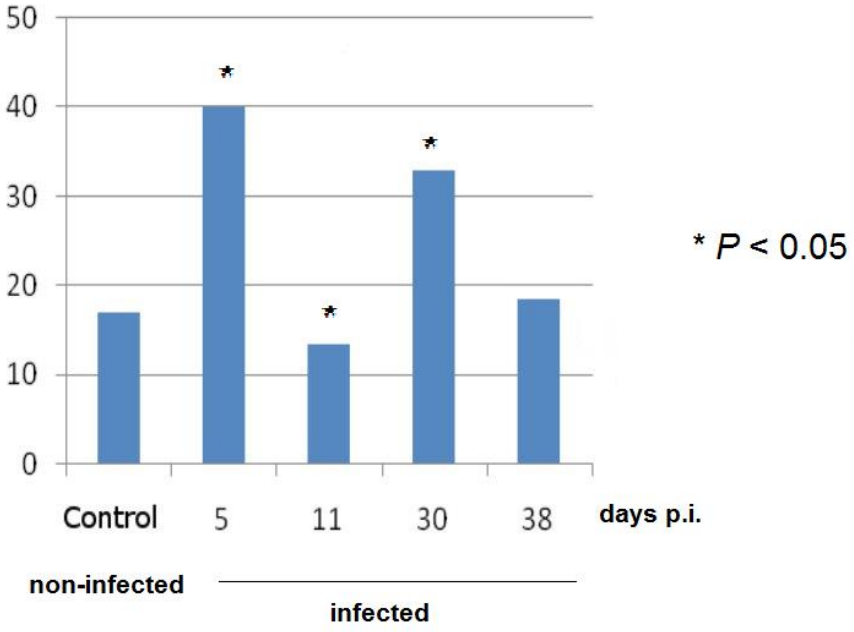

Figure 3. Dynamics of plasma cells in spleen. Histograms represent mean of the number of plasmacytes for each group of rats

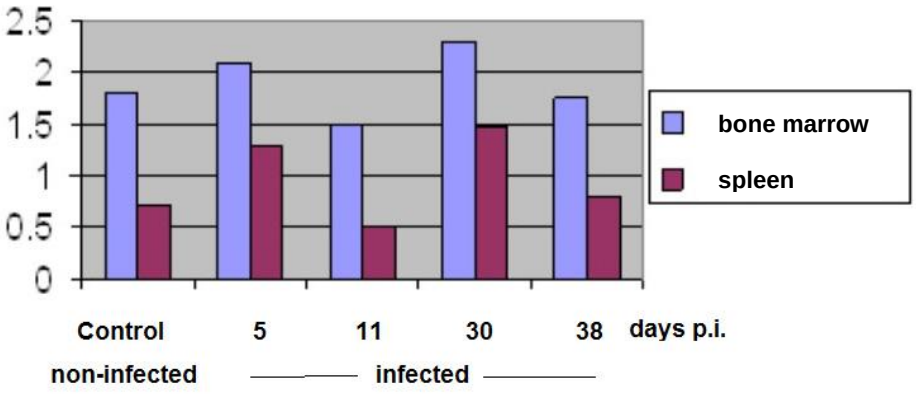

Figure 4. Dynamics of mitotic activity in spleen and bone marrow. Histograms represent mean of the percentage of cells with mitotic activity in spleen (red histograms) and bone marrow (blue histograms).

Migration stage of infection process (6-11th day p.i.) shows a suppression of plasmacytic reaction due to suppressive effect of *Trichinella* larvae on the host. Migrating larvae have the greatest biological activity at the migration period precisely, as it's then that they produce excretory antigens which are suppression factors. Numbers of plasma cells at this stage were lower than in control hosts, i.e.: 13.5 ± 0.45 cells ($P< 0.05$). At the same time a suppression of mitotic activity of marrow cells had been noted - 0.5 ± 0.15%; in control animals - 0.7±1.2%, in marrow – 1.5±0.01% (figure 4). Suppressive effects of *Trichinella* antigens on a host was noted by Dyachenko et al. (1992) and by many other authors later (Givanyan et al., 2011; Maizels et al., 2004; Boyett and Hsieh, 2014; Movsesyan et al., 2009) in experimental *Trichinella* infection.

Early muscle stage of the parasites' development (starting at 30 day p.i.) can be characterized with both increase in numbers of plasma cells in rat spleen and in mitotic activity (figures 3 and 4). It's necessary to note that at all stages of the parasite development numbers of cells with chromosome aberrations remained similar. Indeed, oscillations in these numbers were no greater than 2-5%, which is in accordance with numbers of spontaneous aberrations. It is possible that higher numbers of infective material would allow us to see higher aberration numbers in spleen slides. At 38-40 days p.i. the animals have shown a normalization of plasmacytic reaction and mitotic activity of spleen cells markers (figures 3 and 4). Comparison of data from spleen plasmacytic reaction and mitotic activity shows their simultaneous and parallel changes in infected animals. It should be noted that increase in plasma lineage cells occurs through both immediate stimulating effect of *Trichinella* excretion and secretion antigens on cell proliferation and transformation of lymph cells into plasmacytes necessary for antibodies production.

Conclusion

Experimental *Trichinella* infection have shown undulating course of plasmacytic reaction and cell mitotic activity. A degree of expression of immune maturation of spleen cells directly depended on *T. spiralis* development stage. Stimulating effect of the parasites was pronounced at early intestinal and early muscle stage, and at these periods high levels of blast cells, mitosis and plasma cells have been noted. These cause development of host immunity with various protective mechanisms (Shapiro-Shelef and Calame, 2005). Suppressive effects of *Trichinella* larvae at migrating stage could be seen in decrease of proliferation level and numbers of plasma lineage cells, which caused a suppression of the host organism immune reactivity.

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