Leukocyte composition of pronephros of *Leocottus kesslerii* infected with hemoflagellates of the genus *Trypanosoma* (Kinetoplastea: Trypanosomatida)

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**Abstract.** This study determined for the first time the changes in leukocyte composition of the pronephros (head kidney) of sand sculpin *Leocottus kesslerii*, (Sideleva, 2001) (Lake Gusinoye, Lake Baikal basin, Eastern Siberia, Russia), infected and uninfected with hemoflagellates of the genus *Trypanosoma*. The results indicated that the trypanosomes modulated the development of the immune response of their host (suppression of B cell immunity and granulocytic reactions, activation of the leucopoiesis and monocytopoiesis). Selective suppression of immune response in host with trypanosomes promotes the survival and development of parasites.

1. Introduction

Blood parasites of the genus *Trypanosoma* (Class Kinetoplastea Honigberg, 1963) – flagellate protozoa of many invertebrates and vertebrates [1-5]. In vertebrates, including fish, part of the life cycle takes place in the bloodstream. The blood-sucking leeches – hosts of kinetoplastid flagellates [1, 6-8].

In fish, depending on the intensity of hemoparasite load, various effects are found: the absence of pronounced pathological changes [9-10], anemia [11-15], activation of antibody production, lymphocytosis, eosinophilia [14, 16], leukocytosis [13], pro-inflammatory response [17]. Mortality can occur with high parasitaemia [18-20]. Changes in leukocyte populations are of particular interest because such changes indicate the development of a host immune response initiated by infection with the parasite [16, 21-23].

The head kidney (pronephros) in teleost fish is the main organ for hemo- and immunopoiesis [24, 25]. Therefore, the morphological cellular studies of this organ are important in the study of the immunological aspects of relationships in the parasite-host system. There are no data in the literature on the leukocyte composition of the pronephros in fish trypanosomiasis.

Sand sculpin *Leocottus kesslerii* (Sideleva, 2001) of the family Cottidae (Bonaparte, 1832) is an endemic pelagic-benthic species determines the structure of the ichthyofauna in Lake Baikal and of a number of reservoir fish communities in the lake’s basin. Infection with trypanosomes occurs during horizontal migrations of fish relatively close to the coast. The study of this parasitic system is limited only by infestation data, which are very scarce. Trypanosomes reached a prevalence of 9.1-31% with an intensity of infection of 7.6-40.7 individuals [26]. At the same time, the pathogenic relationship of trypanosomes with the immunological parameters of *L. kesslerii* has not yet been studied.

Therefore, the present study is the first report on the changes in the leukocyte composition in pronephros of *L. kesslerii* naturally infected with *Trypanosoma* sp.
2. Materials and methods
The biological material was obtained from sand sculpin individuals in July 2017. They were caught using a hydrobiological scoop net from Lake Gusinoye (Baikal Basin, Eastern Siberia, Russia), coordinates 51°17’351” N, 106°28’889” E). The morpho-biological analysis has been performed for all the caught fish, including measuring the body length weight and age determination, according to [27, 28].
In each fish anesthetized with benzocaine (1 g/10 L) was drawn of blood from the caudal vein after caudectomy, also was drawn tissue samples pronephros. Flagellates were detected by microscopy (objective 20 X); by counting in 300 fields of view of the microscope on the stained slide with a blood smear from each fish. The parasites have not been identified at the species level (figure 1).

Figure 1. Sand sculpin *Leocottus kesslerii*. *Trypanosoma* spp. (A, B). Bar 10 μm.

In the trypanosome-infected fish group, the infection intensity was 12-190 individuals (mean 54.7±11.32). After the blood collection, the fish were euthanized by severing the spinal cord located behind their head. The pronephros samples were used to prepare prints of organ impressions (three slides per fish). The prints were air-dried and then stained with azure–eosin by the Romanovsky–Giemsa method [29]. A total of 1,000 cells were counted on each print under oil immersion. The leukocytes’ cellular differentiation was identified according to the available data [30]. The leukocytes (blasts, lymphocytes, neutrophils, and monocytes/macrophages) were counted and reported as a percentage.

Data were analysed using the Statistica 6.0 software package. The normality of data distribution was determined using the Shapiro–Wilk test. Data groups not normally distributed were compared using the nonparametric analysis of the Mann–Whitney test. Differences were considered significant at *p* < 0.05, *p* < 0.01.

3. Results and Discussion
For the first time, the leukocyte composition of the *L. kesslerii* pronephros was identified. The leukocytes consisted of three groups of cells: blasts, granulocytes, and agranulocytes (table 1). In infected fishes, the number of blasts in pronephros increases by 1.5 times (*p* < 0.01), which indicates the activation of the proliferation of immature forms of the leukocytes, apparently in response to invasion. Cell elements of different lines (myeloblasts, monoblasts, and lymphoblasts) in the pronephros did not have clear morphological criteria, therefore they were assigned to one group. The lymphoid cell line dominated the leukocyte composition of the pronephros in all sand sculpin individuals (table 1).

The pronephros is the organ that mainly produces immunocompetent cells B lymphocytes [25]. B lymphocytes participate in the reactions of humoral-specific immunity. They produce specific antibodies – immunoglobulins. Immunoglobulins, binding to a foreign antigen, neutralize it or initiate the recruitment of other immunocytes to eliminate the pathogen. Trypanosomes are extracellular parasites that circulate freely in the blood. In this case, it is assumed that the host’s immune response will develop in a humoral manner to effectively destroy protozoa [16, 31]. However, blood parasites have developed strategies to evade the immune response: antigenic variation, regulation of Th1 / Th2
immune responses, and inhibition of antibody production [17, 32], which contribute to the partial survival of trypanosomes. In our study, analysis of the results of lymphopoiesis indicates inhibition of the B lymphocytes immune response in the group of infected fish, which may eventually lead to modest antibody production. This is confirmed by the low counts of immature forms of lymphocytes – prolymphocytes and mature lymphocytes (by 1.3 \[ p < 0.01 \] and 1.2 \[ p < 0.01 \] times, respectively) as compared with uninfected fish. Other authors also reported on suppression of lymphocyte proliferation in infected fish [9, 33, 34] observed were obvious signs of lymphocytic reduction in the head kidney of fish infected by trypanosomes.

Table 1. Leukocyte composition of pronephros (mean±SD and percent prevalence) of sand sculpin (Leocottus kesslerii) infected and uninfected with hemoflagellates of the genus Trypanosoma.

| Parameter            | Uninfected fish | Infected fish |
|----------------------|-----------------|---------------|
|                      | \( n=10 \)      | \( n=10 \)    |
| Blast cells          | 20.8 ± 0.66     | 30.8 ± 0.86\(^b\) |
| Granulocytes         | 28.4 ± 0.46     | 27.7 ± 1.99   |
| Neutrophils          | 20.6 ± 0.61     | 17.8 ± 1.54   |
| Myelocytes           | 7.8 ± 0.33      | 9.9 ± 0.69    |
| Metamyelocytes       | 10.4 ± 0.46     | 9.6 ± 0.67    |
| Bands                | 7.2 ± 0.72      | 5.2 ± 1.25    |
| Segmented            | 1.6 ± 0.22      | 2.2 ± 0.44    |
| Neutrophils          | 1.4 ± 0.22      | 0.8 ± 0.33    |
| Agranulocytes        | 52.0 ± 0.63     | 41.5 ± 1.44\(^a\) |
| Lymphocytes          | 50.8 ± 0.77     | 38.9 ± 1.69\(^b\) |
| Prolymphocytes       | 36.0 ± 0.4      | 30.8 ± 1.25\(^b\) |
| Lymphocytes          | 14.8 ± 0.95     | 8.1 ± 0.61\(^b\) |
| Monocytes            | 1.2 ± 0.18      | 2.6 ± 0.36\(^a\) |
| Monoblasts           | 0.8 ± 0.18      | 1.4 ± 0.36    |
| Monocytes            | 0.4 ± 0.22      | 1.2 ± 0.18\(^a\) |

\(^a p < 0.05; ^b p < 0.01.\)

Granulocytes, monocytes/macrophages provide the cellular form of nonspecific (innate) immunity [35]. These effector cells have the functions of phagocytosis of foreign elements and oxidative mechanisms, produce cytokines and chemokines that differentially regulate the immune response [36, 37]. In L. kesslerii, eosinophils and basophils with parasiticidal properties were not found. Our results do not coincide with the data of [14], which revealed eosinophilia in Abramis brama during trypanosomiasis but are similar to the data on no difference in counts of basophil and eosinophil in the blood of infected and non-infected eels [38]. Granulocytopoiesis in the studied individuals of L. kesslerii was characterized by the presence of only neutrophilic leukocytes at the stages of proliferation and differentiation (promyelocytes, metamyelocytes, stab and segmented neutrophils) (table 1). At the same time, no significant differences between the groups of fish were observed, which probably indicated the failure of these elements in the development of an immune response against trypanosomes. There is evidence of modulation of the host granulocyte response by trypanosomes. Trypanosoma carassii in carp inhibited the functional activity of kidney phagocytes [9, 33] observed a decrease of neutrophils in infected fish.

The system of mononuclear phagocytes in the studied fish was represented by proliferating (promonocytes), maturing and functionally mature cells (monocytes/macrophages) (table 1).
The number of mature monocytes in infected fish exceeded that of uninfected individuals by 3 times ($p < 0.05$). The studies showed that a activation of macrophages by parasite antigens is common in trypanosome infection. Mahasri et al. [38] noted that the monocytes number of blood-derived were above the swamp eels infected by Trypanosoma sp. Other authors reported a similar increase in monocytes in fish also infected with trypanosomes [9]. Oladiran et al. [17] noted an increase pro-inflammatory response in the goldfish (Carassius auratus L.) infected with Trypanosoma carassii. Probably, the activation of the monocytic-phagocytic system is a protective mechanism of the host’s immune response to eliminate part of blood parasites and prevent the development of host death.

4. Conclusions
In conclusion, hemoflagellates Trypanosoma sp. exerted a modulating effect on the development of the immune response of their host L. kesslerii. The following directions of immunomodulation are noted:
- intensification of leukopoiesis;
- suppression of B cell immunity;
- activation of monocytopoiesis;
- lack of immune response from granulocytes.

Selective depression of immune response in L. kesslerii with trypanosomes promotes the survival and development of parasites.

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