Phagocytic and bactericidal activity and morphological parameters of blood platelets in patients with *Trichinella spiralis* infection

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Abstract

Purpose: The production of IgE increases in parasitic invasions, triggering local or systemic inflammatory response with the involvement of blood platelets. The aim of the study was to assess the number and morphological parameters of blood platelets as well as their phagocytic and bactericidal activity in patients with *Trichinella spiralis* infection. It is interesting to investigate the blood platelet response following *Trichinella spiralis* in order to elucidate possible effects on non-specific immunity.

Material and methods: Twenty-six patients with *Trichinella spiralis* (before and after antiparasitic therapy) and forty healthy subjects were examined. The platelet count and morphological parameters were determined using a hematologic analyzer Technicon H-1 System. The platelet phagocytic activity was determined by measuring the percentage of phagocytizing cells and the phagocytic index. The bactericidal activity was assessed measuring the percentage of the bacteria killed by platelets and plasma. The strain *Staphylococcus aureus* ATCC 6538P was used for this purpose.

Results: In patients infected with *T. spiralis* morphological parameters do not change, except for the percentage of large platelets. In the course of trichinellosis the phagocytic index of platelets is statistically significantly decreased and platelet bactericidal activity is impaired, while the bactericidal activity of the plasma is statistically significantly increased, compared to healthy subjects.

Conclusions: The present study has revealed that due to *T. spiralis* infection, the percentage of large, young blood platelets is decreased. The parasitic infection causes impairment of non-specific immunity through decreased bactericidal activity of blood platelets.

Key words: blood platelets, phagocytic activity, bactericidal activity, trichinellosis.

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Introduction

Increased IgE production is the characteristic feature of parasitic invasions. This defect is due to disorders in the regulation of antibody production by T helper cells. Through the release of mediators from mast cells IgE promotes local inflammatory reaction and participates in antibody-dependent cellular cytotoxicity (ADCC) [1]. Parasitic invasions are the source of foreign antigens and exotoxins that trigger local or systemic inflammatory reactions. Blood platelets initiate and maintain inflammatory processes through the secretion of PDGF, platelet activating factor (PAF), platelet factor 4, β-thromboglobulin and IL-1 [2-4]. In the course of some parasitic infections, platelet activation is followed by the release of α-granular contents and granular membranes are fused with the platelet membrane [4]. Cytotoxic properties of blood platelets are induced by such cytokines as IFN-γ, TNF and IL-6. The mechanism of adhesion between platelets and parasites has not been fully elucidated although it is known to depend on platelet surface receptors. With GPIIb-IIIa glycoprotein deficiency, platelets show markedly lower cytotoxic activity against parasites [5].

The aim of the present study was to evaluate platelet count and morphological parameters, and to assess the phagocytic and bactericidal activity of blood platelets in patients with *T. spiralis* infection. It is interesting to investigate the blood platelet response following *T. spiralis* infection in order to elucidate possible effects on non-specific immunity.

Material and methods

The study group included 26 patients (12 women and 14 men, aged 19-65 years) hospitalized in the Department of Infectious Diseases, Medical University of Białystok. The patients (without overweight and hypertension) infected with *Trichinella spiralis* were examined twice (T1 – before treatment, T2 – after antiparasitic therapy). Trichinosis was diagnosed based on the clinical data and immunoserological tests (antibody titre was determined).

Control group (C) consisted of 40 healthy subjects (22 women and 18 men, aged 18-40 years, without overweight and hypertension). The concentration of C-reactive protein was 0-5 mg/l (in each healthy subjects).

As anticoagulants, dipotassium versenate (K_2EDTA – 1.5 mg/ml blood) was used to determine platelet count and morphological parameters, while heparin (50 IU/ml blood) to estimate the phagocytic and bactericidal activity. ACD (a mixture of citric acid and glucose) was added to prevent thromboxan synthesis by platelets, aggregation and secretion, and to reduce plasma pH to 6.5. To determine platelet count (PLT) and morphological parameters (MPV, PDW, LPLT) hematologic analyzer Technicon H-1 System was employed. The phagocytic activity was measured by determining the percentage of phagocytizing platelets and the phagocytic index. The bactericidal activity was assessed by determining the percentage of the bacteria killed by platelets and by plasma [6,7]. *Staphylococcus aureus* ATCC 6538P strain was used to assess the phagocytic and bactericidal activity of blood platelets. Following the Guidelines for Good Clinical Practice all the patients gave consent to the examination.

Student's t test for matched pairs was used for statistical analysis. Values are means ±SD. The means between examined groups were compared using the unpaired Student's t test. All tests of significance were two-tailed, with $P<0.05$, considered to indicate significance.

Results

No statistically significant differences were found in the platelet count between patients with *T. spiralis* infection, both before treatment (T1) and after antiparasitic therapy (T2), and healthy subjects. A statistically significant difference was noted in the mean platelet volume (MPV) between group T2 and C (*Tab. 1*). The lowest MPV was observed in patients after antiparasitic therapy (T2), perhaps due to platelet activation [8], which seems to be confirmed by the slightly increase index of anizocytosis (PDW) noted in this group. However, no statistically significant differences in PDW were found between study groups (T1 and T2) and control group (C). PDW and MPV are means of estimating the thrombocytopoietic activation. The slightly increase of PDW was observed in trichinellosis after antiparasitic therapy (T2), which may point to a slight increase in blood platelet production by megakaryocytes. PDW characterises the intensity of blood platelet production by "megathrombocytes". A statistically significant reduction was noted in the percentage of large young platelets (LPLT), both in group T1 and T2, compared to healthy subjects. This may be the effect *T. spiralis* exerts on the host – blood platelets become activated and undergo exhaustion, and in this way the number of large, metabolically more active platelets gets reduced [9]. Therefore, the lowest percentage of LPLT was observed in the group of patients after antiparasitic therapy (T2).

Table 1. **Statistical analysis of morfological parameters of blood platelets and their number in patients infected with *T. spiralis* before (T1), and after treatment (T2), and in the control group (C)**

Parameters	N-26 T1 X±SD	N-26 T2 X±SD	N-40 C X±SD	P
PLT	218.8±59.7	243.6±66.7	229.2±52.5	T1:T2 $0.4<p<0.5$ T1:C $0.6<p<0.7$ T2:C $0.7<p<0.8$
MPV	8.28±0.9	7.87±0.9	8.66±1.0	T1:T2 $0.3<p<0.4$ T1:C $0.2<p<0.3$ T2:C $p<0.05$
PDW	51.9±5.1	53.4±4.7	48.2±3.7	T1:T2 $0.6<p<0.7$ T1:C $0.7<p<0.8$ T2:C $0.7<p<0.8$
LPLT	2.9±2.7	2.3±2.1	4.8±1.9	T1:T2 $0.1<p<0.2$ T1:C $p<0.05$ T2:C $p<0.05$

The highest percentage of phagocytizing platelets (3.13) was noted in *T. spiralis* patients before antiparasitic therapy (*Tab. 2*). It seems that the presence of the parasite has a stimulatory effect on platelets, increasing their phagocytic activity but decreasing their bactericidal activity. The phagocytic indices in groups T1 and T2 are almost identical, but significantly decreased in comparison to the values noted in healthy subjects (*Tab. 2*). Plasma bactericidal activity in patients with *T. spiralis* infection was significantly higher compared to the values revealed in healthy subjects (*Tab. 2*). Not only was this activity elevated in group T1 (vs control group), but it also slightly increased after antiparasitic therapy (T2). On the contrary, the bactericidal activity of blood platelets decreased statistically significantly both before and after antiparasitic therapy, compared to control group (*Tab. 2*). The percentage of the bacteria killed by platelets in *T. spiralis* patients was three times as low compared to healthy subjects. Application of the antiparasitic treatment only slightly increased the bactericidal activity of blood platelets (*Tab. 2*). Perhaps, the impaired bactericidal activity of blood platelets is compensated by the increased bactericidal activity of the plasma.

Table 2. **Statistical analysis of phagocytic and bactericidal activity of blood platelets in patients infected with *T. spiralis* before (T1), after antiparasitic treatment (T2), and in the control group (C)**

Parameters	N-26 T1 X±SD	N-26 T2 X±SD	N-40 C X±SD	P
Percentage of phagocytic platelets (%)	3.13 ± 1.5	2.27 ± 0.9	2.26 ± 0.6	T1:T2 0.05<p<0.1 T1:C p<0.05 T2:C 0.8<p<0.9
Phagocytic index	1.44 ± 0.3	1.42 ± 0.4	1.83 ± 0.4	T1:T2 0.7<p<0.8 T1:C p<0.05 T2:C p<0.05
Bacteria killed by plasma (%)	28.47 ± 26.6	35.5 ± 30.7	19.8 ± 10.8	T1:T2 0.1<p<0.2 T1:C p<0.05 T2:C p<0.05
Bacteria killed by blood platelets (%)	6.40 ± 6.3	8.03 ± 7.2	20.5 ± 12.9	T1:T2 0.3<p<0.4 T1:C p<0.05 T2:C p<0.05

Discussion

In patients infected with parasites, e.g. *T. spiralis*, Th1 lymphocytes through the released cytokines (IL-2, IFN-γ, IL-12) induce differentiation and proliferation of cytotoxic cells and activate macrophages enhancing their ability to kill parasites [10]. It is suggested that certain manifestations in the pathology of trichinosis are due to cytokine-induced production of nitrogen oxide. Nitrogen oxide kills parasites and its concentration correlates with evident inflammatory reactions observed in the intestines and muscles in *T. spiralis* infection [11,12].

The antibody-dependent antiparasitic immunity involves some mechanisms which cause blockade of the receptors present on the parasite surface. On the one hand, these mechanisms lead to parasite damage through the complement system, but on the other hand increase the production of IgE antibodies [13]. However, the main antiparasitic mechanism is based on antibody-dependent cellular cytotoxicity which is also demonstrated by blood platelets [4,14]. Blood platelets as the effector cells are involved in parasitic diseases. They release various inflammatory mediators, are capable of phagocytosis and get in interactions with the cells of the immune system. In the course of parasitosis, platelets have a cytotoxic effect, but then increased concentrations of IgE, lymphokines (TNF, IFN-γ) and IL-6 are required [15-22].

The clinical course of trichinosis may vary and largely depends on the host response [23,24]. The present study has revealed that *T. spiralis* infection causes a slight decrease in platelet count and volume (MPV), and a statistically significant reduction in the percentage of large platelets, the so-called "megathrombocytes". These patients did not develop coagulation disorders that could result in platelet count decrease and therefore in trichinosis platelets seem to be destroyed mainly on "the periphery". The literature data suggest that platelet count reduction is characteristic of infectious diseases, viral infections and many parasitic infections [3,25-27].

As platelet activation leads to the release of α-granular contents and to thrombocytopoiesis stimulation, it is reflected in morphological parameters [8]. The decrease in platelet volume and in the percentage of large platelets seems to confirm the presence of the platelet-activating factor. The decrease MPV, observed in trichinellosis after antiparasitic therapy (T2), may be connected with their activation and release of platelet factor 4, β-thromboglobulin, platelet derived growth factor, fibrinogen, fibronectin, thrombospondin [2,4,8].

In patients with *T. spiralis* infection, platelet bactericidal properties were evidently impaired, constituting only 30% of the value obtained in healthy subjects. This impairment may be associated with the blocking of bactericidal mechanisms, with changes in arachidonic acid metabolism and with the action of T cells on blood platelets through PCIF lymphokines. Platelets participate in bactericidal mechanisms via peroxidase present in lysosomes and membranes of dense canaliculi and thanks to cationic proteins. Degradation of phagocytized bacteria involves proteolitic enzymes located in the lysosomal fraction: cathepsins and acid phosphatase [3,26,27]. The changes in the bactericidal activity of blood platelets may be related to bactericidal mechanism failure, which is indicated by the increased percentage of phagocytizing platelets and the decreased phagocytic indices. Most likely the impaired bactericidal activity of blood platelets and reduction in the percentage of LPLT are compensated by the increased bactericidal activity of the plasma.

The assessment of morphological parameters and phagocytic and bactericidal activity of blood platelets can be used to evaluate their functional condition and thus to determine platelet involvement in the mechanisms of non-specific immunity in the course of trichinosis.

Conclusions

1. The present study revealed a decrease in the percentage of large, young blood platelets in *T. spiralis* infection.

2. In patients with *T. spiralis* infection non-specific immunity is impaired due to reduced bactericidal activity of blood platelets.

References

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