Contribution of the Complement System to Realization of Biological Fluid Cytotoxicity in Health and Disease

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The cytotoxic effects of human blood serum were studied under normal and pathological conditions. The contribution of the complement system to the realization of this effect was demonstrated. Significant differences in the serum capacity to kill foreign cells were detected for healthy subjects and asthmatics. Bovine sperm suspension can serve as the test object for rapid evaluation of functional activity of the complement system.

Key Words: *blood toxicity index; complement; cytotoxicity; asthma*

Direct toxic effect on the cells, cytotoxicity, is used in pharmacology in testing of new drugs. Cytotoxicity is now used in clinical practice; biological fluids (serum, urine) serve as the "toxic" substances. Their effects on foreign cells (paramecia, bovine spermatozoa, isolated fibroblasts) indirectly characterize the adaptive potentialities of the organism, specifically, the quality of differentiation between the host and foreign objects. Studies in patients with manifest intoxication showed that the complement system could be the main mechanism of the realization of these processes [3].

Bovine sperm suspension served as the test object in our studies. This variant of the cytotoxic test created by BMK-INVEST Company for toxicological studies is suitable for express diagnostics and does not require sterile conditions.

MATERIALS AND METHODS

The study was carried out on the sera from healthy donors (n=145), patients with bronchial asthma (n=188), and athletes (n=112). The measurements were carried out on AT-04 Toxicity Analyzer. The samples were prepared by adding 10 μl serum to 390 μl saline; the control samples contained 400 μl saline. Bovine semen suspension (100 μl) was added to all samples. All solutions and samples were placed in a holder and warmed to 38°C during sample preparation. Capillaries were filled after thorough mixing of the samples.

The spermatozoon mobility was evaluated by changes in the light intensity during cell movement in front of the optical detector. The summary sperm motility was evaluated in each sample and the blood toxicity index (BTI) was calculated by the formula: $Is=(S_{ex}/S_c)\times 100\%$, where S_{ex} and S_c were the mean summary sperm motility in several experimental and control samples. The spermatozoon death in the test was a result of cell injury, the tail remaining intact (which was shown by eosin staining of the cells).

Heparin (5000 U/ml) and furosemide (0.01 g/ml) were used as synthetic blockers of the complement system [4].

RESULTS

Pathological processes are triggered when the defense systems fail to respond to external factors such as entry of foreign objects (bacteria, viruses, cells) not recognized as the host ones, or fragments thereof, for example, bacterial membranes (LPS). The complement system is one of the main systems of innate immunity.

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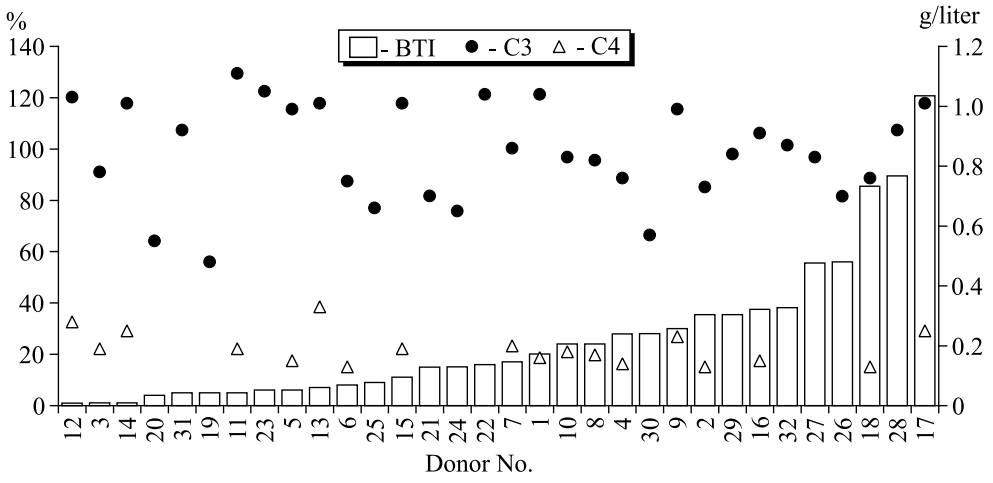

Fig. 1. Serum BTI (%) and C3 and C4 concentrations (g/liter) in donors. The donors are shown in the order corresponding to BTI increase.

Its function consists in identification of foreign agents. It is the main system in cytotoxicity realization.

Evaluation of the complement cascade as the system of cell destruction is difficult because the content of complement components in clinical practice is measured by the immunoturbidimetric method, and this approach precludes evaluation of their functional activity.

Comparison of complement C3 and C4 component concentrations in the sera of healthy subjects, athletes, and patients with asthma and the cytotoxicities of foreign cells revealed no correlations between these parameters (Fig. 1).

Very high and very low concentrations of the complement components have been recorded in the presence of high (up to 15%) serum capacity to kill foreign agents. This is explained by the fact that many of the complement proteins, in addition to participation in the cascade, perform some other physiological functions.

This method for cytotoxicity evaluation is used for the diagnosis and correction of endotoxemia in allergic diseases [2]. Activity of this system decreased under conditions of acute and progressive consumption of the complement or in its deficiency. Significant differences in BTI incidence distribution in normal subjects and patients with allergies were detected. The mean BTI in the patients was 29.4±2.5 *vs.* 1.7±1.7 in donors (****$p<0.0000...$; Student's t test). High BTI in the patients indicated low activity of humoral defense system.

In order to prove the leading role of the complement system in the realization of the serum cytotoxicity, we carried out experiments on the complement cascade blocking.

The term "complement" was initially used to describe the "complementary" activity that is present in the serum and is required for lysis of bacteria recognized by specific antibodies [5]. In our experiments, the serum heated to 56°C for 30 min completely lost its capacity to destroy the test cells.

Heparin (proteoglycane with anticoagulant effects) is widely used in clinical and laboratory practice. It has been described as reactive lysis inhibitor [1], it causes disorders in C1 complex integrity and prevents its effects on C2 and C4 components. This, in turn, leads to inhibition of the classical route of convertase formation, impairs the C3bBb convertase formation for the alternative pathway of complement activation by preventing factor B interactions with C3b. The inhibition constant for heparin *in vitro* is 36.4±1.7 μg/ml [4]. Addition of heparin (5000 U/ml) to the samples prepared by the standard method abolished serum capacity to destroy the foreign cells (Fig. 2).

Furosemide is a synthetic inhibitor of the complement system activation [4]. We used pharmaceutical furosemide with the concentration of 0.01 g substance per ml. Furosemide blocks the complement system cascade less actively than heparin (BTI 45 and 30%, respectively).

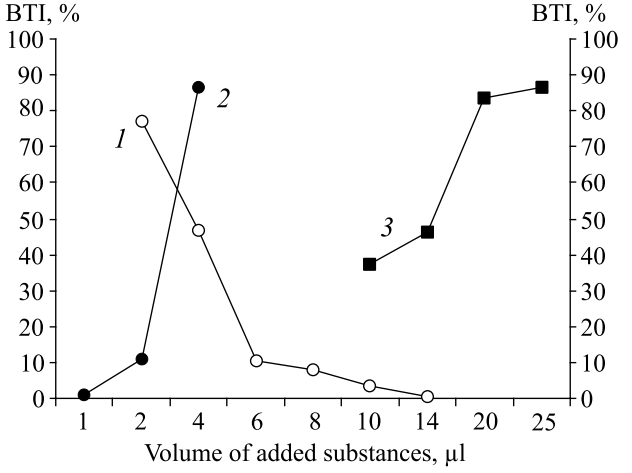

Fig. 2. Relationship between BTI and volume of added serum (*1*), heparin (*2*), and furosemide (*3*).

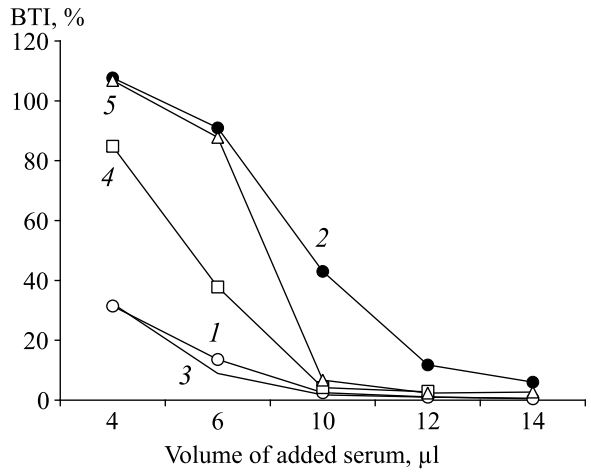

Fig. 3. Changes in BTI after addition of depleted sera. *1*) serum; *2*) C1 reagent; *3*) C2; *4*) C3; *5*) Q1.

In addition, direct blockade of one of the complement components was carried out. Antibodies to C3 from PLIVA-Lachema Diagnostika kit were used. Serum BTI was 23.6% and BTI of serum+antibodies complex 60.8%. Removal of C3 from the serum reduced its reactivity. Incomplete blockade of the complement cascade was due to a rather high level (1-2 mg/ml) of C3 [5].

The available EIA methods for complement proteins show just their content as antigens, while the most informative test is evaluation of their functional activity, characterizing disease development. A series of tests of this kind was developed at G. N. Gabrichevsky Institute of Epidemiology and Microbiology. At the laboratory headed by Dr. L. V. Kozlov, human sera free from a certain complement component (reagents) are used for evaluation of functional activity of this complement component [4].

We used these depleted sera in experiments on the test cells (Fig. 3). Addition of depleted sera allowed evaluation of the amount of serum components needed for lysis of a foreign cell. For reagents Q1 and C3 it was 10 µl, for C1 12 µl (BTI below 15%). However, despite the similarity of the method for cytotoxicity evaluation with bovine spermatozoa and method for evaluation of the functional activity of the complement components by sheep erythrocyte lysis, this method reflected the potentialities of the system for foreign cell elimination depending on the health status.

Hence, BTI is an integral index characterizing organism's capacity to react to bacterial or viral particles and creating a maximally close to the host and at the same time individual model of its reaction to a pathogen. Functional activities of the defense systems in general and, specifically, of the complement system were evaluated by using bovine spermatozoon suspension as the test system for evaluation of serum cytotoxicity.

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