CHARACTERISTICS OF IMMUNE RESPONSE UNDER EXPERIMENTAL MODELS OF ACID BURNS OF THE ESOPHAGUS

It is well known that the immune system is actively involved in the regeneration and healing process of burn wounds. However, unanswered questions remain about the role of humoral immunity in the mechanisms of healing and complications of burn wounds. We have developed an experimental model of the acid burns of the esophagus (ABE) corresponding esophageal burns in children 1-8 years. We studied the features of humoral immunity in rats with ABE. We have developed an experimental model of the acid burns of the esophagus (ABE) solution CCl₃COOH 30% [11].

Materials and methods. In experiments used immature white nonlinear rats (1-month) weighing 90-110 g, were kept on a standard diet vivarium. Work carried out in accordance with the rules of the European Convention for the humane treatment of laboratory animals (European convention the protection of vertebrate animals used for experimental and other scientific purposes – Consul of Europe. Strasbourg, 1986) and the “General Principles of experiments on animals”, approved National Congress of bioethics. The animals experimentally simulated acid burns the esophagus (ABE) solution CCl₃COOH 30% [11]. To obtain IgG fraction from the blood serum,1 ml of serum was layered on a column with protein- A Sepharose (total column volume 5 ml). Nonspecifically bound proteins were washed with 0.05 M Tris-HCl buffer, pH 7.4 in a volume of tenfold of total column volume (50 ml). Elution was carried out using a glycine buffer (0.1 M glycine-HCl, pH 2.2). Samples containing protein were precipitated by chemical burns of the esophagus (BE) of different nature and degree.

The aim of study was to evaluate immune status, which includes determination of the parts of the humoral immune system under the experimental reproduction of acid burns of the esophagus
ammonium sulfate solution (final concentration 50%) and were left at 4 °C overnight. The precipitate was centrifuged at 3000 rpm/min for 30 min. The supernatant was withdrawn and the precipitate was dissolved in 1 ml of 0.05 M Na-phosphate buffer, pH 7.4 [5]. To remove ammonium residues, the solution of antibodies was applied to column G 25 equilibrated with 0.05 M Na-phosphate buffer, pH 7.4 (total column volume 50 ml). Samples containing protein were concentrated, then absorbance was measured and the antibody concentration was calculated. The obtained samples were stored at – 20 °C. The IgG fractions from serum were isolated on the 15th day of the experiment.

The level of antibodies in the blood of animals studied was evaluated by enzyme-linked immunosorbent reaction [4], which was carried out in 96-well microplate (Dynatech, Sweden). In the hole made microplate 100 ml goat IgG monoclonal antibodies against mouse (Sigma, USA).

CIC content in blood serum determined by precipitation of 4.5% solution of polyethylene glycol 6000 (PEG-6000) [8]. The method is based on different solubility Ig monomers composed of IC in the presence of PEG in the environment. Different concentrations of PEG (2.5%, 3.5%, 7%, 10%) cause precipitation of different molecular weight and size of CIC. Low concentrations of PEG precipitated complexes large, high concentrations cause precipitation of low molecular weight compounds. Results reaction photometrical determined using ELISA analyzer (Titertek Multiskan, Finland).

Statistical analysis of the results was performed using the program OriginLab 8.0. Changes considered reliable indicators at p ≤ 0.05; at p ≥ 0.05 changes into account as a trend.

Results: The degree of the pathological states by various organs and results of disease, are depended from severity of inflammation trauma, timely and accuracy of treatment, and age of victim human [5; 6]. In our researches principally was used of inflammation model, which would meet one, that are characteristically to children since 1 to 8 years.

Analysis and generalization of received research results on this model, are allowed reveal a not only factors, that determine the different level of means, and features of regulation mechanisms of the child immune system at the chemical inflammation of esophagus. Mortality from inflammation trauma, are dependent from spread, severity and degree of inflammation.

We were elected under shock and septic-toxemia of inflammation, because the biggest of interest of the dead there in period of septic-toxemia. Causes at the inflammation trauma: sepsis, pneumonia and on it background progressing the multiple organ failure [7].

Analysis of conducted researches, was viewed, that ABE causes of the humoral immunity link change, that consistent with the dates of references [13]. Was established decreased of IgG level, which are the most specific effector of humoral link, on the 1st day of ABE, that responsible of shock stage (Figure 1).

Figure 1. The level of IgG in serum of rats under the experimental simulation of acid burns the esophagus as a percentage relative to control (M ± m, n = 10)

* – p <0.05 compared with control

The average level of antibodies in this group decreased by 1.48 times against the intact control. These changes may indicate that in a chemical burn is the failure of adaptation responses.

One of the indicators of immune status is the level of circulating immune complexes (CIC) in blood. In the process of immunocomplex are important dimensions of immune complexes because most pathogenic immune complexes are small and medium-sized, able to activate the complement system.

Determining the level of immune complexes useful in burn disease, however, problems related to the rationale for determination of circulating immune complexes at ABE have not yet received a clear decision.

We have found that the ABE has been a change of prices on the first day after the burn (Figure 2).
Fig. 2. The level of circulating immune complexes in the blood serum of rats under experimental modeling of acid burns the esophagus as a percentage relative to control at 1 day after the formation of burn wounds. (M ± m, n = 10)

1. macromolecular immune complexes
2. The average molecular immune complexes
3. low immune complexes

* – p < 0.05 compared with control

In the study of high-CIC in animals which ABE induced the tendency to increase their level of performance compared to intact controls. Performance exceeded the reference value of 1.3 degrees in the animals treated with 30% solution of acetic acid.

The level of average molecular CIC also exceeded the reference value of 1.3 degrees, indicating the development of inflammation. At the same time increased the level of low-CIC group of animals with ABE. Performance exceeded the reference value of 1.4 degrees.

So, along with a decrease at the level of IgG in 1st day of ABE seen significant shifts in the molecular composition of immune complexes that manifested itself primarily increased concentration of toxigenic most – middle- and low-CIC.

Figure 1 presents data indicate that on the 21st day of burn disease in rats under experimental modeling of ABE was a significant increase the level of IgG antibodies in serum. It was found that in the group of animals with burns to 30% CCl3COOH, antibody levels exceeded indicators in the control group 1.43 degrees.

Fig. 3. The level of circulating immune complexes in the blood serum of rats under experimental modeling of acid burns the esophagus as a percentage relative to controls at 21st day after the formation of burn wounds. (M ± m, n = 10)

1. macromolecular immune complexes
2. The average molecular immune complexes
3. low immune complexes

* – p < 0.05 compared with control

At the research of high and average molecular CIC in the animals on the 21st day after inflammation (Figure 3) was observed trend to some magnification of this level, in compared with means of intact control.

The means of low molecular CIC in animals with ABE were above than control in 1.25 degrees. In accordance with references, a long circulation of immune complexes in organism, even slight increase of this complexes results to appearance of savings of this complexes in tissues, to increase of aggregation and adhesion of thrombocytes, that results to violation of microcirculation of blood and necrosis [9].

At the development of immunocomplex process, important are the dimensions of immune complexes, because the most pathogenic immune complexes are small and medium size, which are able to activate the complement system, which causes the development of inflammation. Actually, these immune complexes interact with a number of regulatory systems, causing damage response.
Features of the immune system in the 21st day lies in the fact that during our investigation in immature rats (1-month) with ABE was activation of most indicators. Data from clinical observations [1] cannot be considered a change of immunological parameters as evidence of normalization or healing. This can be explained by highiability of most studied indicators and due to the immaturity of physiological systems.

Conclusion. So ABE accompanied by considerable changes in the levels of the humoral immune system. On the 1st day of ABE in immature rats (1-month) took place reduction of IgG and were marked significant shifts in the molecular composition of immune complexes that manifested itself primarily in increased concentration of toxicogenicmost – middle- and low-CIC. On the 21st day of burn disease in rats under experimental modeling of ABE was a significant increase in the level of IgG antibodies in serum. In animals that were burned by 30% solution CCl₄COOH medium and low CIC tended to decrease.

References