

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Immunobiological Value Of The Electric Charge Of Erythrocytes In The Body's Response To Antigenic Effects.

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ABSTRACT

The role of the electric charge of these cells in their participation in the formation of an immune response to antigens of infectious nature (tetanus and botulinum toxoids, the vaccine strain "19" Br. Abortus. A high negative correlation was observed between the electrophoretic mobility (EFM) of erythrocytes and the titer of specific antibodies when immunized with a brucellosis agent ($r = -0.99$ at $p < 0.001$), tetanus ($r = -0.53$ at $p < 0.05$) and botulinum $r = -0.74$ at $p < 0.05$) with toxins, which indicates the importance of the electric charge of erythrocytes in the formation of the immune response of the animal organism. A hypothesis is advanced about the participation of red blood cells in the formation of immunity of the animal, including humans, when exposed to the body of infectious agents.

Keywords: electrophoretic mobility of erythrocytes, cow, pigs, rabbits, tetanic and botulinic toxins, strain of "19" Br. abortus

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INTRODUCTION

The physiology of the immune system in the format of cell biology mainly considers lymphocytes and macrophages, and also, to a lesser extent, other forms of leukocytes. Meanwhile, when entering the body, the antigen has the greatest probability of initial contact with erythrocytes, since the latter are 1-3 orders of magnitude greater than other cells. In addition to a significant number, erythrocytes are characterized by a variety of functions, including adsorption capacity, which predetermines their interaction with foreign agents. The literature data testify to the important role of erythrocyte membranes in the immunological mechanisms of agglutination and lysis [6-9].

Unfortunately, the participation of erythrocytes in the formation of the immune response of the animal organism in the scientific literature has been poorly reviewed, although individual works [2,5] confirm the thesis. Of all the characteristics in this aspect, the most important is the electric charge of the erythrocyte surface [14], especially if the antigen also has a significant surface potential.

Certain structural and functional changes in membranes are revealed upon interaction with antibodies. As one of the most important functions of erythrocytes, their ability to concentrate biologically active molecules on the surface is considered [10]. Representing the latter in this form of lymphocytes and macrophages, red blood cells have a multidirectional effect on the immune response in various physiological and pathological conditions of the organism [11]. These effects are widely used in the formulation of serological reactions (inhibition, haemagglutination, haemadsorption), etc. [15].

There is also an opposite effect, namely the dependence of the electrical characteristics of the erythrocyte surface on immunological responses. Thus, when exposed to antigens of viral and bacterial nature, the charge of erythrocytes, as a rule, decreases [1], which is obviously connected with the processes of their adsorption on the surface [12,13,16]. The facts about the effect of antibodies on the electrical characteristics of erythrocytes are few and contradictory. Unequivocally, we can state the presence of such shifts, which may be the primary link in the activation of these cells.

The foregoing prompted us to investigate the electrophoretic mobility (EFM) of erythrocytes and lymphocytes as an indicator reflecting the magnitude of the electric charge of the surface of these cells in animals of different species under the action of antigens of different nature.

MATERIALS AND METHODS

The experiments were carried out on erythrocytes and lymphocytes (unseparated and divided into T and B cells suspension) of blood of intact and experimentally immunized animals of both sexes (30 rabbits, 10 pigs, 12 cows).

When studying the effect of anatoxins on EPL of blood cells, rabbits were immunized intramuscularly at a dose of 10 IU / ml with botulinum toxoids of types A, B and E and tetanus purified adsorptive anatoxin of the Ufa Research Institute of Vaccines and serum N.I. Mechnikov.

The titre of specific antibodies was detected by indirect immunoassay analysis using antispecial rabbit sera labeled with peroxidase (NF Gamalei Institute of Epidemiology and Microbiology, Moscow). The antigen was adsorbed for 1 hour at 36°C on sensitized and 2 hours on non-sensitized plates. The concentration of antitoxin for the sensitization of the wells was 1 IU / ml. The results of the reaction were taken into account visually and on a photometer "Dynatech MR5000" (Germany) at 492 nm.

Anti-brucellosis immunization was performed with live dry vaccines from strains "19" Br. abortus. Vaccines were diluted in saline solution, so that 1 inoculum dose was 2 ml (2 billion microbial cells) for rabbits and injected subcutaneously in the inner thigh area.

The main method of investigation was cell electrophoresis, performed on an automatic microscope "Parmokvant-2" (Germany).

The measurements were made in an automatic mode with an electric current of 10 mA and a temperature in the chamber of 20 ° C. Before electrophoresis, the cells were washed three times with a suspension medium, which was buffered saline solution. The lymphocytes were isolated by centrifugation in a density gradient of polyvinyl alcohol-verographin, followed by erythrocyte lysis in the precipitate with a 0.83% solution of ammonium chloride. Fractionation and quantitative determination of T- and B-populations of peripheral blood lymphocytes were performed according to I. Lefkowitz, B. Pernis [4], and their percentage ratio by the rosette formation method [3]. To standardize the readings of the device, human erythrocytes were used. Since the suspensions of the cells under investigation are heterogeneous from the EFT, an EFM determination of at least 200-300 cells was performed to correctly interpret the measurement data in each sample and the mean values obtained were further averaged over the number of samples.

The biometric analysis of the obtained data included the calculation of the arithmetic mean, the mean square deviation, the reliability of the sample difference by the Student's test, and the correlation coefficient and the reliability of its evaluation by the Student's criterion.

RESULTS AND DISCUSSION

The comparative analysis of the electrokinetic properties of erythrocytes and blood lymphocytes of a number of animals (Table 1) revealed a large (relative to erythrocytes) EFM of blood lymphocytes in cows (by 9%) of pigs by 18%) and rabbits (by 42%).

Table 1: Mean values of the EFM of blood cells of various animal species

animal	EFM, mcmsm B ⁻¹ c ⁻¹		The difference in EFM, %
	erythrocytes	lymphocytes	
Cow	0,81 ± 0,03	0,89 ± 0,04	-9
Pig	0,61 ± 0,02	0,74 ± 0,03	-18
Rabbit	0,43 ± 0,02	0,74 ± 0,03	-42

This allowed us to formulate a provision on the competitive relationship between these shaped elements for contact with antigens that have entered the bloodstream.

This hypothesis was fully confirmed in the study of the effects of tetanus and botulinum toxoids: less sensitive were in ascending order, rabbits, pigs, cows.

Under the influence of botulinum anatoxins, significant changes in the EEP of lymphocytes were detected only for types A and B. Similar patterns were observed in the dynamics of changes in the titers of specific antibodies, although quantitative changes in the B-population of lymphocytes poorly obeyed the revealed regularities (Table 2).

Thus, the EFM of lymphocytes in suspension, which characterizes the average charge of all types of blood lymphocytes, reflects the functional state of the B-population under the influence of botulinum toxins, rather than their quantitative changes.

Table 2: The state of the lymphocytic link of immunity in rabbits immunized with botulinum toxoid of various types

The condition of the rabbit	Index			
	EFM of lymphocytes, mcmsm B ⁻¹ c ⁻¹ m	number of lymphocytes, %	number of T-lymphocytes, %	number of B-lymphocytes, %
Norm	0,74 ± 0,03	59,5 ± 2,13	60,3 ± 2,13	29,1 ± 2,8
Anatoxin A	0,80 ± 0,03	65,8 ± 5,15	65,0 ± 4,2	31,0 ± 1,4
Anatoxin B	0,82 ± 0,03	89,2 ± 2,33	79,8 ± 7,8	43,3 ± 5,8

Negative correlation of the ESM of erythrocytes and antibody titre when immunized with tetanus toxoid (r = -0.53, p < 0.05) with anatoxin suggests the indirect involvement of erythrocytes in the process of

formation of immunity, in particular, the constitutional one. This assumption is confirmed by data on species differences in the sensitivity of animals to tetanus and botulism. The revealed patterns of changes in the electrokinetic properties of blood cells and their connection with the production of specific antibodies in rabbits under the influence of the anatoxins under consideration make it possible to conduct a targeted search for new, more effective means of prevention and treatment.

Continuing to consider the issue of the protective involvement of erythrocytes in the "pathological correlation", the developing infectious process, we traced the correlation of the ESM of erythrocytes and lymphocytes with the titer of specific antibodies when immunized with the antigens of the brucellosis agent (Table 3).

Table 3: Influence of immunization with brucella strain "19" on antibody titer and EFM (mcm smB⁻¹c⁻¹) of blood cells of rabbits

Index	Termafterimmunization, day			
	Control	30	45	60
EFMLymphocytes	0,76 ± 0,04	0,76 ± 0,05	0,71 ± 0,08	0,72 ± 0,06
EFMerythrocytes	0,40 ± 0,02	0,32 ± 0,02	0,39 ± 0,03	0,39 ± 0,03
AntibodyTiter, L/n	-	2560	320	320

A negative correlation of the changes in the EFM of erythrocytes with the titre of specific antibodies in rabbits ($r = -0.99$, with $p < 0.001$), which are insensitive to brucellosis by animals, was revealed.

CONCLUSION

On the example of 3 species of animals with different ratios of the electric charge of erythrocytes and lymphocytes, a provision is made about the competitive relationship between these shaped elements for contact with antigens that have entered the blood. The high negative correlation between the EFM of erythrocytes and the titre of specific antibodies during immunization with tetanus and botulinum toxoids, as well as the causative agent of brucellosis, indicate the significant role of red blood cells in the formation of the immune response of the animal organism.

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