can thus be concluded from these investigations that peripheral blood lymphocytes of patients with IHD possess more viscous membranes, whose orderliness increases proportionally to the increase in the cholesterol content in the cells. An increase in viscosity of the membranes is one of the more important causes of depression of the function of these cells in IHD, and disturbances of the redistribution of Ca^{++} ions are evidently one mechanism realizing the effect of modification of membrane structure on cell function.

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ANTIBODY FORMATION TO AUTOLOGOUS ERYTHROCYTES AFTER IMMUNIZATION OF NORMAL MICE AND OF MICE TOLERANT TO THE IMMUNIZING ANTIGEN, WITH RAT ERYTHROCYTES

T. B. Prigozhina, N. V. Chulkova, L. N. Fontalin, E. V. Nagurskaya, and I. Yu. Chernyakhovskaya

UDC 612.017.1.06:612.438.4

KEY WORDS: tolerance; autoimmunity; erythrocytes.

A method of inducing an autoimmune response to erythrocytic antigens in mice by repeated injection of a cross-reacting antigen, namely rat erythrocytes (RE), was developed in 1973 [10]. For a long time (over 3 months) antibodies to autologous erythrocytes were found in animals immunized in this way. Several parameters of the response to autologous erythrocytes have been investigated in the USSR [1, 2] and in other countries [8, 9], but the conditions of formation of this autoimmune response have not been adequately studied.

In the investigation described below this problem was investigated by comparing the effectiveness of induction of the autoimmune response in mice capable of synthesizing antibodies to RE, and in animals specifically areactive to that antigen.

EXPERIMENTAL METHOD

Male CBA/CaLacSto and BALB/B/c mice and (CBA × C57BL/6)F_1 hybrids, and August rats were obtained from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR; male CC57BR/Mv mice were obtained from the "Rappolovo" Nursery, Academy of Medical Sciences of the USSR. For immunization, 2×10^8 RE were injected intraperitoneally 4-5 times with intervals of 7-10 days between injections.

The overall antibody titer to RE in the serum was determined by the hemagglutination test on the 7th-9th day after the last immunization. Antibodies of the IgG class were determined after preliminary incubation of 50 µl of test serum in a dilution of 1:5 with 50 µl of a 1% solution of mercaptoethanol ("Serva") in buffered physiological saline (pH 7.4). The

Laboratory of Immunological Tolerance, N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 3, pp. 324-327, March, 1989. Original article submitted October 27, 1987.

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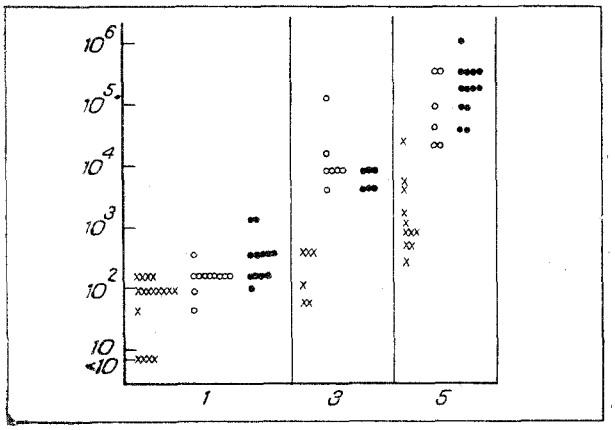

Fig. 1

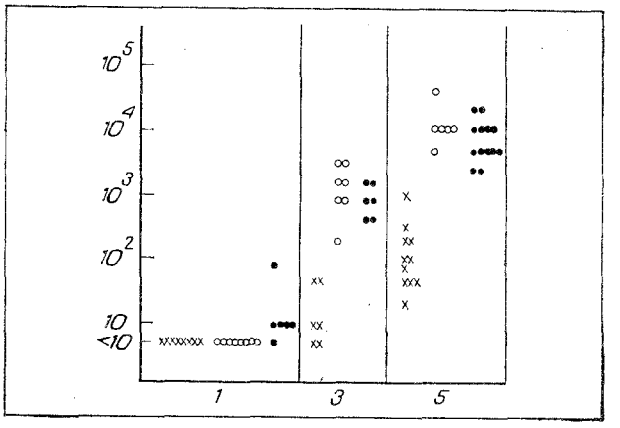

Fig. 2

Fig. 1. Titer of antibodies to RE in mice receiving complete tolerogenic treatment (crosses), subjected to nonspecific immunosuppression – thymectomy and CP (empty circles), and in mice not receiving any preliminary treatment (filled circles), after 1st, 3rd, and 5th immunizations. Here and in Figs. 2 and 3: abscissa, serial number of immunizations; ordinate, titer of antibodies.

Fig. 2. Titer of IgG-antibodies to RE in animals subjected to tolerogenic treatment (crosses) and control animals (empty and filled circles).

control sample was incubated with physiological saline. Autoantibodies to mouse erythrocytes (ME) were detected by the direct test in [7]. The isotype of the antibodies to autologous erythrocytes was judged by inhibition of Coombs' test by affinity-purified mouse IgG. Different quantities of IgG were added to a working dilution of anti-immunoglobulin serum 30 min before addition of the erythrocytes. The anti-immunoglobulin serum and affinity-purified mouse IgG were generously provided by E. V. Sidorova (Moscow Research Institute of Virus Preparations, Ministry of Health of the RSFSR).

Tolerogenic treatment was carried out by the method described previously [3, 4]. Adult mice were thymectomized, and 1 month after the operation they were given an intravenous injection of 10^8 spleen cells from August rats, followed 24 h later by an intraperitoneal injection of 200 mg/kg of cyclophosphoamide (CP) (from Saransk Medical Preparations Factory). Mice were used in the experiments 2 months after the end of tolerogenic treatment. Intact animals and thymectomized mice, receiving 200 mg/kg of CP each 1 month after the operation (CP mice) served as the controls.

EXPERIMENTAL RESULTS

The intensity of the autoimmune response to repeated immunization with RE varied strongly in mice of the different lines [8-10]. In the present experiments antibodies not only to RE, but also to ME, were found after four injections of RE in all the 40 (CBA × C57BL/6)F_1 hybrids tested (100%), in 27 of the 33 CBA mice (80%), in seven of the 10 BALB/c mice (70%), and in two of the 10 CC57BR mice (20%). All subsequent experiments were performed on (CBA × C57BL/6)F_1 hybrids.

The results of repeated immunization of mice with RE were compared with the results of the same immunization of mice subjected to tolerogenic treatment and tolerance to RE. The writers showed previously that ability to synthesize specific hemolysins in response to a single injection of RE was depressed in tolerant mice for 4 months or longer [6]. A similar rule also was found in the case of hemagglutinin production (Fig. 1). The difference between the intensity of production of antigen-specific agglutinins in tolerant and control animals also continued during subsequent immunizations. Titers of antibodies to RE in tolerant mice increased five-tenfold during this period, compared with 50- to 100-fold in control animals.

Assessment of IgG antibody production (Fig. 2) showed that CP leads to transient nonspecific inhibition of synthesis of antirat antibodies after the first immunization. During subsequent immunizations animals of both control groups synthesized antibodies of the IgG class with equal intensity. Antibodies also appeared in the tolerant animals, but their titers were an order of magnitude lower than the antibody titers of the control mice. Incidentally, the

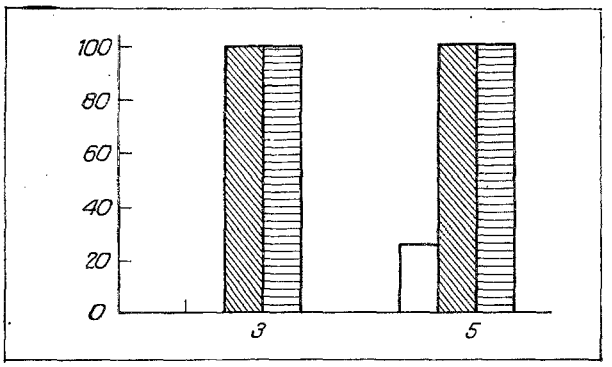

Fig. 3. Antibody formation to autologous erythrocytes in mice tolerant to RE (unshaded column), rats subjected to nonspecific immunosuppression (obliquely shaded), and normal animals (horizontal shading) after 3rd and 5th immunizations. Ordinate, antibody titer (in % of control, taken as 100).

titer of IgG-antibodies during the immune response to RE in all mice investigated was an order of magnitude lower than the total antibody titer (Figs. 1 and 2). Deviation from this rule was found only for a short time after the first immunization of mice receiving CP. The impression was obtained that both tolerogenic and nonspecific treatment influence the intensity of the immune response, but not the ratio of the IgM/IgG titers.

The data on preservation of differences in antibody production to RE in tolerant and control animals even after repeated immunizations can be compared with previous results indicating prolonged (more than 6 months) persistence of heterotropic transplants of the neonatal heart of August rats in tolerant mice. The transplant is a permanent source of foreign antigens in the recipients body. Its long existence, as well as the incomplete emergence from a state of immunologic tolerance during repeated immunization with RE, is evidence of the stability of the tolerant state.

The next step was to study antibody production to autologous erythrocytes during repeated immunization of tolerant and control mice with RE. After the first immunization no such antibodies were found in any of the groups of animals. After the 3rd immunization (Fig. 3) autoantibodies appeared in all 13 control animals, but in none of the 13 tolerant mice. After the 5th immunization most (10 of 13) tolerant animals likewise did not produce autoantibodies. Thus nonspecific methods of immunosuppression (CP and thymectomy) affected neither the time of appearance of autoantibodies nor the number of animals synthesizing autoantibodies, whereas complete tolerogenic treatment delayed the appearance of autoantibodies in the blood serum of the mice and sharply reduced the proportion of animals synthesizing autoantibodies.

The overwhelming majority of antibodies to the crossed mouse-rat determinant, detected in response to ME by Coombs' method belonged to the IgG class. This was shown by complete inhibition of Coombs' test by 2 μg/ml of affinity-purified mouse IgG. Similar results also were obtained by other workers, who showed that autoantibodies to ME are IgG-antibodies which do not fix complement [8, 9]. Conversely, the majority of antibodies to RE belonged to the IgM isotype. The reasons for these differences are not clear. It can be tentatively suggested that the rapid switching of cells synthesizing autoantibodies from IgM production to production of IgG that do not bind complement, is a protective mechanism, restricting the after-effects of the autoimmune reaction. Anemias due to complement-fixing IgM-autoantibodies are known to be particularly severe and to be infrequently found [11]. The concrete mechanisms of exclusion of B cells, capable of producing such antibodies, from the immune response require further study.

The interconnection between areactivity to foreign antigens (RE) and to crossed antigen (determinant), revealed by the present investigation, suggests that both phenomena are based on a common mechanism. The writers showed previously that areactivity of tolerant animals to RE is due to activation of suppressor T cells carrying the I-marker [5].

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DETERMINATION OF SERUM ANTIPLATELET ANTIBODIES IN PATIENTS WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA BY ELISA

A. A. Koval', A. V. Mazurov, D. V. Vinogradov, A. V. Rudin, V. S. Repin, V. G. Savchenko, and L. I. Idel'son

UDC 616.155.294-07:616.153.962.4-097-02:616.155.25]-078.333

KEY WORDS: idiopathic thrombocytopenic purpura; platelets; autoantibodies; enzyme-linked immunosorbent assay (ELISA).

Improvements in methods of recording antiplatelet antibodies (AB) in patients with idiopathic thrombocytopenic purpura (ITP) have led to improvements in the diagnosis and evaluation of the effectiveness of treatment of this disease. Direct and indirect methods of determination of antiplatelet AB are distinguished. In the first case the quantity of antibodies associated with platelets is determined, in the second case the quantity of antiplatelet AB in the patient's serum. Various methods have been suggested for recording antiplatelet AB. Agglutination tests are nowadays considered to be inefficient. These tests do not reveal incomplete AB and they are attended by the difficulty of setting up a precise negative control, due to the ability of platelets to aggregate [1]. Dixon and co-workers [3] were among the first to develop a method of quantitative determination of antiplatelet AB based on inhibition of complement-dependent lysis of erythrocytes, correlating with the use of antiglobulin AB. Immunofluorescence [8] and radioimmune methods [2, 5] of recording AB associated with platelets and circulating AB also have been successfully used. However, immunoenzyme methods and, in particular, enzyme-linked immunosorbent assay (ELISA) are most widely used at the present time [3, 7]. Advantages of ELISA include the possibility of simultaneous determination of AB in several samples, quantitative estimation of AB, relative simplicity, and sufficiently high reproducibility.

This paper describes an indirect method of ELISA for recording serum antiplatelet AB on the basis of their binding with healthy human platelets immobilized on plastic.

Institute of Experimental Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR. Department of Hematology, Central Postgraduate Medical Institute, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. I. Vorob'ev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 3, pp. 327-329, March, 1989. Original article submitted March 5, 1988.

0007-4888/89/0003-0359$12.50