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There Is No Relationship between the Activities of Mono- and Polyclonal Antirhesus Immunoglobulins *In vitro* and *In vivo*

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Monoclonal anti-D antibodies induce lysis of rhesus-positive erythrocytes in the antibody-dependent cytolysis test with blood mononuclear cells, the activity of the best antibodies being 10-15 times higher than of polyclonal IgG. The activities of anti-D antibodies in the cytolysis test with monocytes and lymphocytes varied: only one monoclonal antibody was highly active in both tests, whereas polyclonal IgG caused no lysis of erythrocytes in the cytolysis test with monocytes but was active in the same test with lymphocytes. Monoclonal antibodies intravenously administered to rhesus-negative recipients after transfusion of rhesus-positive erythrocytes stimulated their clearance, but its rate was slower than with polyclonal antibodies. Erythrocytes sensitized *in vitro* with monoclonal antibodies were completely removed from circulation within 3 h. It is not clear the results of which of the studied tests correlate with the capacity of anti-D immunoglobulins to block rhesus sensitization.

Key Words: *antirhesus immunoglobulin; monoclonal antibodies; antibody-dependent cytolysis; erythrocyte clearance; hemolytic disease of newborns*

Passive immunization of rhesus-negative women with antirhesus immunoglobulin during the first days postpartum effectively prevents rhesus sensitization and drastically decreases the incidence of hemolytic diseases of newborns. Although the prophylaxis has been carried out all over the world for about 30 years, the mechanism of immunosuppression is not clear [3]. It is believed that the effect is caused by accelerated clearance of erythrocytes (ER) of a rhesus-positive fetus from maternal circulation due to interactions between cells expressing Fcg-receptors with Fc-fragments of IgG antibodies on sensitized ER. *In vitro* target cells are destroyed by monocytes carrying FcgI-receptor and by K-cells with FcgIII-receptor by means of antibody-dependent cytolysis (ADC) [7,9]. The ADC test is considered as an accurate model of the mechanism of ER destruction in the organism [2,4].

Creation of monoclonal antirhesus immunoglobulin [1] involved study of its functional activity in comparison with the currently used polyclonal preparations whose efficacy is proven. However, lythic activity of immunoglobulins in variants of ADC did not correlate with their capacity to accelerate elimination of rhesus-positive ER from the circulation of rhesus-negative subjects.

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MATERIALS AND METHODS

Three human monoclonal anti-D IgG1 antibodies (Mab) (G-7, G-12, and G-48), which are produced by human/mouse heterohybridomas, and standard polyclonal anti-D IgG (LFB, lot 54050091) were used. For standardization, the immunoglobulins were diluted to the same initial concentration of 1250 ng/ml specific antibodies with a further dilution of 1:5. All experiments were carried out with the same mixture of mononuclear cells from 3 donors isolated on a cutting density gradient and frozen in aliquotes. Three variants of ADC were performed: with total mononuclear cell fraction, with adhesive cells (monocytes), and with nonadhesive cells (lymphocytes). Total cell fraction was placed 0.5×10^6 per well in a flat-bottom 96-well plate in 100 μl RPMI-1640 (Flow) with 10% fetal calf serum (Biokhim, Russia) and antibiotics (complete medium). For separation, mononuclears were plated in a dose of 10^6 per well, incubated for 1.5 h at 37°C in CO_2 incubator, after which nonadhering cells (about 50%) were transferred into a new plate, and to adhered cells 100 μl complete medium was added. Target cells were a mixture of rhesus-positive group A(II) ER washed three times. 100 μl of the sediment was incubated for 1 h at 37°C with 250 μCi ^{51}Cr and then washed three times. For ADC with monocytes, ER were presensitized. For this, 50 μl of the corresponding antibodies was incubated for 1 h at 37°C in a 96-well U-shaped plate with 50 μl of labeled ER (3×10^5/well). The plate was centrifuged, the supernatant discarded, and 100 ml of complete medium was added to ER. For ADC with total cells or lymphocytes, ER were treated with 0.1% bromelain solution for 10 min at room temperature and washed three times. To 100 μl of total mononuclears or lymphocytes 50 μl of diluted antibodies and 3×10^5/well labeled bromelain-treated ER in 50 μl of complete medium were added. To monocytes 3×10^5/well sensitized ER in 100 μl complete medium were added. The plates were incubated overnight at 37°C in a CO_2 incubator and the supernatant radioactivity was then measured. Mixtures of effector cells and ER without antibodies were the spontaneous lysis control; for determining the maximum lysis, ER were incubated with H_2O overnight. Specific lysis (SL) was calculated from the formula:

$$SL=\frac{^{51}Cr_{ad}-^{51}Cr_{sl}}{^{51}Cr_{ml}-^{51}Cr_{sl}}\times100\%,$$

where $^{51}Cr_{ad}$ is the chromium release in ADC test, $^{51}Cr_{sl}$ is the chromium release in spontaneous lysis, and $^{51}Cr_{ml}$ is the maximum chromium release. Three repeats per point were made.

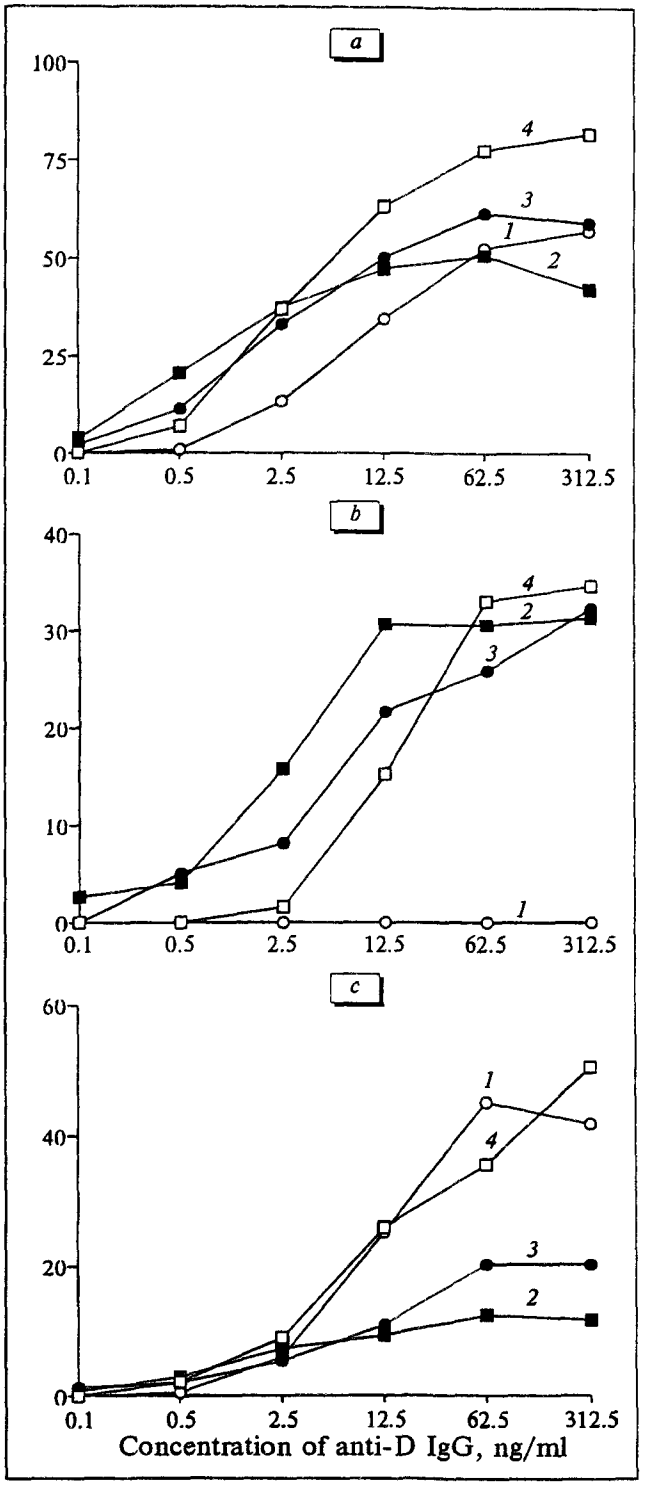

Fig. 1. Lysis of rhesus-positive erythrocytes by total mononuclear cell fraction (*a*), adhesive (*b*) and nonadhesive (*c*) cells in the antibody-dependent cytolysis test mediated by mono- and polyclonal antibodies. Polyclonal IgG (*1*); monoclonal IgG: G-48 (*2*), G-7 (*3*), and G-12 (*4*). Ordinate: specific lysis, %.

ER labeled with ^{51}Cr were intravenously injected to donors in a dose of 4 μl. To study autologous ER

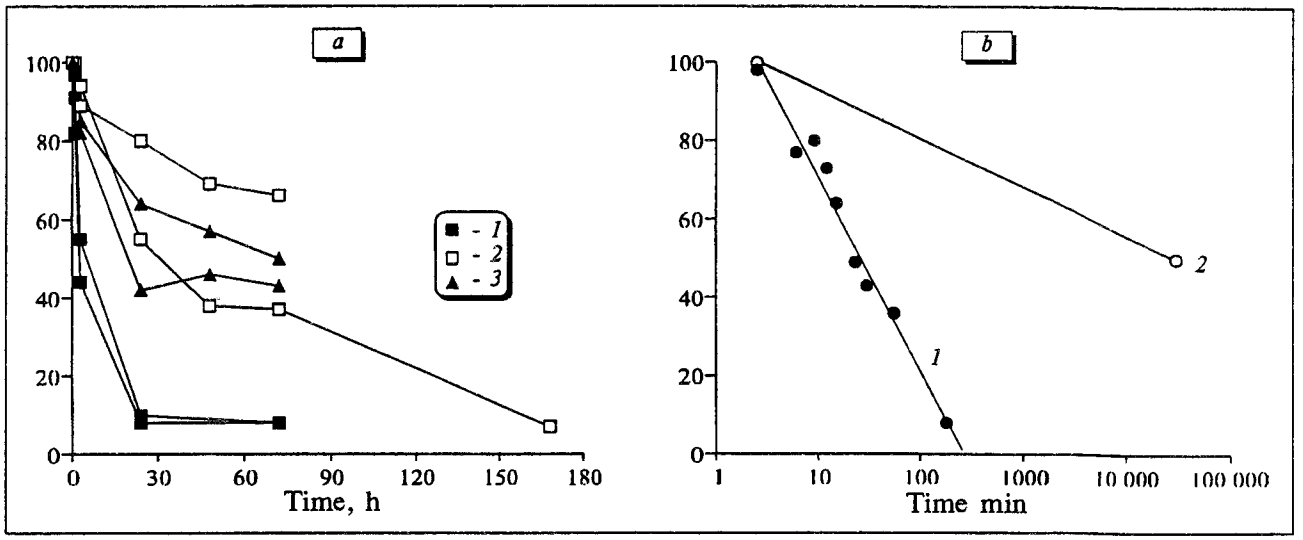

Fig. 2. Accelerated clearance of rhesus-positive erythrocytes from the circulation. *a*) after intravenous injection of different antirhesus immunoglobulins. Polyclonal IgG, 150 mg (*1*); monoclonal IgG in doses of 150 (2) and 300 mg (*3*). *b*) after transfusion of erythrocytes sensitized with monoclonal antibodies (*1*) *in vitro;* time course of elimination of non-sensitized erythrocytes is given for comparison (*2*). Ordinate: residual radioactivity in the blood, % of radioactivity 5 min after transfusion of erythrocytes.

clearance, these cells were presensitized with 150 μg Mab for 1 h at 37°C. For study of the clearance of allogenic rhesus-positive ER, rhesus-negative volunteers were intravenously injected with 150-300 μg of anti-D immunoglobulin 5 min after transfusion of ER. Blood was collected from the vein immediately before injection of immunoglobulin and 1 and 3 h and 1, 2, 3, and 7 days after it.

RESULTS

The activity of antirhesus IgG in different ADC variants is shown in Fig. 1. The greatest (80%) cytolysis was observed in experiments with total mononuclears, ED_{50} for Mab varying from 5 (G-12) to 12.5 (G-7 and G-48) ng/ml, while ED_{50} for polyclonal preparation was about 62.5 ng/ml (Fig. 1, *a*). Monocytes caused much lesser cytolysis (no more than 35%), polyclonal IgG was inert in this test, and Mab were equally effective (Fig. 1, *b*). By contrast, in the test with lymphocytes polyclonal IgG and Mab G-12 were the most active (Fig. 1, *c*). The data demonstrate that different ADC variants give results different in principle: in two variants the polyclonal preparation was notably inferior to all monoclonal ones, whereas in the test with nonadhesive cells it was (together with G-12 antibodies) obviously more active than other Mab. These differences cannot be explained by saturation of rhesus-positive ER with anti-D antibodies, because we have found (data not shown) that the same number of D-epitopes on ER membrane is available for all mono- and polyclonal IgG: about 20,000 per CDe/cDE phenotype ER and 9000 per CDe/cde ER [8].

The clearance of rhesus-positive ER in volunteers under the action of antirhesus IgG is demonstrated by Fig. 2. Our protocol (transfusion of ER followed by antibodies), in contrast to that used by other researchers [6], was maximally similar to clinical situation: a puerpera is preventively injected with anti-D after delivery, that is, after fetal ER enter the circulation. Although the polyclonal preparation more rapidly than Mab eliminated ER on day 1, after 7 days the level of remaining ER was the same after both preparations (Fig. 2, *a*). This results may be explained not by different mechanisms of clearance of ER sensitized by poly- or monoclonal antibodies, but by nonoptimal dose of Mab, because ER sensitized with G-48 antibodies in saturating concentration *in vitro* were completely eliminated in 3 h (Fig. 2, *b*).

Thus, we demonstrated for the first time that ADC test yields results contradicting those obtained *in vivo*. It is not clear which test reflects the capacity of antibodies to block the immune response and prevent rhesus sensitization. Just like in trials of polyclonal immunoglobulin [5], only trials in a large group of volunteers will help assess the prophylactic efficacy of monoclonal antirhesus IgG.

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DNA Damage in Mononuclear Blood Cells of Patients with Systemic Lupus Erythematosus

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Alkaline electrophoresis of DNA in individual cells (the DNA comet method) showed that the mean DNA damage is increased in blood mononuclear cells and the proportion of cells containing damaged DNA is higher in patients with systemic lupus erythematosus than in healthy donors; the number of hypodiploid cells is increased, indicating intensified apoptosis.

Key Words: *systemic lupus erythematosus; DNA damage; blood cells*

Cytogenetic studies showed that the incidence of spontaneous mutations and *in vitro* sensitivity of blood cells to mutagens in patients with systemic lupus erythematosus (SLE) [9] are increased [1]. The production of potentially mutagenous active oxygen species and oxidative damage to DNA are higher in the patients than in healthy subjects [4,6,14].

On the other hand, the occurrence of apoptosis increases in SLE and in some other autoimmune diseases [5,8,12,17]; apoptosis is a mechanism for elimination of defective aging cells and cells with numerous DNA injuries [13]. Apoptosis in such cases is associated with increased excretion of degraded DNA and histones into the extracellular space, which promotes the development of an autoimmune reaction [8]. Autoimmune antibody attack enhances DNA damage and accelerates the progression of the disease [10,11,13].

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The aim of this study was to assess DNA damage in peripheral blood mononuclear cells of SLE patients by alkaline electrophoresis of individual cell DNA.

MATERIALS AND METHODS

Blood was collected from normal subjects and patients with SLE (ACR diagnostic criteria [16]) - 13 women and 1 man hospitalized at the Institute of Rheumatology routinely treated by prednisolone (daily dose of 5-40 mg) without cytostatics.

Mononuclear cells were isolated from heparinized blood after 2- to 3-fold dilution with RPMI-1640 by centrifuging in Ficoll-Verograffin gradient (1.076-1.079 g/cm^3). Isolated cells were washed and suspended to a concentration of 10^6/ml in RPMI-1640 with 10% bovine serum. According to the Trypan Blue exclusion test, >95% cells were viable.

Cells were immobilized in agarose with a low melting point (agarose concentration 0.67%) used as a thin gel layer on a slide [3]. The final concentration of cells was $50\text{-}100\times10^3$/ml. Immediately after gel hardening, the slides were submerged into lysing

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