Changes in Substrate Adhesiveness in the Course of IgG- and C-Dependent Adhesion of Polymorphonuclear Leukocytes

I. V. Chelyshev and A. N. Mayanskii

UDC 616.155.3-008.939.624-07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, № 6, pp. 641-642, June, 1993
Original article submitted January 23, 1993

Key Words: *desorption; neutrophil adhesive reaction*

Understanding the mechanisms of polymorphonuclear leukocyte (PMNL) adhesion, a stage necessary for the realization of the effector potential of these cells, is one of crucial importance for the regulation of the neutrophil-dependent reaction [8]. PMNL are fixed to membranes with plasma membrane components acting as adhesins. Some of them provide ligand-receptor type interactions, while others are nonselective and make use of hydrophobic and electrostatic contacts. Adhesion is a stepwise process in which initiation, stabilization, and desorption stages may be distinguished [3]. Desorption is connected with cell mortality and appears to be an active process resulting from adhesive contact destabilization. To verify this hypothesis the authors used models of receptor-dependent (IgG- and C3b-mediated) and receptor-independent (electrostatic) neutrophil adhesive reactions, the regularities of which have been previously studied [3]. One of the possible desorption mechanisms, sorbent neutrophil-dependent modificator at the site of adhesive contacts, is analyzed here.

MATERIALS AND METHODS

PMNL were isolated from heparin-treated venous blood of normal subjects by centrifugation in a Ficoll-Verografin density gradient (Pharmacia, Sweden, and Spofa, Czechoslovakia) [4] and suspended in Hanks solution with 0.1% of human albumin (Reanal, Hungary). There were at least 98% PMNL in the isolated fraction, their viability being at least 96%, as indicated by the trypan test. Sepharose 4B (Pharmacia) bound to aggregated human IgG (Research Institute of Epidemiology and Microbiology, Nizhny Novgorod, Russia) was used as the substrate for IgG-dependent adhesion, C3b-dependent adhesion was investigated using Sephadex G-25 "Fine" (Pharmacia) conjugated with complement factor C3b. The preparation methods for these substrates have been described previously [3]. Receptor-independent adhesion was studied using DEAE Sephadex A-25 (Pharmacia). Prior to the investigation, Sephadex DEAE granules were kept at 100°C for an hour in Hanks solution, and after washing three times they were resuspended in the same solution.

PMNL were incubated with adsorbents for 30 min at 37°C in ratios providing the maximal intensity of the reaction: 95-100% of granules fixing at least 3 PMNL (positive granules).

For C3b Sephadex and IgG Sepharose this ratio was 300 cells per granule, for DEAE Sephadex 125 cells per granule. After three washings the granules with the cells adsorbed on them were resuspended in the initial volume and incubated at 37°C for 4 h until complete desorption of cells was attained. After washing of the granules, the sorption-desorption

Department of Microbiology, S. M. Kirov Medical Institute, Nizhny Novgorod. (Presented by A. D. Ado, Member of the Russian Academy of Medical Sciences)

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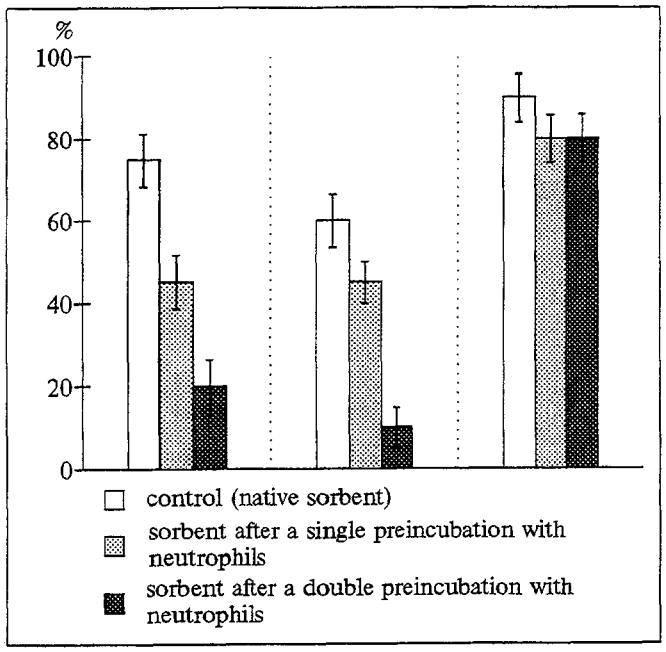

Fig. 1. Changes in adhesiveness of various sorbents after preincubation with neutrophils. Ordinate: share of sorbent granules with at least 3 neutrophils.

procedure was repeated once again in the same mode. PMNL were then added to the samples, 100-125 cells per granule, and after 30 min incubation at 37°C the percent content of positive granules was estimated. In control tests adsorbents incubated with Hanks solution were used. In some experiments exposure with PMNL was replaced by a 6-h treatment of adsorbents with supernatant obtained after a 4-h PMNL incubation with IgG Sepharose.

The Sepharose-fixed IgG content was assessed by enzyme immunoassay with anti-IgG conjugated with horseradish peroxidase (Sigma, USA). The results were assessed by luminol-dependent chemiluminescence [1].

RESULTS

The data presented in Fig. 1. show that after PMNL desorption the capacity of IgG Sepharose and C3b Sephadex for fixation of a new portion of cells was reduced. After two preincubations with PMNL, the IgG- and C3b-dependent adhesion values were, respectively, 16.0±9.0 and 11.0±5.4% vs. 74.0±9.5 and 67.0±4.9% in the control ($p<0.05$). The adhesion values were unchanged in experiments with a sorbent electrostatically fixing the cells (DEAE Sephadex). This means that the substrate ligand sites which fixed PMNL lost their activity in the course of adhesion. One may assume that the detected changes were related to PMNL factors (proteolytic enzymes, biooxidants) capable of IgG and C3b degradation [6-8]. During activation, PMNL are known to release their products into the environment and thus attack large objects inaccessible to phagocytosis. This seemed all the more likely because IgG- and C3b-dependent adhesion activated PMNL, thus mobilizing their effector potential [5].

Such a possibility was verified in experiments when, instead of exposure with PMNL, IgG Sepharose was treated with supenatant obtained after 4-h incubation of PMNL with the same sorbent. Figure 2 shows that this procedure did not reduce adhesion. The effects of the factors reducing substrate sorption capacity seemed to be restricted to the zone of cell ligand-receptor contacts with the target and were not observed beyond this zone. Substrate modification could be explained by removal of ligand molecules or their qualitative changes. These suppositions were checked using a model of PMNL IgG-dependent adhesion. Enzyme immunoassay showed that the content of Sepharose-conjugated IgG was unchanged after incubation with PMNL (Fig. 3); the sorbent's capacity for fixing PMNL wa significantly reduced in the same experiments. These results indicate that reduced adhesiveness of the sorbent was caused not by ligand molecule elimination but by changes in the active sites not involving the IgG-specific epitopes. It is possible that besides structural degradation of ligand sites they could be screened by

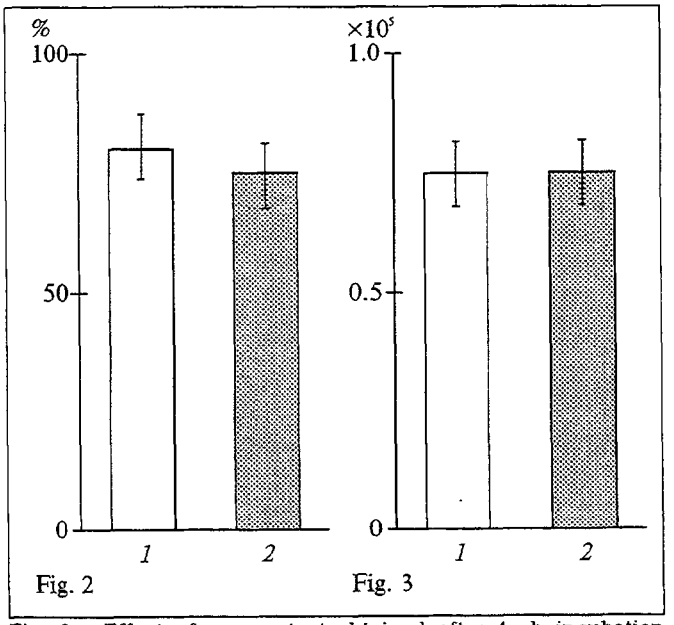

Fig. 2. Effect of supernatant obtained after 4-h incubation of neutrophils with IgG Sepharose on IgG Sepharose adhesiveness. 1) control; 2) IgG Sepharose exposed for 4 h to supernatant of a mixture of neutrophils with IgG Sepharose incubated for 4 h.

Fig. 3. IgG content on IgG Sepharose before and after two 4-h incubations with neutrophils, as shown by enzyme immunoassay with anti-IgG conjugate. 1) intact IgG Sepharose; 2) IgG Sepharose exposed to neutrophils. Ordinate: chemiluminescence intensity (cpm).

plasma membrane receptors which split off from the cells during desorption. The receptor "shedding" phenomenon after complementary structure fixation [2] underlies this hypothesis (which remains, however, to be verified).

Our conclusions based on model experiments may be of far-ranging importance. The endothelium is the most significant object for PMNL adhesive reactions in the body. Overcoming this barrier, the cells interact in succession with enditheliocytes and basal membrane components, adhering to them and disengaging themselves from the adhesive contacts. It is possible that here, too, the neutrophil-mediated modification of ligand structures determines the pattern of events.

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A Method for Primary Selection of Immunomodulators by Peritoneal Exudate Macrophage 5'-Nucleotidase Activity

G. B. Kirillicheva, M. S. Solov'eva, I. G. Baturina, and M. A. Tumanyan

UDC 615.275.4.015.4:612.017.1].07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, № 6, pp. 642–645, June, 1993
Original article submitted December 28, 1992

Key Words: *immunomodulators; 5'-nucleotidase; macrophages*

The problem of immunity modulation as acquired great significance of late because of the ever-increasing prevalence of immunodeficiency states of various origin. Quite a number of both natural and synthetic immunity modulators have been proposed to stimulate the organism's natural nonspecific resistance. The number of such modulators in increasing, complicating the investigation of the biological activity of these agents and the selection of the most effective of them.

N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences, Moscow (Presented by A. D. Ado, Member of the Russian Academy of Medical Sciences)

We have developed a method for primary selection of immunomodulato based on measuring the level of ecto-5'-nucleotidase (5'-n) activity of peritoneal exudate macrophages (PEM) [1]. The possibility of using this metabolic characteristic to assess the immunomodulating efficacy of drugs is based on the detected relationship between the type of immunomodulating effect and the trend of enzymological parameter changes. The activity of 5'-n has been found to change in different directions when exposed to immunosuppressives and immunostimulants.

Drugs characterized by immunostimulating activity are found to reduce PEM 5'-n activity, whereas immunosupressives, on the contrary, increase it. Drugs

0007-4888/93/0006-0723$12.50