EFFECT OF CONCANAVALIN A ON DIFFERENT TYPES OF ADHESIVE REACTIONS OF HUMAN NEUTROPHILS

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Reconnaisance reactions of phagocytes frequently take place on a basis of lectin-carbohydrate contacts [3, 10]. Many receptors, which are complex proteins, contain in their structure carbohydrate components, which largely determine specific recognition of the ligand [6]. Glycoprotein receptors control two basic types of regulation of phagocytes. The first type is connected with cytokines, eicosanoids, derivatives of complement, immunoglobulins, and hormones. The second path proceeds through adhesive contacts (for example, in the "neutrophil–endothelium" system) through a family of membrane molecules, some of which are identified with the aid of monoclonal antibodies and whose structure has been studied [9]. The possibility cannot be ruled out that in both cases the receptors work according to the principle of lectin–carbohydrate interactions.

The aim of this investigation was to study the relations of different variants of receptor-dependent (IgG- and C3b-mediated) and receptor-independent (adsorption on hydrophobic objects, on surfaces with an electrostatic charge) adhesion of human neutrophils, and exposure to concanavalin A (con A), one of the most thoroughly studied lectins, which binds selectively with carbohydrate radicals of the "α-D-mannose, α-D-glucose" type. It was shown previously that con A can interact with neutrophils, causing activation of respiratory metabolism [12].

EXPERIMENTAL METHOD

Human blood neutrophils were isolated on a two-step Ficoll–Verografin density gradient [5] (the purity of the populations 98%, their viability over 96%). IgG- and C3b-dependent adhesion of neutrophils was reproduced on sepharose 4B and Sephadex G-25 granules ("Pharmacia," Sweden), conjugated with aggregated IgG- and C3-factor of human complement respectively. The method of obtaining and standardization of the IgG- and C3b-sorbents and establishment of their ligand specificity were described previously [1, 4]. Hydrophobic adhesion of neutrophils was studied in a system with polymethyl methacrylate beads (PMA, diameter 60 μ). As the object on which to study cell adhesion to a surface with positive charge, we used DEAE Sephadex A-25 ("Pharmacia," Sweden). Adhesion of neutrophils was reproduced as follows. To 0.1 ml of a suspension of neutrophils in Hanks' solution ($2.5 \cdot 10^6$ ml^{-1}) we added 0.08 ml of a suspension of one of the sorbents: IgG-sepharose, C3b-Sephadex, DEAE Sephadex A-25 ($2 \cdot 10^4$ granules/ml), and PMA ($4 \cdot 10^3$ granules/ml). After incubation (37°C, 30 min, Periodic shaking) the samples were examined under the microscope under a coverslip (magnification 80), and the percentage of granules on which three or more neutrophils were adsorbed ("positive" granules) was estimated. To study the effect of con A, 0.02 ml of a solution of con A (up to a final concentration of between 5 and 100 μg/ml) was added to each system; 0.02 ml of Hanks' solution was added in the control. In a separate series of experiments, the neutrophils and IgG-sepharose were treated separately with con A, for which purpose neutrophils or IgG-sepharose were incubated with con A (50 μg/ml) for 30 min at 37°C, washed three times with Hanks' solution, and used for the reactions as described above.

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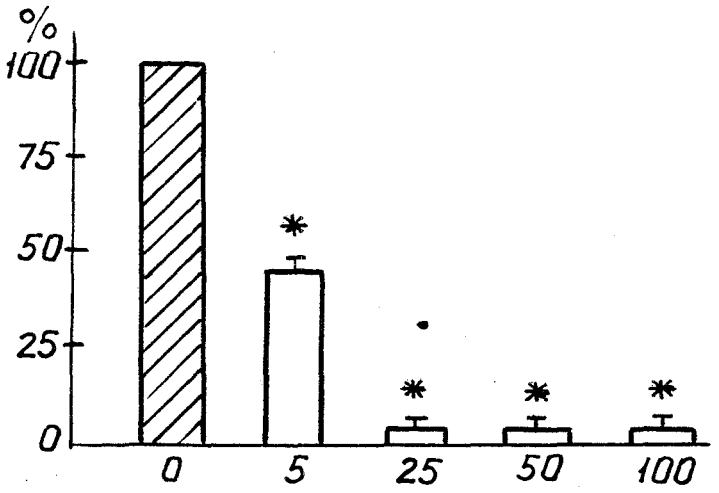

Fig. 1

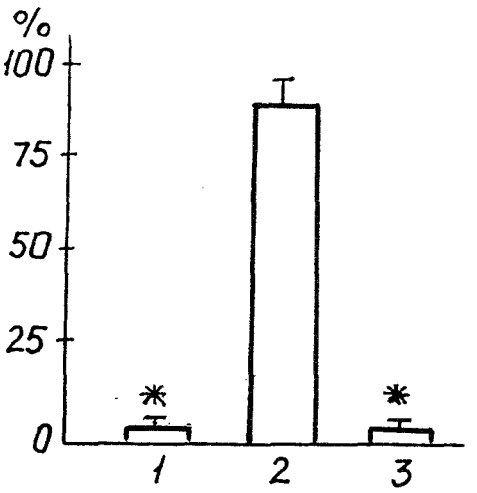

Fig. 2

Fig. 1. Inhibition of adhesion of human neutrophils on IgG-conjugated sepharose by concanavalin A. Results of eight experiments with neutrophils from different donors are shown. Abscissa, concentration of con A (in μg/ml); ordinate, number of "positive" IgG-sepharose granules (in % of control). Asterisk indicates significant differences compared with control ($p < 0.001$).

Fig. 2. Effect of concanavalin A (50 μg/ml) on IgG-dependent adhesion of human neutrophils. Separate treatment of cells and IgG-sepharose (based on results of five experiments with neutrophils from different donors). Abscissa, 1) addition of con A to mixture of neutrophils and IgG-sepharose; 2) preincubation of con A with IgG-sepharose, 3) preincubation of con A with neutrophils; ordinate, number of "positive" IgG-sepharose granules (in % of control). Asterisk indicates significant differences compared with control ($p < 0.001$).

EXPERIMENTAL RESULTS

The number of positive granules in the experiments without con A was 71.1 ± 5.4% for IgG-sepharose, 73.8 ± 5.9% for C3b-sepharose, 92.4 ± 5.1% for DEAE Sephadex A-25, and 78.4 ± 5.1% for PMA.

Con A in concentrations of between 25 and 100 μg/ml completely blocked IgG-mediated adhesion of neutrophils; in a concentration of 5 μg/ml the degree of inhibition of IgG-adhesion was 54.1% (Fig. 1). In experiments with other sorbents con A, even in maximal concentration (100 μg/ml) had no significant effect on adhesion.

The results of separate treatment of neutrophils and IgG-sepharose with con A (50 μg/ml) are given in Fig. 2. Preincubation of IgG-sepharose with con A had no significant effect on subsequent adhesion of neutrophils. Conversely, treatment of neutrophils with concanavalin A completely suppressed their binding with IgG-sepharose. This indicates that the target for con A was the neutrophil, but not aggregated IgG, adsorbed on sepharose.

Inhibition of IgG-mediated adhesion was due not only to the toxic effect of con A, for more than 94% of cells remained viable after incubation for 30 min (according to the trypan blue test).

The results of these experiments indicate that con A inhibits adhesion of human neutrophils. The effect is specific for IgG-mediated reactions and is due to the action of con A directly on the neutrophils. The effect of con A on neutrophils is known from investigations by other workers, who studied mainly stimulation of the respiratory reactions of the cells [12]; it was also shown that erythrocytes, treated with con A, form rosettes with human neutrophils [11]. Con A, fixed to sepharose, facilitates adhesion of neutrophils, which, with respect to their functional consequences (stimulation of the hexose monophosphate shunt), exceed C3-dependent adhesion [12]. This proves that neutrophils possess sites for binding con A, but does not enable their relations to the reconnaisance structures of the cell to be judged. Information has been obtained that con A can interact with Fcγ-receptors of human neutrophils. It has been shown, in particular, that con A reacts on the neutrophil membrane with the site for 3G8 monoclonal antibodies [11], which are a marker of FcγIII receptors (cD 16) [7]. Isolated FcγIII (but not FcγII)-re-

ceptors bind con A; the reaction is specifically blocked by α-methylmannoside [8]. It can accordingly be assumed that suppression of IgG-mediated adhesion by con A is coupled with blockade of the Fcγ-receptors of the neutrophil, due to screening of the carbohydrate radicals that are complementary to this lectin. This is confirmed by selectivity of the effect, which was absent not only in reactions with nonspecific sorbents, but also in experiments with another receptor-dependent substrate (adhesion to C3b-Sephadex). This means, first, that realization of IgG-adhesion depends exclusively on interaction with homologous receptors, not requiring connection of adhesive molecules, which are essential for other types of sorption. Second, this confirms the structural differences between the two main categories of "immune" receptors (Fcγ- and C3-receptors), emphasizing their functional capacity. A wider choice of lectins is required in order to characterize the spectrum of carbohydrates participating in the structural and functional organization of the receptor apparatus of the cells and, in particular, the Fcγ-receptors of the neutrophil. In this way a solution can be found to the problem of how specific the effect of con A is, and what is the role of different carbohydrates in expression of Fcγ-receptors and their individual types. It would also be interesting to study its effect on other IgG-mediated reactions, and also on the quantitative parameters of Fcγ-reception. This would enable the nature of the blocking effects, on which not only structural, but also functional inhibition of receptors may be based [2], to be judged.

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