IgG- and C3-DEPENDENT ADHESION OF NEUTROPHILS IN SYSTEMS WITH ALLOGENEIC AND XENOGENEIC LIGANDS

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Receptors for IgG and the C3 factor of complement are represented in various types of cells [3, 5, 10]. Phagocytes have been best studied in this respect, and interaction with immunoglobulins and complement is a very important method of realization of their effector potential. IgG and C3 behave as ligands for a spectrum of cellular receptors, which differ from one another in structure [4, 7, 10], epitopic profile [4, 5, 7, 10, 11], and affinity to submolecular elements of the ligand [5, 10]. For instance, at least four varieties of C3 receptors have been counted, each reacting predominantly with particular ligand centers of C3, which are revealed as it undergoes proteolytic degradation [10]. Different types of Fc_γ-receptors also are known and can be differentiation with the aid of monoclonal antibodies [5, 7], by their degree of affinity for different IgG subclasses [5], their interaction with soluble and aggregated forms of IgG [10], and sensitivity to priming agents [9].

One definite gap in our knowledge of recognition reactions of cells of the immune system is the almost complete absence of information about species differences in ligand-receptor interactions. This is partly due to the fact that in order to characterize Fc_γ- and C3-binding activity of human lymphocytes and phagocytes, it is usual to use preparations based on xenogeneic substrates (for example, EA- and EAC-rosette-formation with rabbit antibodies, mouse complement, etc.), i.e., the method itself implies rejection of the probability of intraspecific specificity of contacts of this type. When developing a model with which to study receptor-dependent adhesion of neutrophils, we directed our attention to the characteristics of adhesive reactions in systems with allogeneic and xenogeneic IgG.

The investigation described below revealed species-specificity of IgG- and C3-dependent reactions of human and rat neutrophils.

EXPERIMENTAL METHOD

Human and rat blood neutrophils were isolated on a two-step Ficoll–Verografin density gradient [2, 6] and the purity of the population obtained was 98% and its viability not below 96%. Rat exudative neutrophils were obtained from the peritoneal cavity 3 h after intraperitoneal injection of 5 ml of a 10% sterile solution of peptone; the cells, after washing and resuspension in Hanks's solution, contained no fewer than 85% of neutrophils (viability over 97%). Adhesion of the neutrophils induced through C3- and Fc_γ-receptors was reproduced on Sephadex G-25 and sepharose 4B granules ("Pharmacia," Sweden), conjugated with the C3-factor of complement and with aggregated human or rat IgG respectively. The method of obtaining C3- and IgG-sorbents was described previously [1]. Granules of the sorbents ($2 \cdot 10^4$ ml^{-1} were mixed in equal volumes (0.2 ml of each) with neutrophils ($2 \cdot 10^6$ ml^{-1}) and incubated for 30 min at 37°C, with periodic shaking. After washing to remove unbound neutrophils the percentage of sorbents (magnification 50) to which three or more cells were adherent was counted (Fig. 1). Each result is the average for a series of 10-20 experiments; the significance of the mean values and their differences was tested by Student's test.

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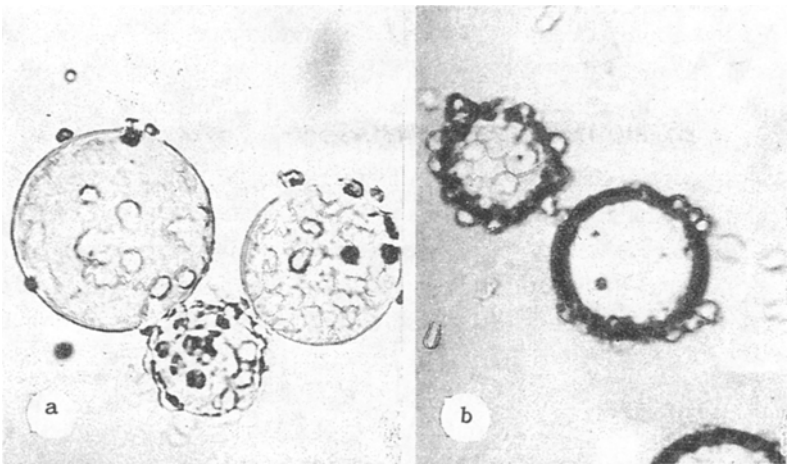

Fig. 1. Adhesion of human neutrophils to IgG-sepharose (a) and C3-Sephadex (b). Versions of allogeneic system (human IgG and C3): magnification 50.

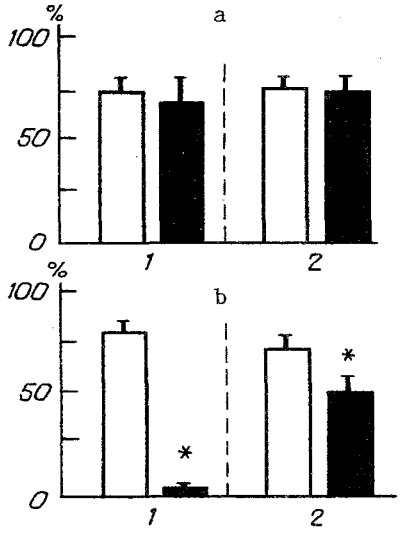

Fig. 2. Adhesion of human (a) and rat (b) neutrophils on IgG-sepharose (1) and C3-Sephadex (2) in allogeneic (unshaded columns) and xenogeneic (black columns) systems. Asterisk indicates that differences are significant. Ordinate, percentage of granules with three neutrophils or more.

EXPERIMENTAL RESULTS

The indicator of reactions of human neutrophils in an allogeneic IgG-system (interaction with sepharose, conjugated with human IgG) was 74.0 ± 3.7%. Approximately the same results were obtained for a xenogeneic system (sepharose bound with rat IgG), namely 69.1 ± 8.1% (Fig. 2). Rat neutrophils (peritoneal exudate, peripheral blood) were not adsorbed on sepharose conjugated with human IgG; in an allogeneic system the indicators of the reaction reached a value of 78.4 ± 2.8% (Fig. 2).

Human neutrophils reacted about equally in allogeneic and xenogeneic C3 dependent systems: the results were 76.1 ± 4.2 and 75.9 ± 6.3% respectively at $p > 0.05$ (Fig. 2). Reactions with rat neutrophils in an allogeneic C3-system followed a much more intensive course than in a xenogeneic system: 68.4 ± 2.8 and 45.9 ± 5.3% respectively (Fig. 2).

A number of general conclusions can be drawn from these results. First, the cell receptors can differentiate species-specific features of functionally similar ligands. In our experiments this was most evident for Fc_γ-receptors of rat neutrophils: they determined IgG-dependent adhesion in the allogeneic system but were inactive in reactions with human IgG. Second, the ability of the cells to receive xenogeneic mediators of the immune system differed in intensity in animals of different species. In our experiments the advantage of allogeneic combinations was exhibited significantly for rat neutrophils, but to a lesser degree for human neutrophils. We constantly produced adhesion of human neutrophils on a sorbent conjugated with xenogeneic (rat) IgG, but we always obtained negative results in the reaction of rat neutrophils with human IgG One explanation of this unexpected observation may be that human neutrophils have a broader spectrum of receptors receiving different structural details of IgG, Indirect confirmation of this is given by studies [4, 7] which showed that the Fc_γ-receptors of human neutrophils have a more complex set of epitopes than the same structures in the mouse: human and murine Fc_γ-receptors reacted with IV3 monoclonal antibodies, but only human receptors bound IV3, 3G8, and 4F7 monoclonal antibodies. It may be recalled also that xenogeneic immunoglobulins (rabbit antibodies) are usually used to indicate the Fc_γ-receptors of human phagocytes; the use of an allogeneic preparation does not change the result significantly [8]. Another explanation also may be put forward: ligand loci of IgG are nonhomogeneous; some of them, moreover, are complementary to species-specific receptors, whereas others go beyond the bounds of intraspecific homology Both these hypotheses require verification.

Finally, another conclusion which may be drawn from this study is that species-specific features are unequally expressed for different types of ligand-receptor interactions in the immunity system. We observed this for reactions of the neutrophil with derivatives of the C3-factor of complement which, unlike IgG-dependent adhesion, were manifested in both versions of xenogeneic test systems. This may perhaps reflect the common evolutionary roots of the material substrate of nonspecific immunity and the more marked structural conservatism of this system.

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