EFFECT OF IMMUNE COMPLEXES ON ELECTROPHORETIC MOBILITY OF SHEEP'S RED BLOOD CELLS

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Estimation of the electrophoretic mobility (EPM) of cells loaded with immune complexes (IC) with different physicochemical properties is the most widely used indirect method of assessing the charge of IC. The electric charge is one of the most important characteristics of the complexes because its magnitude and sign directly influence the localization of IC in the body [1, 8, 9]. Complexes deposited in tissues can undergo essential changes and, in particular, antibodies, antigens, and the components of complement may become attached to them [4, 10]. The presence of complexes in tissues depends mainly on anatomical and physiological factors and not on immunologic specificity [12]; consequently, the physical properties of IC are decisive in the process of deposition.

The aim of this investigation was to determine the effect of concentration of model immune complexes differing in molecular weight on the electrophoretic mobility of sheep's red blood cells (SRBC).

Heat-induced aggregates of human IgG (IgG*), obtained by heat treatment of the aggregates at 63°C for 20 min, were used as model immune complexes. Depending on the initial IgG concentration (from 0.5 to 6 mg/ml) aggregates were obtained in this way with a relative molecular weight (M/M_0, where M and M_0 denote molecular weights of the heat-induced aggregate and immunoglobulin respectively) of between 10 and 28, determined by laser nephelometry [6]. The concentration of these heat-induced aggregates in solution was estimated as in [3]. To separate the complexes from free IgG the method of 3, 4, and 10% PEG precipitation [13] was used. The standard suspension of SRBC in a concentration of 4×10^6 cells/ml was loaded with heat-induced aggregates at 37°C in the course of 45 min by the method of Levin et al. [5]. The same suspension of SRBC, but not loaded with IC, was used as the control. EPM of the cells was measured on the "Parmoquant-2" instrument (Carl Zeiss Jena, East Germany), with an electrophoretic current of 8 mA and at 20°C. The EPM of 50 cells was recorded in each test. Mean values of mobility are given in this paper.

Dependence of EPM of SRBC loaded with IgG* aggregates with different molecular weights, previously isolated by 3% PEG precipitation, on the concentration of complexes in solution, is shown in Fig. 1. Clearly with an increase in the IgG* concentration in the solution, linear relationships were observed for all the complexes used. The maximal increase in EPM of SRBC was observed in the case of loading with heat-induced aggregates of average molecular weights. The greatest rate of change of EPM of SRBC with an increase in concentration of the aggregates occurred on complexes with greater molecular weight.

In the next series of experiments dependence of EPM of SRBC on the size of the complexes loading them, isolated by 3, 4, and 10% PEG precipitation, with different initial IgG* concentrations in the solution, was obtained (Fig. 2). It follows from the results given that in the case of 3 and 4% PEG-isolation the greatest change in EPM was observed when complexes with average molecular weights were used (Fig. 2a, b). Deviation of M/M_0 from mean values toward an increase or decrease led to weakening of the effect of IC on EPM of SRBC. A different type of dependence was recorded for aggregates obtained with the use of 10% PEG precipitation (Fig. 2c). In this case an increase in the relative molecular weight of IC caused a decrease of EPM of SRBC loaded with these complexes. The magnitude of the change in EPM also depended on the initial IgG concentration. For nearly all sizes of heat-induced aggregates tested their action of EPM of the cells was stronger the higher initial protein content in the solution.

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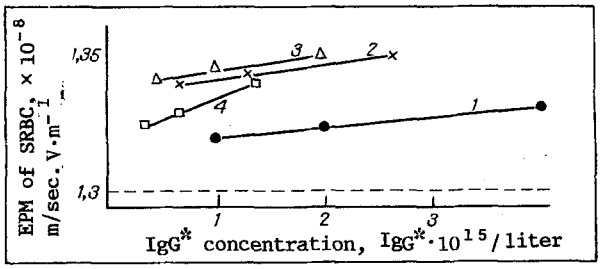

Fig. 1. Effect of concentration of heat-induced aggregates isolated by 3% PEG precipitation on EPM of sensitized SRBC for heat-induced aggregates of mean relative molecular weights (M/M_0): 1) 10, 2) 15, 3) 20, 4) 28.

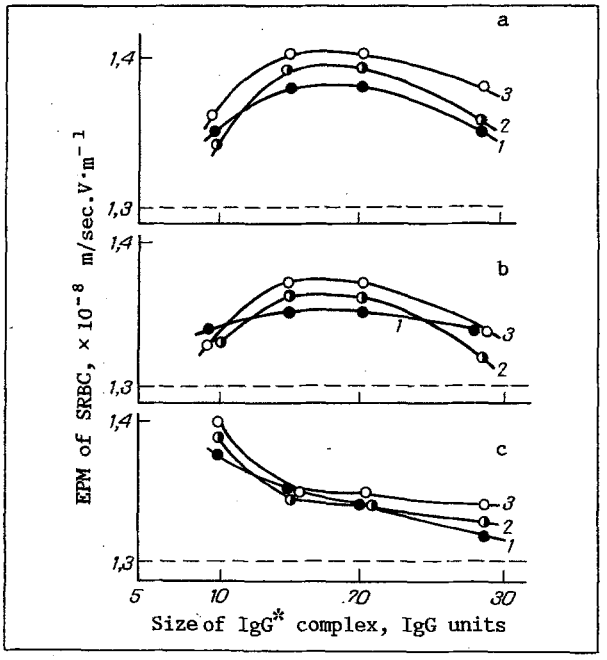

Fig. 2. Effect of size of IgG* complex, isolated by PEG precipitation, with different concentrations of PEG on EPM of SRBC: a) 3% PEG, b) 4% PEG, c) 10% PEG. Loading SRBC with IgG* complexes took place with their concentration as protein: 1) 0.25, 2) 0.5, 3) 1.0 g/liter.

The increase in EPM of SRBC with an increase in concentration of IgG* aggregates, and the nonlinear dependence of EPM on the molecular weight of the aggregate are evidently linked with the specific spatial structure of the complexes, incorporating different quantities of IgG. During aggregation a significant change is observed in the secondary, tertiary, and quarternary structures of IgG [2]. New intra- and intermolecular, hydrogen and Van der Waals bonds appear, and previously hidden additional active groups revealed on the surface of the aggregate. It can accordingly be postulated that with an increase in the number of IgG molecules present in the aggregate the density of the surface negative charge of the aggregate increases. Nonspecific binding of such aggregates, by an as yet unknown mechanism, with the membrane of SRBC may lead to an increase in its total surface negative charge and to a corresponding increase in electrophoretic mobility of the cells. This may probably explain the linear dependence obtained for EPM of SRBC on the concentration of complexes. The nonlinear dependence of EPM of SRBC on the molecular weight of IC is evidently attributable to instability of structure of large aggregates. This view is confirmed by our data on laser nephelometry [3] and data in [11]. The attachment of such aggregates (M/M_0 over 19) to an erythrocyte may lead to collapse of the complexes on the membrane and to a decrease in EPM of the cells.

The reduction of EPM of SRBC loaded with aggregates isolated at 4% compared with 3% PEG precipitation (Fig. 2a, b) can be explained on the grounds that with an increase in the PEG concentration, complexes of ever diminishing molecular weight are precipitated from the solution. With 10% PEG precipitation, unbound IgG, whose adsorption on the erythrocyte membrane leads to screening of the intrinsic negative charge of the membrane, is partly precipitated from solution. Since at physiological pH values the surface charge of the globulin molecules is 15-20 times less than the density of the surface charge of the erythrocytes [7], adsorption of free IgG of the membrane leads to a decrease in EPM of SRBC.

Thus the total charge of erythrocytes loaded with immunoglobulin aggregates depends both on the number of complexes adsorbed on it and on the dimensions of these complexes, the second of these factors being decisive.

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