INHIBITION OF PLATELET AGGREGATION BY IMMUNE COMPLEXES. II. EFFECT OF PREFORMED IMMUNE COMPLEXES ON PLATELET AGGREGATION

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Comparison of the results of clinical studies of parameters of immune complexes (IC) and of platelet aggregation (PA) in patients with coronary atherosclerosis showed negative correlation between the level of circulating IC detectable in a 7% solution of polyethylene glycol (PEG) and the degree of PA. It was accordingly decided to undertake a detailed study of the effect of IC on various parameters of PA in man.

The aim of the present investigation was to study the character of aggregation of human platelets when present in physiological concentrations *in vitro*, in response to addition of xenogenic IC and of IC formed with autologous plasma proteins, both in optimal proportions of antigen and antibodies for the formation of soluble IC and with an excess either of antigen or of antibodies.

EXPERIMENTAL METHOD

Citrated plasma and blood serum were obtained from nine patients with ischemic heart disease and three healthy subjects. PA was determined by the method in [2] in a "Chrono-Log" aggregometer (USA). The IC level was estimated by laser nephelometry [1]. Soluble IC with autologous proteins were obtained by adding different quantities (15, 30, and 90 µl) of monospecific rabbit antisera against human IgG and IgM (from Behringwerke, West Germany) to 0.45 ml of platelet-containing plasma and incubating the mixture for 1 h at 37°C. Xenogeneic IC were prepared by similar incubation of guinea pig blood serum with rabbit antiserum against guinea pig γ-globulin (from Behringwerke). To 10 µl of whole guinea pig serum or serum diluted in the ratios of 1 : 4 and 1 : 16, 100 µl of antiserum was added. The maximal IC level in 3.5% and 7% PEG solutions were obtained by the use of guinea pig serum in a dilution of 1 : 4. Xenogeneic IC in a volume of 30 µl were added to 0.45 ml of platelet-containing plasma and incubated for 30 min at 37°C immediately before the PA test was set up. Control samples of

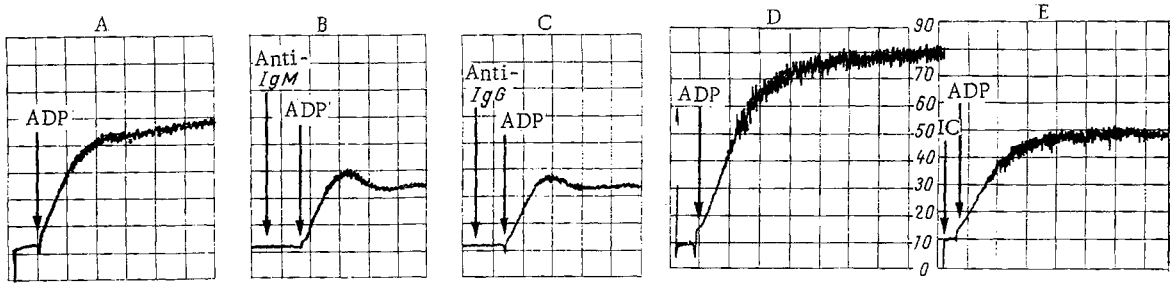

Fig. 1. PA induced by ADP (A, D) and its inhibition by IC with autologous IgM—anti-IgM (B) and IgG—anti-IgG (C) IC and by xenogeneic IC (E).

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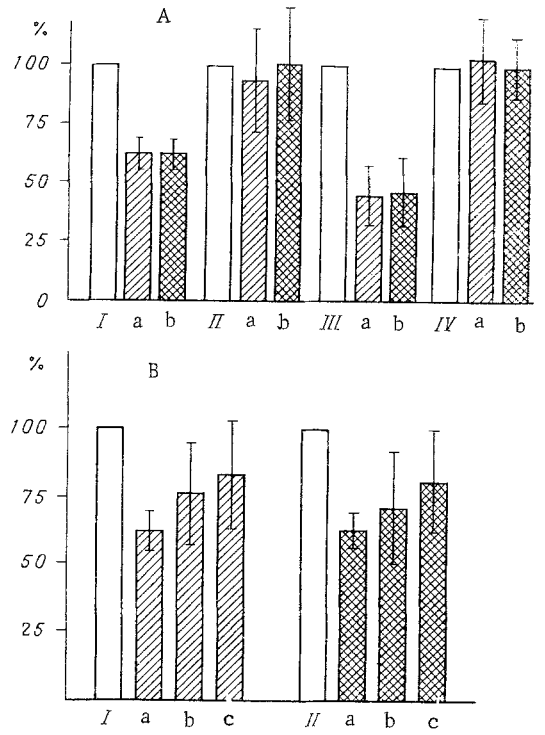

Fig. 2. Inhibition of PA under the influence of IC with autologous immunoglobulins. A) Inhibition of PA by IC: a) with autologous IgG—anti-IgG; b) with autologous IgM—anti-IgM (n = 12). I) Degree of aggregation; II) size of aggregates; III) aggregation time; IV) aggregation velocity. B) Inhibition of degree of PA by IC: a) with equivalent antigen—antibody ratio; b) in excess of antigen; c) in excess of antibodies (n = 3). I) IgG—anti-IgG; II) IgM—anti-IgM.

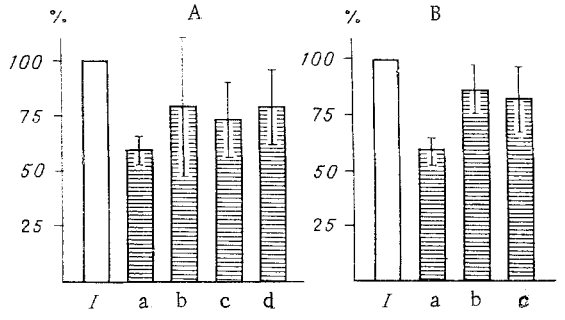

Fig. 3. Characteristics of inhibition of PA by xenogeneic IC. A) Inhibition of PA by xenogeneic IC (n = 5). a) Degree of aggregation, b) size of aggregates, c) aggregation time, d) aggregation velocity. B) Inhibition of degree of PA by xenogeneic IC (n = 3). a) With equivalent antigen—antibody ratio, b) in excess of antigen, c) in excess of antibodies.

plasma (without addition of IC) also were incubated at 37°C for 30 min. To determine any possible effect of complement on interaction between IC and platelets, in some experiments platelet-enriched plasma was adjusted to a physiological concentration with medium RPMI-1640 (from Serva, West Germany) or with autologous serum (native, inactivated at 56°C for 40 min, activated by corpuscular IC, and activated by zymosan).

EXPERIMENTAL RESULTS

Incubation of platelet-containing plasma with anti-IgG and anti-IgM did not lead to spontaneous PA in any experiment. However, on addition of ADP to these samples of plasma, a considerable and significant reduction in the degree and time of PA was observed, compared with values in control samples of plasma to which antisera against immunoglobulins had not been added (Figs. 1 and 2). On incubation with the optimal quantity of anti-IgG (30 µl) the degree of aggregation was reduced to 62 ± 3.3% of the initial PA level without antisera, the aggregation time was reduced to 44 ± 5.7%, and the size of the aggregates to 94 ± 10.2%; conversely, the rate of aggregation was increased to 104 ± 8.3% of the initial value. Similar changes were found with anti-IgM antiserum. In the presence of an excess of antigen (addition of 15 µl antiserum) and of antibodies (90 µl antiserum) PA was inhibited less than when antigen and antibody were present in optimal proportions (Fig. 2). An excess of antibodies caused least inhibition of PA.

Maximal inhibition of PA was observed also after addition of xenogeneic IC formed with antigen and antibodies in optimal proportions, i.e., in a dilution of 1 : 4 (Figs. 1 and 3). Inhibition of PA also was reduced by an excess of antigen and of antibodies.

Dilution of the platelet-containing plasma with medium RPMI-1640 and also with sera containing activated or inactivated complement did not lead to inhibition or intensification of PA and did not affect inhibition of PA by immune complexes.

When the methods described previously were used, induction of human PA was observed only in the case of the action of precipitated IC or aggregated γ-globulin, with prolonged incubation for 18-20 h at 5°C, and with visual assessment of the degree of aggregation [5-7]. Under these conditions the direct aggregating action of immunoprecipitates and cryoglobulins on platelets cannot be ruled out. Analysis of ADP-induced PA in the aggregometer revealed an additional potentiating effect only of insoluble IC [3], and also of aggregated IgG_1, IgG_2, IgG_3, and IgG_4, but not of IgE, IgA, IgD, or IgM [4], on aggregation.

Inhibition of PA by soluble IC is thus evidence of the existence of a hitherto unknown phenomenon of platelet blocking by a high concentration of soluble immune complexes; this is evidently of great physiological importance for protection of the endothelial layer of blood vessels and for its resistance to platelets.

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