INTERACTION OF FIBRONECTIN WITH POLYMORPHONUCLEAR LEUKOCYTES UNDER NORMAL CONDITIONS AND IN ANAPHYLACTIC SHOCK

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Fibronectin, a high-molecular-weight glycoprotein circulating in blood and other biological fluids, is known as an opsonin with a broad spectrum of action [2, 6]. Receptors for fibronectin have been on the cell surface of peripheral blood macrophages [4, 5] and alveolar macrophages [3]. It has been suggested that fibronectin can play the role of opsonin for phagocytic reactions involving polymorphonuclear leukocytes (PNL) also, although the participation of fibronectin in responses of neutrophilic phagocytosis remains virtually unstudied. Moreover, the role of fibronectin in the pathogenesis of reactions of delayed-type hypersensitivity, which are accompanied by marked changes in structure and function of phagocytes, is not known.

In the investigation described below interaction of fibronectin with PNL was studied under normal conditions and during bacterial sensitization at the height of anaphylactic shock.

EXPERIMENTAL METHOD

Interaction of fibronectin with PNL was studied by the method developed by the writers previously [1]. Purified homologous fibronectin was bound with gelatin-sepharose granules (FGS), and these were then incubated with PNL under different conditions. After 30 min a sample of the suspension was taken for microscopic investigation in a drop. Gelatin-sepharose granules without fibronectin (GS), albumin-sepharose, and pure sepharose 4B were used as the controls.

Fibronectin was obtained from blood plasma or serum by affinity chromatography on GS [6]. The purity of the preparation was verified electrophoretically.

To obtain PNL, blood from healthy donors was collected into a flask with heparin in a final concentration of 5 U/ml. The heparinized blood was layered above a Ficoll–Verografin mixture with specific gravity of 1.114 g/ml and centrifuged at 250 g for 35 min. The layer of PNL was drawn off and washed twice with Hanks' solution without Ca^{++} and Mg^{++} ions. Contaminating erythrocytes were removed by lysis in hypotonic (0.1%) NaCl solution. Homogeneity of the cell suspension was tested in films stained by the Romanovsky–Giemsa method. The viability of the cells was assessed by their ability to take up trypan blue. PNL accounted for 93-98% of all the cells, and 87-95% of them were viable.

PNL of animals (rabbits weighing 2.5-3 kg) were obtained by sedimentation of arterial blood taken on EDTA and mixed with dextran T-500. After incubation for 1 h at 4°C the supernatant, containing leukoytes and a few contaminating erythrocytes, was drawn off. The leukocytes were washed twice by centrifugation in a solution of rabbit albumin (1% albumin, 0.01 N EDTA in Hanks' solution). The final residue was suspended in a 1% solution of albumin in Hanks' solution to a concentration of 2×10^7 cells in 1 ml, and layered above a Ficoll–Verografin mixture with specific gravity of 1.114. After centrifugation for 35 min at 250g the layer of neutrophils (87-95% purity) was drawn off, viability was tested, and the cells

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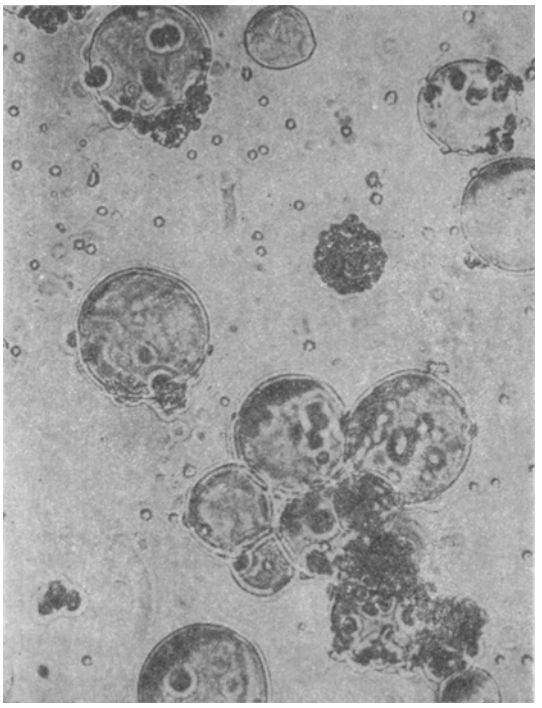

Fig. 1

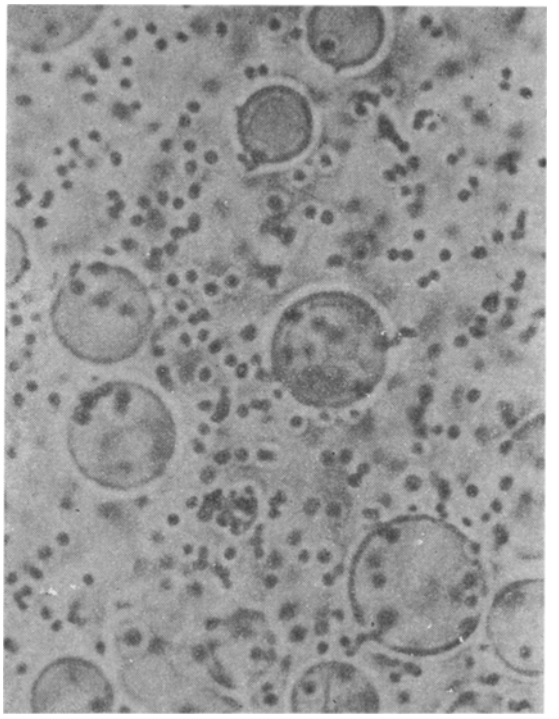

Fig. 2

Fig. 1. Adhesion of intact rabbit neutrophils to FGS granules in a drop (200×).

Fig. 2. Absence of adhesion of rabbit neutrophils taken at the height of anaphylactic shock to FGS granules in a drop (200×).

were suspended in 1% albumin up to a concentration of 2×10^6 cells/ml. Rabbit PNL were isolated in the presence of EDTA in order to prevent the cells from aggregating.

The PNL were trypsinized by incubation in Hanks' solution containing trypsin in a concentration of 1 mg/ml at 37°C for 30 min, after which the cells were washed with a 1% solution of albumin in Hanks' solution. The viability of the cells after incubation with trypsin was 80-85%.

The rabbits were sensitized by subcutaneous injection of a preparation of ultrasonically disintegrated Staphylococcus aureus, containing 1 mg protein and mixed with 10 mg of dextran sulfate and 0.5 mg of dry autoclaved BCG vaccine. On the 21st-25th day anaphylactic shock was induced in the sensitized animals by intravenous injection of disintegrated Staph. aureus cells in a dose of 5 mg protein/kg body weight. The anaphylactic reaction reached its peak intensity after 5-10 min. Blood was taken from the carotid artery before and 7 min after injection of the reacting dose of antigen.

EXPERIMENTAL RESULTS

Incubation of intact human peripheral blood neutrophils with FGS granules in the presence of Ca^{++} and Mg^{++} led to two types of interaction: most PNL were present in the form of aggregates, and a minority of the cells were attached to FGS granules, causing the individual granules to adhere to each other through the cell (Fig. 1). Heparin had no appreciable effect on these processes. Most rabbit neutrophils, whether in intact animals or in sensitized animals before injection of the reacting dose of antigen, were attached to FGS granules, and spread out over their surface. Adhesion of human and rabbit PNL to FGS definitely depended on bivalent cations: in the presence of Ca^{++} ions (0.01 M) the fraction of bound cells was 40-50%, and in the presence of Mg^{++} ions (0.01 M) it was 80-90%. Addition of EDTA (0.05M) to the incubation medium depressed aggregation and adhesion of the neutrophils to FGS granules.

At the height of anaphylactic shock the rabbit PNL lost their ability to adhere to FGS granules irrespective of whether bivalent cations were present or not (Fig. 2).

On incubation of human PNL with GS granules no visible changes took place. However, after 15-20 min some neutrophils were adherent to the surface of the GS granules. In the presence of heparin interaction of the cells with GS granules was accelerated and was completed in the course of 1-2 min. Bivalent cations had no effect on this process. No aggregation of neutrophils was observed. Unlike human PNL, rabbit neutrophils obtained either from intact or from sensitized animals, did not react with GS. Cells obtained at the height of anaphylactic shock were unable to bind with GS granules, as before.

Treatment of the PNL with trypsin suppressed all the interaction of the cells described above both with one another and with FGS and GS granules.

Control incubation of intact human and rabbit PNL with albumin-sepharsoe and pure sepharose 4B granules led to adhesion of single cells, which was independent of the presence of heparin and of Ca^{++} and Mg^{++} ions.

The ability of intact human and rabbit neutrophils to bind with FGS granules is evidence that regions with high affinity for fibronectin are present on the surface of these cells. These regions consist evidently of proteins, for interaction of this kind disappears after treatment of the cells with trypsin. Considering the existence of receptors for fibronectin on the surface of monocytes [4, 5], it can also be postulated that receptors for fibronectin exist on the surface of neutrophils. This marked dependence of adhesion of PNL to FGS on CA^{++} and Mg^{++} ions indicates that chelate complexes may be formed between the receptor proteins and fibronectin.

The fact that during anaphylactic shock, under conditions of bacterial sensitization, the receptors lose their ability to bind with fibronectin deserves special attention. This observation can be interpreted in different ways. Very probably a change in the functional state (desensitization) of the receptors takes place under the influence of mediators of allergic reactions, such as lymphokines, histamine, serotonin, etc. The possibility likewise cannot be rules out that receptors, together with ingested particles and fragments of membranes, exist within the cell in the form of phagosomes, or they are detached together with regions of the membrane during secretion of lysosomal granules into the extracellular space. In anaphylaxis both these processes are known to take place. In addition, receptors can be destroyed by lysosomal hydrolases, just as, in the present experiments, trypsin had a similar action. As regards possible saturation of the receptors with fibronectin adsorbed from the blood plasma, this is not confirmed by the absence of interaction between "shock" PNL and gelatinized granules. Irrespective of the mechanism of this phenomenon, it can be postulated that a definite role in the pathogenesis of sensitization and anaphylaxis is played by fibronectin and its cell receptors. This role is linked above all with disturbance of the mediator function of fibronectin in processes of cell adhesion and phagocytosis. A change in the functional state of the fibronectin receptors is a new consequence of the well-known fact that the outer membrane of the PNL is reorganized in delayed-type hypersensitivity reactions.

As regards the ability of intact human PNL to bind with GS granules, this is most likely due to the presence of fibronectin, adsorbed from the blood plasma and (or) synthesized by neutrophils on the surface of the cells. The absence of interaction between rabbit PNL and GS in the present experiments can be explained on the grounds that the rabbit PNL were isolated in the presence of EDTA, which causes dissociation of fibronectin from the cell surface.

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