ALVEOLAR MACROPHAGE–FIBRONECTIN INTERACTION IN SENSITIZATION AND ANAPHYLACTIC SHOCK

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One of the main functions of cells of the mononuclear phagocyte system is phagocytosis, which involves the participation of receptors on the outer membrane. Besides such well studied receptors as Fc and C3, the presence of receptors for fibronectin, a blood glycoprotein with high opsonic activity [3], also has been demonstrated on the surface of monocytes which are immediate precursors of macrophages. The presence of structures capable of interacting with fibronectin also has been demonstrated on the surface of neutrophils [1] and alveolar macrophages [2], evidence that this protein participates in phagocytosis. Meanwhile the character of interaction of fibronectin with macrophages, especially when their functional state is altered under pathological conditions, has not been studied.

The aim of this investigation was to study the effect of sensitization and anaphylactic shock on interaction between alveolar macrophages (AM) and fibronectin and to assess the effect of specific antigen and histamine on this interaction.

EXPERIMENTAL METHOD

AM were obtained from rabbits by washing out the bronchi [5] and centrifugation of the washings in a mixture of Ficoll and Verografin (ρ = 1.082 g/ml) and washing three times in Hanks' solution without Ca^{++} and Mg^{++}. The resulting suspension contained 95-98% of AM, the viability of which, as shown by the methylene blue exclusion test, was 90-95%; all manipulations with AM were carried out in siliconized glassware.

Animals weighing 2-2.5 kg were sensitized by subcutaneous injection of bovine serum albumin (BSA, from Reanal, Hungary) in a dose of 0.0035 g/kg three times at intervals of 24 h. Anaphylactic shock was induced on the 21st day after the beginning of sensitization by intravenous injection of 0.007 g/kg of the same antigen. The AM were obtained at the height of anaphylactic shock (7-10 min after injection of the antigen).

Granules of fibronectin-gelatin-sepharose (FGS), obtained by adsorbing fibronectin from rabbit serum on gelatin-sepharose (GS) [6], were used as the fibronectin-coated surface. After being mixed with serum the granules were washed in a siliconized glass column measuring 1 × 5 cm with 0.05 M Tris-HCl buffer, pH 7.4, and then with 1 M NaCl in the same buffer, with monitoring of E_{280} up to the value of E_{280} = 0.01. Before use the FGS granules were equilibrated with Hanks' solution free from Ca^{++} and Mg^{++} ions. Control elution showed that under the influence of 4 M urea mixed with 1 M NaCl, 0.6 mg of fibronectin bound with 1 ml of GS; the purity of the fibronectin was estimated by immunoelectrophoresis and SDS-electrophoresis before and after reduction of the disulfide bonds. During immunoelectrophoresis by the method of Grabar and Williams, using polyvalent goat antiserum against rabbit blood serum proteins (from the N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR), besides the main fibronectin fraction, traces of albumin and gamma-globulins also were found as impurities. During SDS-electrophoresis with 4% polyacrylamide gel (PAG) fibronectin was found as a fraction with mol. wt. of 450,000 daltons. After rupture of the disulfide bonds with 2-mercaptoethanol (from Lola-Chemie, Austria) this fraction disappeared, but under these circumstances a protein appeared with mol. wt. of 220,000 daltons,

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TABLE 1. Interaction of AM with FGS Granules (in %, M ± m)

Incubation medium	AM					
	of intact animals	of sensitized animals	of "shocked" animals	of sensitized animals + 0.01% BSA	of sensitized animals + 10^{-3} M histamine	of sensitized animals + 0.01% HSA
Hanks' solution without Ca^{++} and Mg^{++}	8±2	8±1	7±1	6±2	7±3	8±2
with Ca^{++} and Mg^{++}	62±12	46±5	8±0,5	10±3	9±2	43±3
with Mg^{++} and heparin	35±13	30±4	11±1	11±3	10±2	32±1

Legend. Here and in Table 2 index of adhesion is shown: It was calculated as the ratio of the number of cells adherent to granules to their total number, counted on no fewer than 200 cells.

TABLE 2. Interaction of AM with GS Granules (in %, M ± m)

Incubation medium	AM					
	of intact animals	of sensitized animals	of "shocked" animals	of sensitized animals + 0.01% BSA	of sensitized animals + 10^{-3} M histamine	of sensitized animals + 0.01% HSA
Hanks' solution without Ca^{++} and Mg^{++}	9±2	7±3	5±1	6±1	7±1	6±2
with Ca^{++} and Mg^{++}	17±2	12±2	6±2	7±1	9±1	12±1
with Ca^{++}, Mg^{++}, and heparin	23±1	21±3	6±1	7±1	8±1	20±2

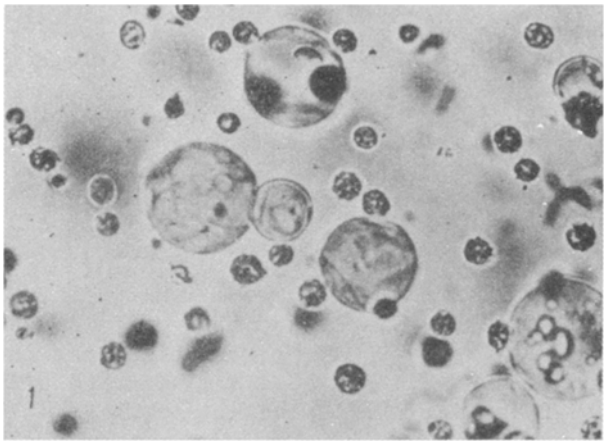

Fig. 1. Interaction between AM obtained from intact animals and FGS granules (200×).

and it corresponded completely in its structural characteristics to fibronectin. The degree of purity of the preparation, which was determined after densitography of the gels after electrophoresis, was 90-95%.

For the control, GS granules, albumin-sepharose (AS) and pure sepharose 4B, equilibrated with the same solution, were used.

To 0.1 ml of a suspension of intact or trypsinized AM (10^5 cells) 0.1 ml of a suspension of FGS granules or the control granules was added, and the total volume was made up to 0.5 ml, by adding Ca^{++} and Mg^{++} in a final concentration of 0.02 M and (or) 10 U/ml of heparin to the system. The ratio of the number of cells to the number of granules in the mixture was about 10:1. After incubation at 37°C for 30 min the suspension was withdrawn for microscopic examination in a drop.

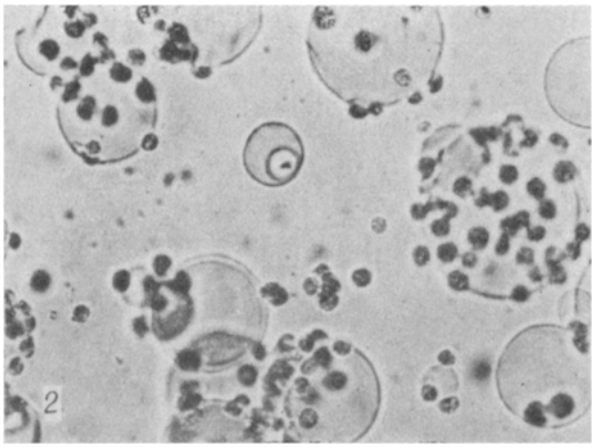

Fig. 2. Absence of binding of AM obtained from animals at height of anaphylactic shock with FGS granules (200×).

The AM were trypsinized by incubation of the cells in Hanks' solution containing trypsin (from Spofa, Czechoslovakia) in a concentration of 1 mg/ml (16-18 U), after which the cells were washed with Hanks' solution.

The AM obtained on the 21st day after the beginning of sensitization, without injection of the reacting dose of antigen, were incubated for 30 min with one of the following solutions: 0.01% BSA, 0.01% human serum albumin (HSA, from Reanal), or 10^{-3} M histamine dihydrochloride (from Polfa, Poland). The cells were then washed three times with modified Hanks' solution and suspended in the same solution up to a concentration of 10^6 cells/ml.

EXPERIMENTAL RESULTS

Data on interaction between AM of the intact and sensitized animals and FGS and GS, respectively, are given in Fig. 1. Incubation of AM obtained from intact rabbits with FGS granules led to the most marked adhesion of the cells to the granules when Ca^{++} and Mg^{++} ions were added to the medium; AM obtained from sensitized animals behaved in the same way (Table 1).

Alveolar macrophages of intact and sensitized animals adhered more completely to GS granules when Ca^{++} and Mg^{++} ions and heparin were added simultaneously to the medium (Table 2). Trypsinization of the cells depressed both types of interaction.

AM obtained at the height of anaphylactic shock, unlike intact cells, virtually did not adhere to FGS and GS granules irrespective of addition of Ca^{++} and Mg^{++} ions or heparin (Fig. 2). AM obtained from animals on the 21st day of sensitization, incubated with BSA or histamine solution (primed AM) behaved similarly. Contact with HSA did not affect adhesion of these AM to a surface covered with fibronectin or gelatin.

AM of intact, sensitized, "shocked" animals, and also primed AM did not bind with AS granules or sepharose 4B granules whether bivalent cations and heparin were present or not. The cells and granules were located on different planes and exhibited no mutual affinity.

AM obtained from animals with a fully developed picture of anaphylactic shock were thus unable to bind with fibronectin, whereas AM from intact and sensitized animals could interact in this way. The specificity of binding of AM with fibronectin was confirmed indirectly by the absence of adhesion of the cells under analogous conditions to AS and sepharose 4B granules, although binding may perhaps be based on heterogeneous and noncovalent interactions. The structures responsible for adhesion were evidently protein in nature, for binding disappeared after treatment of the cells with trypsin. On this basis it can be concluded that, on the surface of AM there are sites with affinity for fibronectin, which become insensitive as a result of the development of anaphylactic shock. The similarity of this phenomenon to that observed on incubation of AM with BSA or with histamine is evidence of a possible role of specific antigen and mediators of anaphylaxis in the phenomenon of desensitization of the cell membrane to fibronectin. This conclusion is confirmed by the fact that AM of sensitized animals remain capable of fibronectin-mediated adhesion after incubation with HSA.

Loss of the ability of AM at the height of anaphylactic shock to adhere to GS granules is another important fact. Interaction of AM of intact and sensitized animals with GS granules, dependent on heparin and sensitive to the action of trypsin, confirms the presence of membrane-bound fibronectin on the surface of AM, as was demonstrated previously [7].

These phenomena may be connected with the following processes which accompany anaphylaxis: 1) sites on the cell membrane responsible for adhesion may be absorbed inside the cell during endocytosis; 2) these sites may be shed during the secretion of biologically active substances by macrophages; 3) surface proteins, which include membrane-bound fibronectin, may undergo digestion through the action of proteases, secreted by macrophages and other cells; 4) during interaction of AM with antigen or histamine a redistribution of surface receptors takes place along with, possibly, a conformational change in the sites of affinity for fibronectin into an inactive form. These processes evidently take place simultaneously, and as a whole they lead to inability of AM to interact with fibronectin and with gelatinized granules.

Loss of the ability of the cells to interact with fibronectin after treatment with histamine is in agreement with data on the mutual influence of Fc- and H-receptors [4], which amounts, in particular, to temporary depression by histamine of the ability of Fc-receptors to bind their own ligand.

The investigations show that anaphylactic shock causes changes in the functional state of the outer cell membrane of AM, expressed as disturbance of interaction between macrophages and fibronectin. This phenomenon is probably due to the action of anaphylaxis mediators, primarily histamine, on macrophages, although the possibility cannot be ruled out that AM are subject to the influence of nonspecific processes which accompany the shock reaction.

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