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FIBRONECTIN AND ITS RECEPTORS ON THE SURFACE OF POLYMORPHS

O. D. Zinkevich, R. I. Litvinov, and M. S. Kuravskaya

UDC 612.112.31:612.115.1

KEY WORDS: fibronectin; polymorphs; cell surface.

The surface of monocytes has receptors for fibronectin [2], a fact that is closely linked with its function as a nonspecific opsonin. There is reason to suppose that fibronectin can perform the role of opsonin also during phagocytosis of disintegrated tissues, collagen, and fibrin by polymorphonuclear neutrophils. However, the role of fibronectin in the regulation of neutrophilic phagocytosis has not yet been studied and, in particular, there is no information on the existence of receptors for fibronectin on the surface of polymorphs.

The object of this investigation was to study interaction between fibronectin and polymorphs, and so to obtain evidence of the existence of receptors for fibronectin and fibronectin itself on their surface.

EXPERIMENTAL METHOD

Since fibronectin binds with cells only when in an immobilized state [4], as fibronectin-covered matrix we used fibronectin–gelatin–Sepharose (FGS). Gelatin–Sepharose (GS) was obtained by the method in [6] by attaching gelatin (from Kepf, West Germany) to CnBr-activated Sepharose 4B (from Pharmacia, Sweden). After equilibration of the column with GS (2·5 cm) with buffered physiological saline (BPS), pH 7.4, containing 0.1 M sodium citrate, 150 ml of fresh pooled human citrated blood plasma was passed through it. After the column had been rinsed with BPS and 1 M urea in BPS to remove plasma (verified at E_{280}), the column was equilibrated with Hanks' solution containing (20 units/ml) or not containing heparin. As a result, pure (95%) fibronectin was found in the absorbed state on the GS column, as was confirmed by the results of electrophoresis in polyacrylamide gel in the presence of sodium dodecylsulfate after elution of the protein with 4 M urea.

Human polymorphs were obtained from heparinized blood (20 units/ml) by centrifugation in a Ficoll–Verografin mixture (ρ = 1.114 g/ml). In this way a suspension of viable (as regards ability to phagocytose trypan blue) polymorphs of 98% purity was obtained.

A suspension of FGS granules (0.1 ml) was mixed with a suspension of polymorphs with a concentration of $2 \cdot 10^6$ cells/ml in 2% albumin solution in buffered Hanks' solution with heparin (20 units/ml) or without it. The mixture was incubated for 30 min at 37°C with mixing, after which it was examined under the microscope. GS granules and pure Sepharose 4B, treated in the same way, were used for the control.

In some experiments EDTA was added to the reaction mixture in a final concentrattion of 0.05 M.

The polymorphs were trypsinized by incubating them in Hanks' solution containing trypsin (from Spofa, Czechoslovakia) in a concentration of 1 mg/ml, at 37°C for 30 min, followed by rinsing to remove the enzyme.

Laboratory of Immunology and Biochemistry, Research Institute of Epidemiology and Microbiology, Kazan'. Department of Biochemistry, Kazan' Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Fedorov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 7, pp. 86-88, July, 1982. Original article submitted February 19, 1982.

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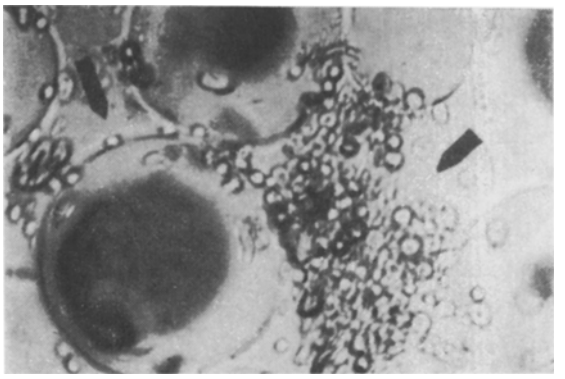

Fig. 1. Incubation of intact neutrophils with FGS granules. Arrows indicate aggregates of polymorphs and a single cell attached to a granule. 200 ×.

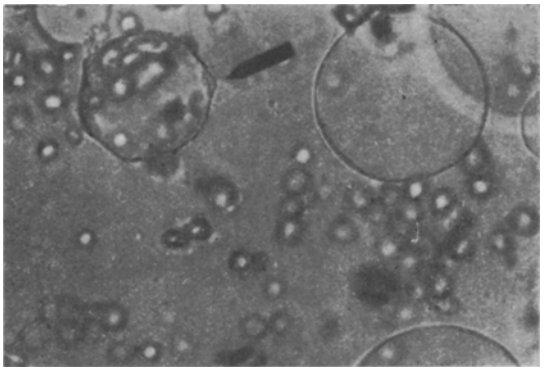

Fig. 2. Incubation of intact polymorphs with GS granules in the presence of heparin. Arrow indicates cell spread out on gelatinized surface. 200 ×.

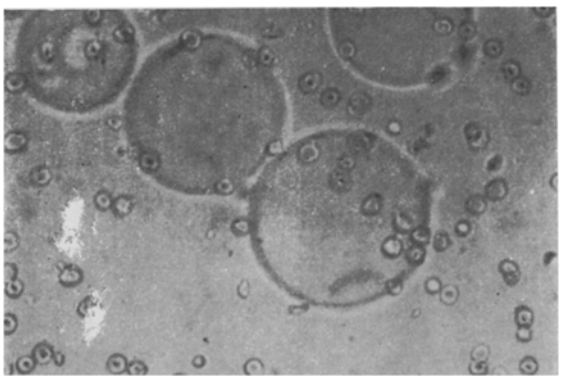

Fig. 3. Incubation of intact neutrophils with Sepharose 4B granules in the presence of heparin. 200 ×.

EXPERIMENTAL RESULTS

Incubation of intact polymorphs with FGS granules led to two types of interaction: most of the polymorphs were in the composition of aggregates; a minority of the cells was attached to FGS granules, causing agglutination of the separate granules together through the cell (Fig. 1). Heparin had no appreciable effect on these processes.

On incubation of the polymorphs with GS granules no visible changes took place initially. However, after the lapse of 15-20 min some polymorphs were attached to the surface of the GS granules or actually spread out on them. In the presence of heparin interaction between cells and GS granules took place much more rapidly and was complete after 1-2 min. Under these circumstances no aggregation of the polymorphs was observed (Fig. 2).

Incubation of polymorphs with Sepharose 4B granules led to no visible changes in the state of the cells or granules (Fig. 3).

Addition of EDTA to the incubation medium caused inhibition of polymorph aggregation in the presence of FGS, although the ability of the cells to adhere to GS granules was unimpaired. Treatment of the cells with trypsin suppressed all types of interaction of the cells with one another and with FGS and GS granules.

The most probable interpretation of these facts is that fibronectin and receptors for it are present simultaneously on the surface of the polymorphs. On interaction between cells and FGS granules, the receptors cause dissociation of fibronectin from GS and adsorb the protein on the cell surface, which leads to aggregation of the polymorphs. Some cells adhere to GS granules freed from fibronectin (just as in the control with pure GS), evidently because of the presence of surface-bound fibronectin, for no other proteins capable of interacting specifically with gelatin exist in blood. This is the most likely explanation of the combination of aggregation of the polymorphs and their adhesion to granules during interaction between cells and FGS.

The fact that trypsinization of the cells suppressed all forms of interaction confirms the presence of protein molecules on the surface of the polymorphs responsible for specific interaction of the cells with gelatin and fibronectin.

On the basis of these observations, suppression of fibronectin-induced aggregation of the cells by the action of EDTA without suppression of their adhesion to GS granules allows the following suggestion to be put forward. At least two contact centers evidently exist in the fibronectin molecule, one of which is responsible for binding with gelatin, the other for binding with cell receptors. The former interacts independently of bivalent ions (but depends on the presence of heparin), whereas the presence of Ca^{++} and/or Mg^{++} is essential for binding of fibronectin with receptors. The hypothesis of the existence of these two centers, in particular, explains why spontaneous aggregation of polymorphs does not take place. Evidentily the surface-bound fibronectin is facing only that part of the outside of the molecule which contains the gelatin-binding heparin-dependent region. Fibronectin adherent to GS and other gelatinized surfaces, on the other hand, interacts with cells only with that part of its molecule that contains a Ca^{++} (Ma^{++})-dependent receptor center. This hypothesis is in full agreement with the hypothetical domain structure of fibronectin [5].

These conclusions explain data in the literature on the high affinity of polymorphs for fibrin threads [3] and collagen fibers [1]. They also confirm the hypothesis that fibronectin is an opsonin in the reaction of neutrophilic phagocytosis of damaged tissues and of fibrin and collagen fragments, i.e., that it participates in cleansing of the wound surface.

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