ROLE OF ALVEOLAR MACROPHAGES IN FIBRINOGEN AND FIBRIN CATABOLISM

D. M. Zubairov, F. B. Subkhankulova, and G. B. Evranova

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Alveolar macrophages synthesize tissue thromboplastin, factor V, and plasminogen activator, especially during maturation of cells or their stimulation by antigens [6, 10]. Precipitation of fibrin and the indurative character of the inflammatory reaction are linked with the precoagulant properties of macrophages, and lysis of fibrin is associated with plasminogen activator production. It is suggested that alveolar macrophages can also carry out phagocytosis of fibrin on entering the air space of the respiratory passages [4].

For a more complete understanding of the catabolism of coagulable proteins and other processes taking place during edema of the alveoli and lobar pneumonia, it was decided to study interaction of alveolar macrophages with fibrin and fibrinogen.

EXPERIMENTAL METHOD

Alveolar macrophages were obtained from 10 rabbits by washing out of the lungs [9] followed by centrifugation in a Ficoll–Verografin gradient and washing off in Hanks' solution. The resulting suspension contained $3 \cdot 10^6$ cells/ml, of which 95-98% were alveolar macrophages, whose viability was estimated by their ability to take up methylene blue.

Homologous fibrinogen was isolated from 140 ml of rabbit blood plasma by the method described previously [7]. Labeling with fluorescein isothiocyanate (FITC) was carried out on rabbit fibrinogen, also by the method described previously [1], and the products were additionally purified by gel-filtration on a column measuring 2.5 × 50 cm containing acrylic P-50, equilibrated with 0.05 M phosphate buffer, pH 7.35, containing 0.15 M NaCl. Purified FITC-fibrinogen was lyophilized and dissolved before use in Hanks' medium without calcium and magnesium salts, which were added only after the FITC-fibrinogen had dissolved. FITC-fibrin was obtained from FITC-fibrinogen by specific coagulation with thrombin (Kaunas Bacterial Preparations Factory, 10^4 activity units/mg). Low-molecular-weight degradation products of FITC-fibrin in the incubation medium were determined by their absorbance at 495 nm [5] after precipitation of proteins with 20% TCA solution.

FITC-albumin was obtained from bovine serum albumin (Olaine Factory) by the method described above [5], purified like fibrinogen by gel-filtration, and then lyophilized.

Alveolar macrophages were incubated with the test protein in Hanks' solution for 30 min and 1, 2, and 4 h at 37°C. After incubation the cells were washed in the same solution, centrifuged, and investigated in the ML 4.2 4V fluorescent microscope. The intensity of fluorescence was measured by a V5-7 electrometric amplifier.

EXPERIMENTAL RESULTS

It was first etablished in control experiments that alveolar macrophages, incubated in 0.1% FITC-albumin solutin, take up this protein. The process becomes well marked after only 30 min, and after 2-3 h diffuse fluorescence of the cells developed (Fig. 1).

In the same way as albumin, the alveolar macrophages also took up homologous FITC-fibrinogen from the incubation medium. After 1 h of contact with a 0.1% solution of FITC-fibrinogen, diffuse fluorescence of the cells developed as a rule (Fig. 2). Incubation of alveolar macrophages in medium not containing Ca^{++} and Mg^{++} delayed uptake of FITC-fibrinogen (Table 1).

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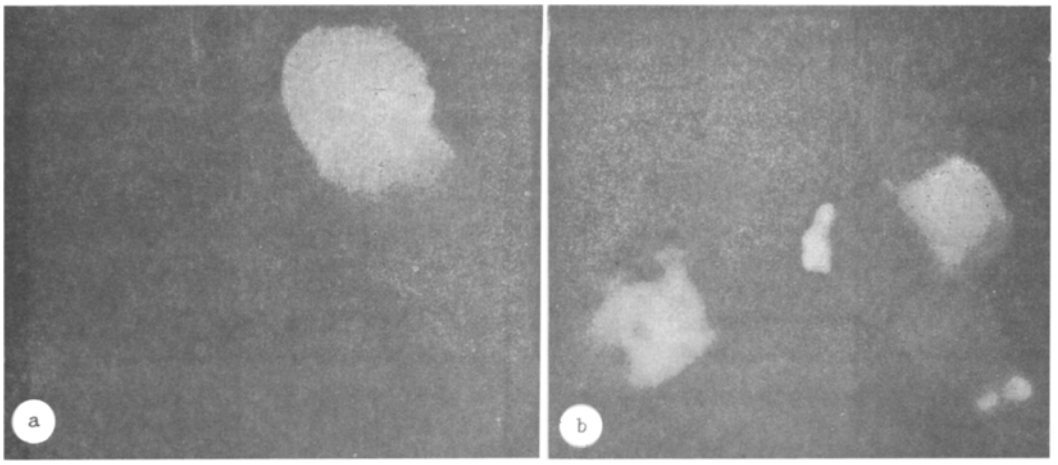

Fig. 1. Rabbit alveolar macrophages. Incubation with FITC-albumin for 30 min (a) and 2 h (b). Here and in Figs. 2 and 3: objective 90, ocular 10.

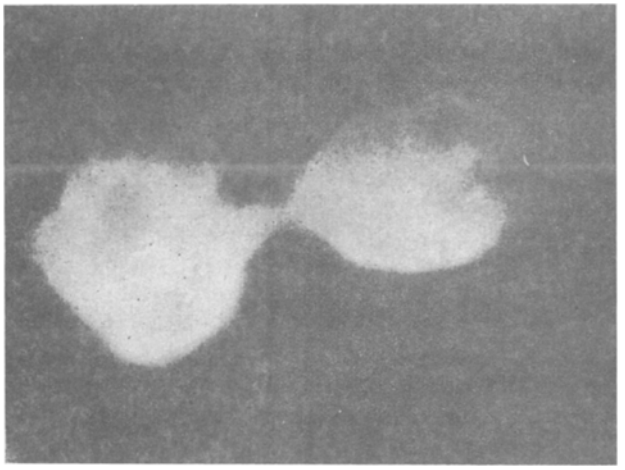

Fig. 2. Rabbit alveolar macrophages. Incubation with FITC-fibrinogen for 1 h.

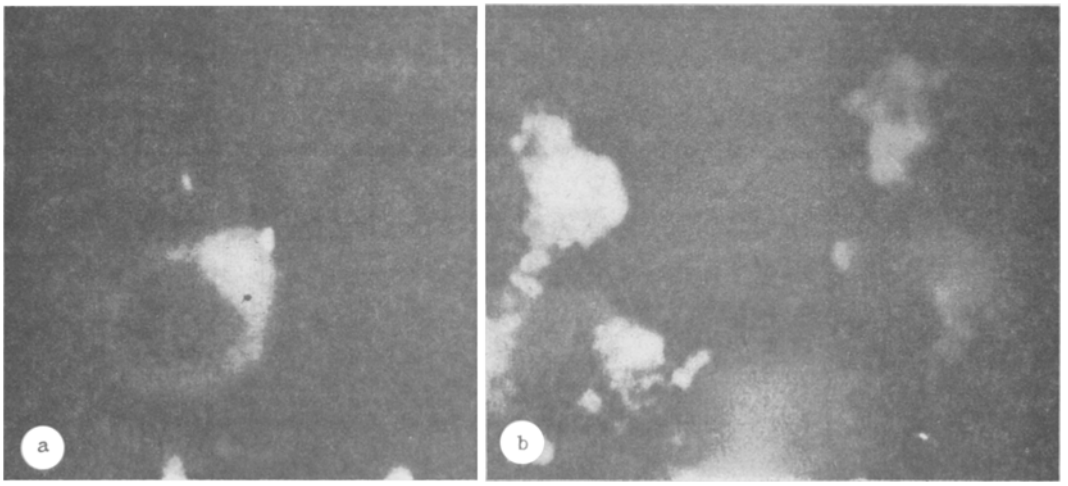

Fig. 3. Rabbit alveolar macrophages. Incubation with FITC-fibrin for 2 h (a) and 4 h (b).

TABLE 1. Effect of Ca^{++} and Mg^{++} on Uptake of FITC-Fibrinogen by Alveolar Macrophages (M ± m)

Incubation Medium	Incubation time, min	Intensity of fluorescence, units	p
Hanks' solution	30	7,2±0,5	—
Hanks' solution without Ca^{++} and Mg^{++}	30	4,8±0,5	<0,01

To study interaction of alveolar macrophages with fibrin, finely divided FITC-fibrin, obtained by passing the preparation through a Potter–Elvehjem homogenizer, was used. A 0.4% suspension of FITC-fibrin, whose particle size was commensurate with that of the alveolar macrophages themselves, or smaller, was used.

In the course of incubation of the alveolar macrophages with FITC-fibrin after 30 min adhesion of fibrin granules to the surface of 43.7 ± 4.9% of cells took place, 80.3 ± 1.3% after 2 h, rising to 83.3 ± 1.8% of cells after 4 h (Fig. 3). An increase in the number of fibrin particles adherent to cells after incubation for 30 min and for 2 and 4 h was statistically significant ($p < 0.002$).

Fluorescence of macrophages after contact with particles or conglomerates of fibrin appeared as early as after 30 min, but more slowly than in the case of interaction with soluble proteins (FITC-fibrinogen and FITC-albumin). The intensity of fluorescence (4.8 ± 0.5 units) was less than in the case of interaction with fibrinogen (8.8 ± 0.7 units; $p < 0.001$). After incubation for 2 h the intensity of fluorescence increased to 5.4 ± 0.6 units, and after 4 h to 7.0 ± 0.8 units (Fig. 3c). The intenisty of fluorescence of pure FITC-fibrin was 11.1 ± 0.8 units. Clearly the intensification of fluorescence during interaction of alveolar macrophages with FITC-fibrin was less than with contact with FITC-fibrinogen. After incubation for 4 h, even in cells which had no visible contact with FITC-fibrin particles, fluorescent pinocytotic vesicles were observed along the periphery of the cytoplasmic membrane. Meanwhile Hanks' incubation medium, after addition of 20% TCA in the ratio of 1:1, had no absorbance at 495 nm. This means that after interaction of this duration of the alveolar macrophages with FITC-fibrin, no low-molecular-weight degradation products of this protein were formed.

The writers showed previously that during incubation of Kupffer cells with fibrinogen, proteolytic degradation of the latter takes place with the formation of high-molecular-weight products of fibrinogenolysis [2]. The present investigation showed that the process of fibrinogenolysis can take place not only extracellularly, under the influence of plasminogen activator, produced by alveolar macrophages, but also intracellularly, with the participation of lysosomal enzymes of macrophages.

Penetration of fibrinogen into the cell depends on the presence of bivalent cations, and it can accordingly be postulated that it is not limited to diffusion along the concentration gradient, but also takes place by pinocytosis which, like many active intracellular processes, is dependent on Ca^{++}.

During 4 h of interaction of alveolar macrophages with FITC-fibrin, the fluorescent label penetrated into the cells more slowly than during incubation with FITC-fibrinogen. In the case of a protein with almost identical amino-acid composition and identical antigenic determinants, and differing only in size, the particular features distinguishing endocytosis of the monomer and polymer forms of the protein were demonstrated. Fibrin degradation through the participation of alveolar macrophages evidently takes place mainly extracellularly, under the influence of the secreted plasminogen activator, which converts plasminogen, adsorbed on fibrin, into the active proteolytic enzyme plasmin. The soluble high-molecular-weight fibrin degradation products formed may undergo pinocytosis. It can be concluded on the basis of these results that degradation of fibrin threads is juxtamural in character. The solution to this problem requires special electron-microscopic investigations.

Thus through the production of tissue thromboplastin and factor V [8], macrophages are involved in specific coagulation catabolism of fibrinogen, which according to our data [3] accounts for about 30% of the pool of this protein in mammals, and also nonspecific, noncoagulation catabolism which, as the present investigation has shown, begins with pinocytosis of species-specific fibrinogen.

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OXIDOREDUCTASE ACTIVITY OF AN UNUSUAL ESTROGEN-BINDING PROTEIN OF RAT LIVER

A. N. Smirnov and E. G. Kazanova

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The writers previously found a sex-dependent protein in the liver cytosol of male rats which, unlike sex hormone receptors, interacts specifically and to a comparative degree with two groups of steroids at once: estrogens and androgens [3]. Later this unusual estrogen-binding protein (UEBP) was fully purified by affinity chromatography on estradiol-sepharose and described [4, 8]. The results of experiments in vitro and in vivo point to the functioning of this protein as a modulator of reception and metabolism of its ligands by a process of labile deposition [1, 5, 7, 9, 12]. With regard to those ^{3}H-steroids that have been investigated (estradiol, estrone, estriol, testosterone, and androstenedione) UEBP did not exhibit hydroxysteroid-dehydrogenase and reductase activity under the conditions used [7]. By the use of chromatographic procedures to purify UEBP in order to isolate an estrophilic nonreceptor protein from the soluble fraction of rabbit liver, we obtained a highly purified preparation of steroidophilic hydroxysteroid dehydrogenase (NADP-dependent), active on androgens and gestagens as substrate.

Accordingly, in the present investigation the presence of oxidoreductase activity was again tested in UEBP, using a broad spectrum of steroid compounds as potential substrates.

EXPERIMENTAL METHOD

A highly purified preparation of UEBP was isolated from the liver cytosol of mature male rats by fractionation with ammonium sulfate, gel-filtration, and ion-exchange, affinity chromatography on estradiol-sepharose, as described previously [4, 8]. The resulting protein preparation preserved its hormone-binding properties characteristic of UEBP. Purity of the preparation was verified by polyacrylamide gel electrophoresis under denaturing conditions [10] and revealed the presence of only one polypeptide band, staining with Coomassie, with mol. wt. of about 31,000. The enzyme activity of the preparation was determined by measuring fluorescence of NADPH formed during oxidation, in the presence of NAD^+, $NADP^+$, NADH, or NADPH ("Reanal," Hungary), utilized for reduction of steroid substrates. Depending on the structure of the steroid substrates (on the presence of hydroxy and keto groups or of a double

Laboratory of Endocrinology, Faculty of Biology, M. V. Lomonosov Moscow University.
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