

ULTRASTRUCTURAL CHARACTERISTICS OF AUTOLOGOUS ROSETTE FORMATION IN VIVO

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KEY WORDS: autologous rosette formation; erythrocytes; lymphocytes; macrophages; polymorphonuclear leukocytes

The phenomenon of autologous rosette formation has attracted increasing attention of research workers in recent years. Although the study of this process in vitro has revealed a broad spectrum of subpopulations of immunocompetent cells, capable of adhering to autologous erythrocytes, the interest of research workers has been concentrated chiefly on characteristics of receptors of human peripheral blood lymphocytes, the special features of contact between the cells in autologous rosettes, and the analysis of the general principles of this process and its place in the order of other immunologic reactions only just beginning [2]. An essential addition to the study of this problem can be made by the investigation of autologous rosette formation in vivo, actually in the tissues, permitting not only the characterization of autologous rosettes as cellular associations with the different kinds of rosette-forming cells that are possible, but also to correlate this process with changes in the tissues in pathology. Yet virtually no such studies have been undertaken. The aim of the present investigation was accordingly to study autologous rosette formation in the gastric mucosa of patients with chronic ulcer, by an electron-microscopic method.

EXPERIMENTAL METHOD

The experimental material consisted of biopsy specimens from the gastric mucosa at the edge of a chronic ulcer and in the region of the postulcerative scar, obtained from nine patients with gastric ulcer during diagnostic endoscopy. Tissue specimens for electron-microscopic study were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4), postfixed in 1% OsO₄, dehydrated in alcohols, and embedded in Araldite. To characterize the intercellular matrix and the juxtamembranous layer of cells the histochemical reaction for glycosaminoglycans with ruthenium red was carried out [8]. Ultrathin sections, after double staining with uranyl acetate and lead citrate, were examined in the JEM-1200EX electron microscope and photographed with magnification of 5000-50,000.

EXPERIMENTAL RESULTS

Extravasally situated erythrocytes, identical in the density of their cytoplasm with erythrocytes in the lumen of the vessels, were found among granulation tissue cells of the chronic gastric ulcer and in the region of the postulcerative scar. In virtually all of the extravascular erythrocytes the plasma membrane was well defined and sometimes formed small festoons. On the surface of the erythrocytes and in the interstitial connective-tissue substance surrounding them glycosaminoglycans were discovered in the form of ruthenium-positive granular or thin fibrillar material, accompanied by dense osmiophilic clumps of protein nature and of different sizes. Wide interaction of the extravasal erythrocytes with different types of cells in the tissue was observed: with lymphocytes, macrophages, polymorphs, and fibroblast-like cells (Fig. 1). Each cell could make contact with three or four erythrocytes, to form autologous rosettes. At the same time, cells connecting one or two erythrocytes were also quite frequent. A characteristic feature of the extravascular erythrocytes was their polymorphism: they varied in shape from regularly round to intricate, with numerous constrictions and isthmuses. Mainly the less deformed erythrocytes took part in intercellular interactions. Deformed erythrocytes also made contact with lymphocytes, and because of their twisted outlines, they had several small zones of contact with the even surface of the lymphoid cell or with its processes (Fig. 1a). Membranes of interacting cells were preserved

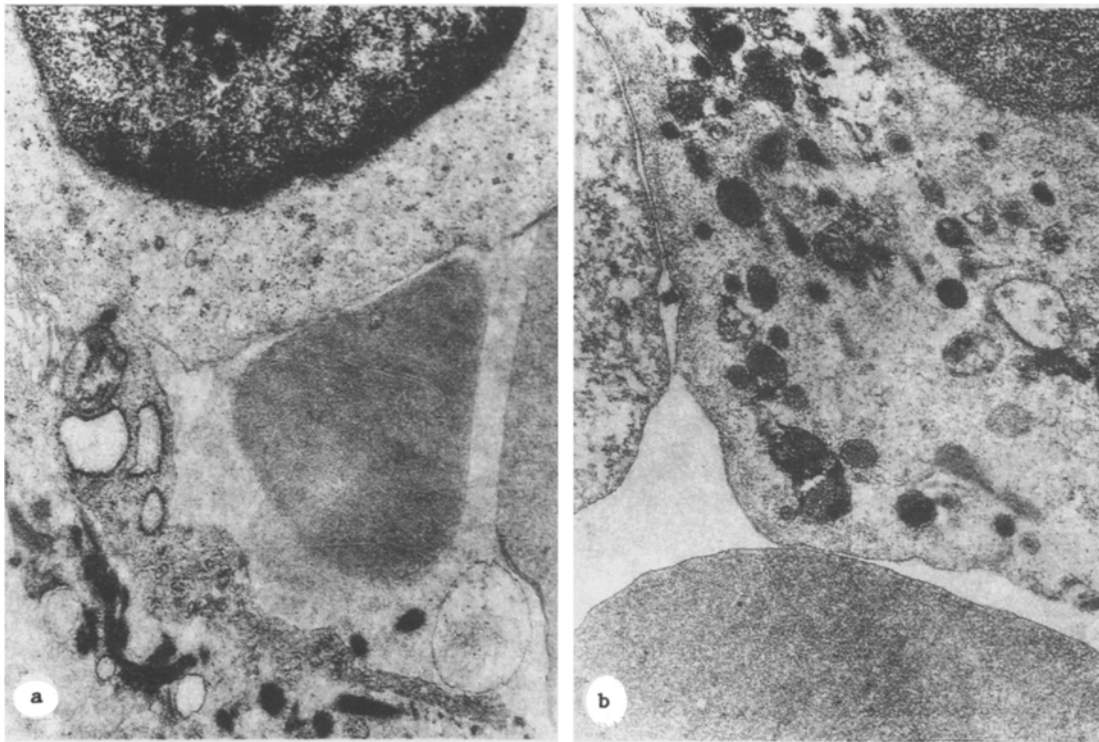


Fig. 1. Autologous rosette formation in gastric mucosa close to chronic ulcer. Rosette-forming cell is: a) lymphocyte, 22,000 \times ; b) macrophage, 27,000 \times .

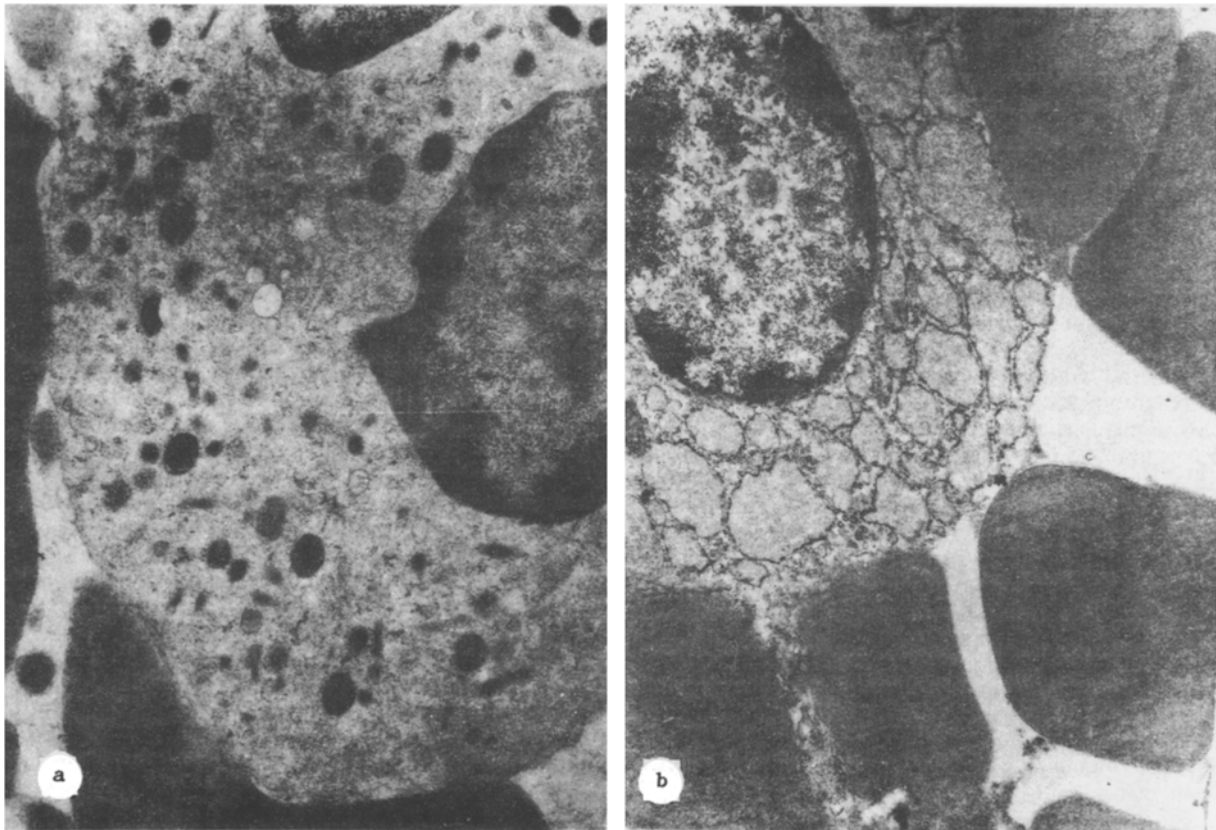


Fig. 2. Autologous rosette formation in gastric mucosa close to chronic ulcer. Rosette-forming cell is: a) polymorphonuclear leukocyte, 25,000 \times ; b) fibroblast-like cell, 10,700 \times .

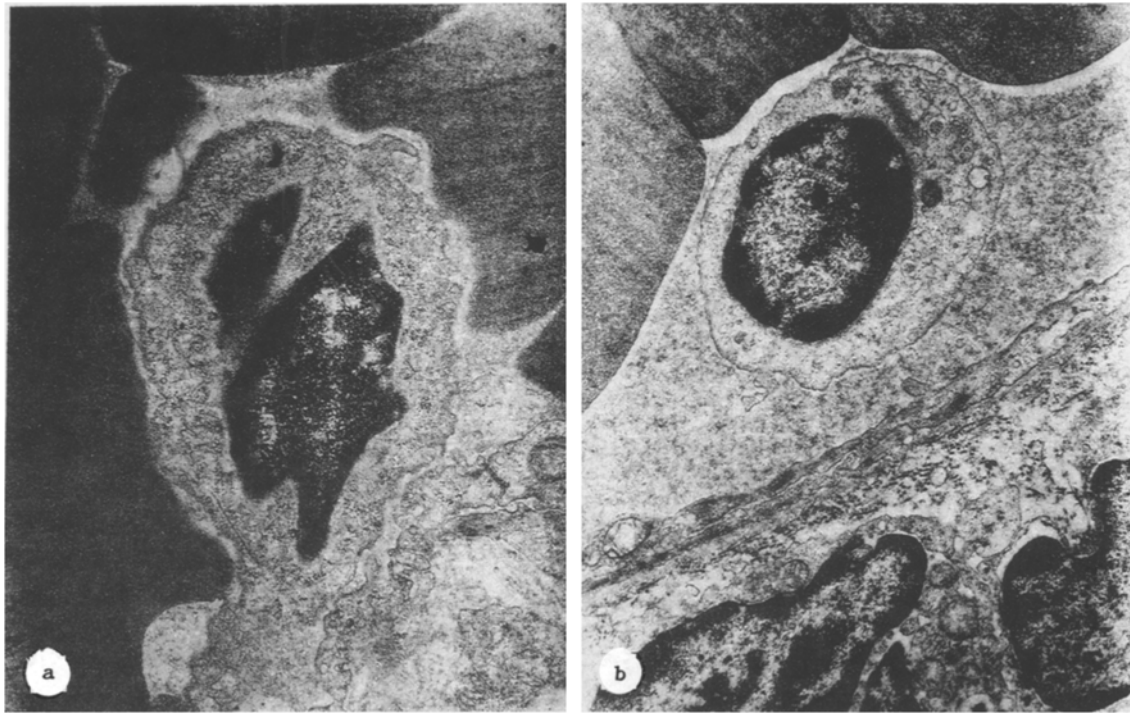


Fig. 3. Autologous rosette formation in lumen of blood vessel in gastric mucosa close to chronic ulcer.
Rosette-forming cell is: a) endotheliocyte, 13,500 \times ; b) lymphocyte, 10,500 \times .

in areas of contact. Small zones of condensation with increased osmiophilia were discovered in the plasma membrane of the lymphocyte. Lymphocytes forming autologous rosettes differed in their degree of differentiation. Both small cells with a round nucleus and a narrow border of cytoplasm, containing solitary organoids, and activated cells, with an irregularly shaped nucleus and cytoplasm containing mitochondria, a rough endoplasmic reticulum, a Golgi lamellar complex, and centrioles, were discovered.

Macrophages are another type of cell forming rosettes with autologous erythrocytes (Fig. 1b). They contained primary and secondary lysosomes, pointing to phagocytic activity of the cell. A characteristic feature of erythrocytes in contact with macrophages was their round or oval shape. Among them there were some erythrocytes with signs of hemolysis. The interacting cells made contact by areas of their surface of different sizes. The plasma membrane of both cells usually was intact. Small areas of increased density were found in the plasmalemma of the macrophages. Sometimes the cytoplasm adjacent to the membrane was a little condensed.

Autologous rosette formation was associated much less frequently with polymorphonuclear leukocytes and fibroblast-like cells. Areas of adhesion of erythrocytes to polymorphs were quite large in size (Fig. 2a). The plasma membrane of the leukocyte in these rosettes was partly destroyed. However, the cell as a whole preserved its structural specificity: its lobed nucleus and characteristic granularity of the cytoplasm. By contrast, fibroblast-like cells, with several erythrocytes adhering to them, had distinctive structural features in the form of partial lysis of the nuclear chromatin, hyperplasia and hypertrophy of the rough endoplasmic reticulum, and degranulation of the membrane. The plasma membrane was intact in zones of contact, but outside them it was partially or completely destroyed (Fig. 2b).

Autologous rosette formation also was observed in the capillary lumen. The rosette-forming cells in this case were endothelial cells, protruding into the lumen (Fig. 3a), and lymphocytes (Fig. 3b). In both cases the plasma membrane of the contacting cells was preserved.

The investigations revealed interaction between different kinds of cells and extravasally situated erythrocytes in the tissue of a chronic gastric ulcer, with autologous rosette formation. Contact between the cells was based mainly on the property of their surfaces, in which special proteins and whole zones, namely receptors responsible for this interaction, are found [5, 6]. The areas of condensation of the plasmalemma of the rosette-forming cells which we discovered may perhaps reflect this phenomenon. The number of receptors and their types vary from cell to cell and determine the particular features of function of the cells under concrete conditions [4, 10]. Intercellular interactions also are mediated through the surrounding microenvironment [7]. In chronic

gastric ulcer, glycosaminoglycans, proteins, and metabolites, promoting autologous rosette formation, accumulate in the interstitial substance of the connective tissue. The process of autologous rosette formation reveals the special features of the rosette-forming cells: the presence of immature T lymphocytes [9], widening of the range of function of polymorphonuclear leukocytes [3]. Meanwhile autologous rosette formation exerts its influence on processes taking place in the tissue of the ulcer itself. For instance, interaction of T lymphocytes with autologous erythrocytes is accompanied by release of a factor stimulating cell proliferation [1], which is connected with regeneration of the mucosa. Thus autologous rosette formation in the tissue of a chronic gastric ulcer reflects profound changes in local metabolism and probably depends on those changes.

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INTRACELLULAR AND EXTRACELLULAR CATHEPSIN D ACTIVITY IN THE LIVER DURING CIRRHOSIS AND INVOLUTION

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Acid endopeptidase or cathepsin D, the principal lysosomal enzyme, reflects the ability of cells to hydrolyze protein and plays an important role in the process of intracellular proteolysis [3, 11]. Cathepsin D can also produce lysis of collagen and other components of the intercellular matrix [5-7, 9, 12]. The enzyme has also been found extracellularly in connective tissue in vitro [8], although its involvement in extracellular catabolism has not been proved [4].

To discover the role of cathepsin D in the resorption of fibrous tissue we investigated the distribution of the enzyme in the liver at the ultrastructural level in cirrhosis and during the first 3 weeks of its involution.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 150 g. Cirrhosis of the liver was induced by subcutaneous injection of 50% CCl₄ in olive oil in a dose of 0.3 ml/100 g body weight twice a week for 15 weeks. Samples of liver for study were

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