transplantation of sarcoma Ad-12 cells in doses of 10^2 and 2×10^2, whereas in hamsters of the experimental group (receiving a preliminary injection of starch suspension) tumors appeared in 53-72% of cases after transplantation of the same numbers of malignant cells (the stimulation of tumor growth phenomenon). Thus, animals naturally resistant to small numbers of tumor cells, after appropriate treatment become sensitive to inoculation with these cells.

The results of this investiation are evidence that the appearance of tumors in hamster after subcutaneous transplantation of small doses (10^2, $2 \cdot 10^2$) of Sarcoma Ad-12 cells after preliminary injection of starch suspension is associated with removal of mobile macrophages from the location of tumor cells into the peritoneal cavity..

Local or general mechanisms through which tumor growth is stimulated are not yet known, and may be various. However, there is no doubt that after removal of mobile macrophages, the primary antitumor resistance of the animal may be modified. Consequently, an essential role in the effective proteetion of the organism against small numbers of tumor cells is played by nonspecific resistance, which may be effected through macrophages.

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EXPRESSION OF VIMENTIN AND PREKERATINS IN SOLID AND ASCITES VARIANTS OF ZAJDELA's HEPATOMA

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KEY WORDS: intermediate filaments; hepatoma; intercellular junctions; extracellular matrix.

Cells of various tissues contain intermediate filaments (IF) consisting of subunits specific for the given type of tissue [9]. Primary and transplantable epithelial tumors, including liver tumors, still contain IF proteins characteristic of epithelium, namely prekeratins (PK), and they do not express the IF protein specific for connective tissue, namely vimentin [4, 7]. The only exceptions are certain forms of carcinomas of the salivary glands and kidneys [6, 12]. By contrast with this, besides PK, as a rule vimentin filaments also are found in ascites forms of carcinoma [10]. The reasons for this anomalous expression of vimentin in ascites forms of carcinoma are not known. The solution to this problem must be found before we can understand the mechanisms both of normal morphogenesis and of the disturbances of morphogenetic processes in tumor growth.

EXPERIMENTAL METHOD

Zajdela's ascites hepatoma (ZAH), obtained from the Collection of Tumor Strains, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, was transplanted intraperitoneally into adult noninbred male rats. The transplantation was repeated every 5

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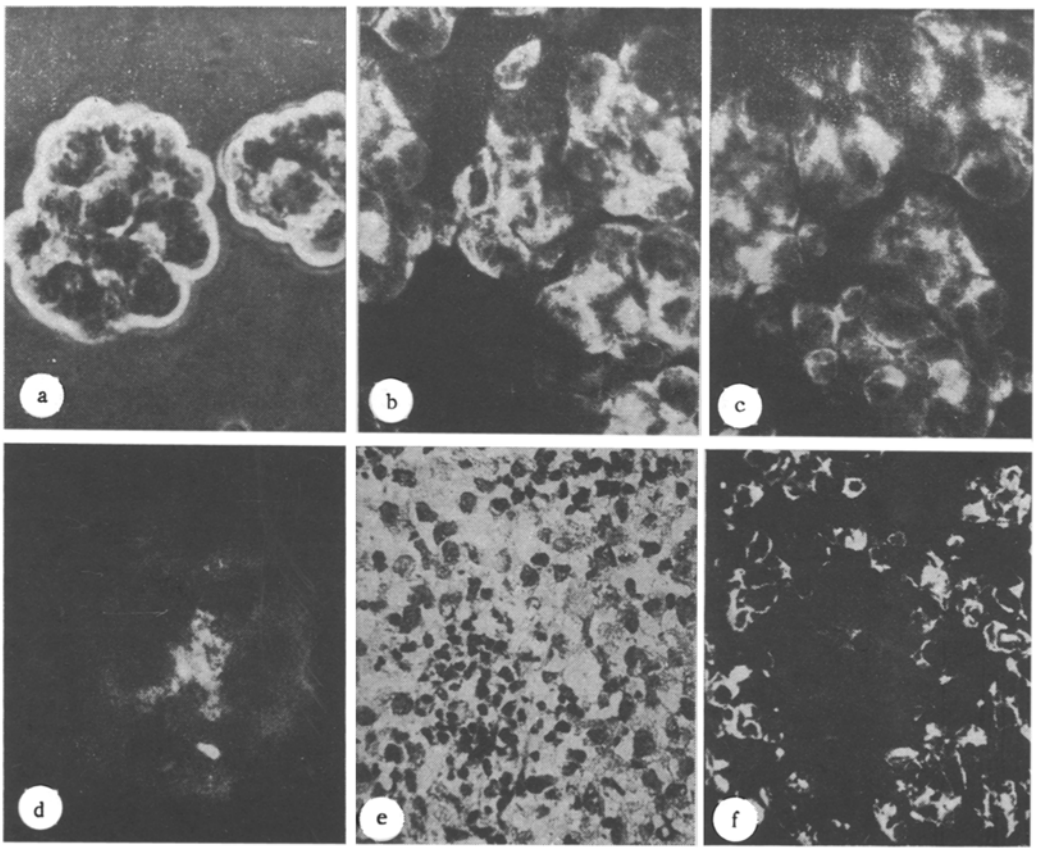

Fig. 1. Immunofluorescence staining of frozen sections of ascites aggregates of ZAH (a-d) and of metastases of ZAH cells in paratracheal lymph nodes (e, f). a) Ascites aggregate under phase contrast. Staining with antibodies against PK_{49} (b) and vimentin (c) reveals these proteins in all cells of the aggregates; laminin (d) is distributed in the form of granules on the apical surface of the aggregates; e) staining a section of a metastasis in the paratracheal lymph node with hematoxylin. Large pale cells are ZAH cells; f) staining serial section with antibodies against PK_{49}. PK contained only by tumor cells, lymphoid cells do not stain. Magnification: a-c) 500, d) 600, e, f) 300 ×.

days. Rats were killed 5-6 days after intraperitoneal transplantation of ZAH. Frozen sections were cut from aggregates floating in the ascites fluid and from the paratracheal glands. The residue of tumor cells from the ascites fluid was resuspended in Eagle's medium in the ratio of 1 volume of cells to 0.5 volume of medium, and 1 ml of this thick suspension as injected subcutaneously into male rats weighing 50-100 g. The rats with tumors were killed after 10, 15, 20, and 25 days, one part of each tumor was frozen in liquid nitrogen for immunohistological investigation, and the other part was transplanted subcutaneously into other male rats, using the standard procedure. Immunofluorescence staining of IF in the solid tumors, metastases, and ascites fluids was done on frozen sections 5-7 μ thick. For this purpose pieces of tissue measuring 0.5 × 0.5 cm were embedded in a 7% solution of gelatin and frozen in liquid nitrogen. The tumor cells were first sedimented by centrifugation (10 min, 100 g) and the residue was frozen in 7% gelatin solution. The frozen blocks and sides with sections were kept at −70°C. Before staining the sections were fixed with 4% formalin solution for 2-3 min. The staining procedure, by the indirect immunofluorescence method, was described previously [2]. Monoclonal antibodies of the following clones were used: E_2 — against PK with mol. wt. of 55 kilodaltons (kD) (PK_{55}), E_3 — against PK with mol. wt. of 40 kD (PK_{40}), C_{12} — against PK with mol. wt. of 49 kD (PK_{49}), and clone 30 — against vimentin. The antibodies used were characterized in detail by the writers previously [3]. Antiserum against basement membrane protein (laminin), generously provided by A. V. Lyubimov (All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR), also was used.

EXPERIMENTAL RESULTS

Translated ZAH cells in the ascites fluid associated with aggregates varying in size from

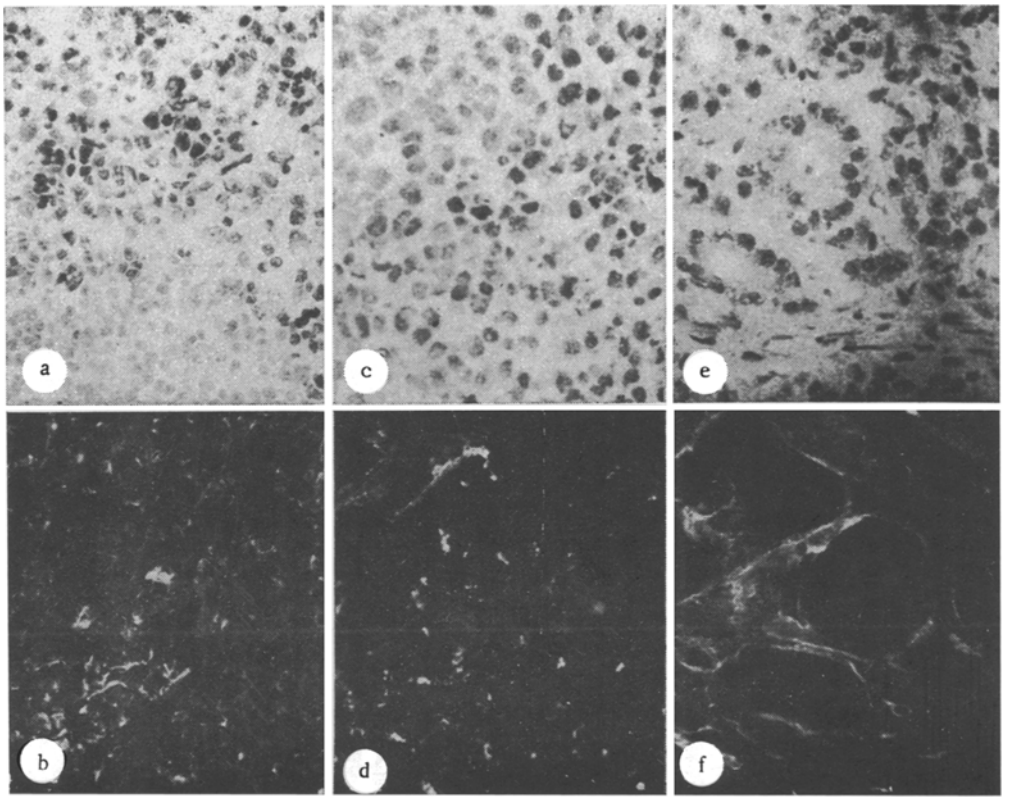

Fig. 2. Immunofluorescence staining of frozen sections through metastases of ZAH cells into paratracheal lymph nodes (a, b) and through solid tumors (c-f). a) Staining of section through ZAH metastasis in lymph node. Serial sections stained with antibodies against vimentin (b) shows that this protein is present both in ZAH cells and in lymph nodes; c) staining sections of "structureless" tumor with hematoxylin. Tumor cells do not form definite structures; laminin (d) found in the form of granules or short bands; e) adenocarcinoma formed by ZAH cells, stained with hematoxylin. Cells form glandular structures; laminin (f) visible in the form of bands (basement membranes) on the basal surface of the cells. Magnification: a, b) 250, c-f) 300.

10 to 100 cells and in shape from spherical to rod-like. These aggregates have a definite structure and consist of spheres or cylinders with an inner cavity (Fig. 1a). Tight junctions and desmosomes, typical of simple epithalia [1], were found between the cells of these aggregates. Staining frozen sections of ascites aggregates with monoclonal antibodies to PK_{40}, PK_{49}, PK_{55}, and vimentin showed that filaments of both prekeratin (containing all PK tested) and vimentin type exist in all ascites cells (Fig. 1b, c). Staining the same sections with antibodies to laminin showed that this glycoprotein accumulates in the form of granules near the "apical" surfaces of the cells in the lumen of the aggregates (Fig. 1d), but does not form a covering for the "basal" surfaces of the cells as in normally organized epithelium.

Depending on the method of transplantation of ZAH cells three varieties of solid structures could be obtained. After intraperitoneal transplantation in rats metastases developed frequently in the paratracheal lymph nodes. Metastasizing cells were not organized into regular structures but consisted of chaotic accumulations among lymphoid cells (Fig. 1e). After subcutaneous injection of ZAH cells, tumors of two types were formed at the site of injection. In most cases these were solid undifferentiated carcinomas — type I tumors (Fig. 2). Carcinomas with this morphology as a rule regressed spontaneously in 20-30 days. Tumors whose cells were organized into tubular adenomatous structures (type II tumors) appeared much less frequently after subcutaneous transplantation (Fig. 2e, Fig. 3d).

Staining with monoclonal antibodies against different PF proteins showed that PK_{40}, PK_{49}, and PK_{55} are present both in metastasizing ZAH cells (Fig. 1f) and in cells of tumors of types I and II (Fig. 3b, f). In the type II tumors the prekeratin filaments had a strictly regular distribution, for they were localized mainly beneath the cell membranes and formed

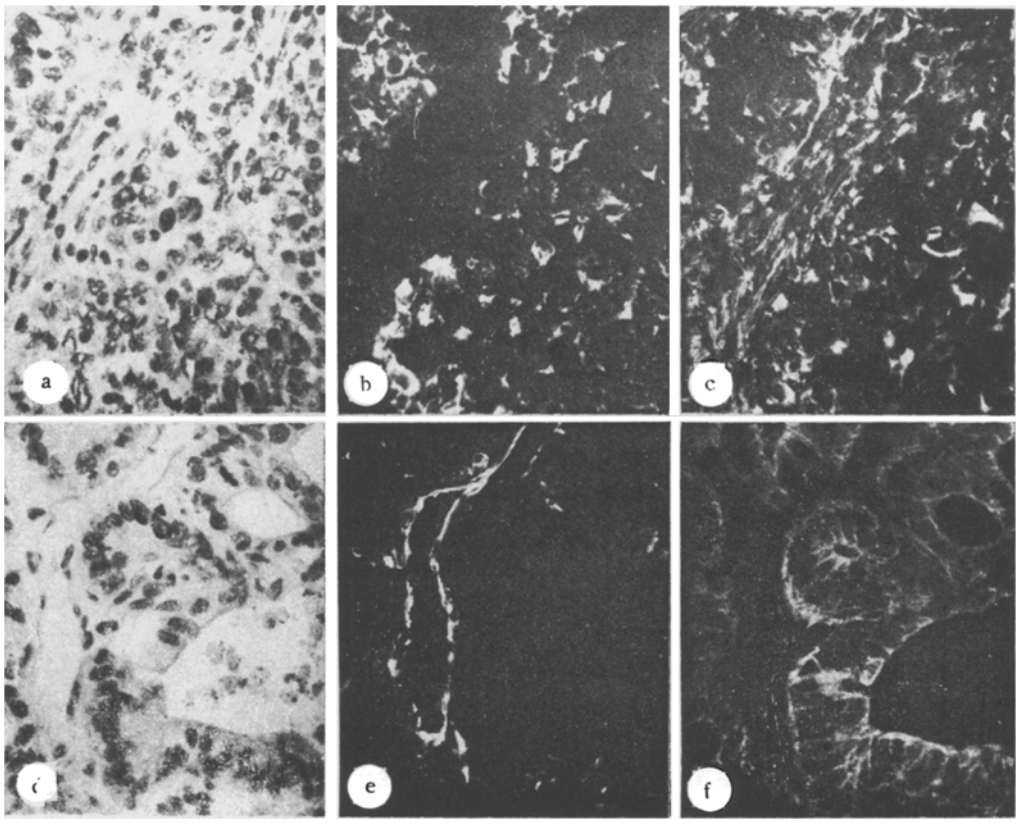

Fig. 3. Immunofluorescence staining of frozen sections of solid ZAH tumor. a-c) Serial sections of the "unstructured" variant: a) stained with hematoxylin; b) with antibodies against PK_{49}; c) against vimentin. All epithelial cells contain both proteins; d-f) serial frozen sections through ZAH of adenocarcinoma type; d) stained with hematoxylin, e) with antibodies against vimentin, f) against PK_{40}. Epithelial tumor cells contain filaments of PK but not of vimentin. 300 ×.

concentrations in their apical part. Meanwhile prekeratin filaments were distributed more or less chaotically in type I tumor cells. Cells of metastases and type I tumor cells, moreover, contained vimentin (Fig. 2b, Fig. 3c). On the other hand, type II tumors, whose cells formed adenomatous structures, were completely without vimentin (Fig. 3e). Staining with antibodies to laminin showed that this glycoprotein is present in type I tumors in the form of granules and short bands between the cells (Fig. 2d). Conversely, in type II tumors the cells were arranged on a continuous "membrane," which stained with antibodies to laminin (Fig. 2f).

Inhibition of vimentin expression thus correlates with morphological "normalization" of structures formed by ZAH cells. We do not yet know what are the factors which cause this normalization. It was reported previously that transfer of ascites carcinoma cells into the conditions for solid growth inhibited their expression of vimentin [11]. Incidentally, in the system studied transition from the ascites into the solid form did not itself lead to a change in synthesis of PF proteins. The progenies of ZAH cells ceased to express vimentin only when they formed sufficiently highly organized structures. Besides the purely morphological features of orderliness of structure, to distinguish them from ascites aggregates and solid tumors of the I type, type II tumors were characterized by a regular intracellular distribution of prekeratin filaments and a regular arrangement of the extracellular matrix protein, laminin. We know that organ cultures of the liver, while preserving orderly intercellular junctions and extracellular matrix, have no vimentin filaments, unlike monolayer hepatocyte cultures which have no specific matrix, no orderly system of junctions, and express vimentin [2]. Another example of this kind has been described in the literature. Cells of the developing distal endoderm of the mouse embryo, which lose the junctions between them and migrate along Reichert's basement membrane, begin to express vimentin in addition to PF of prekeratin type, which were already expressed by their precursor cells (of the primary endodern [8]).

Expression of vimentin filaments in epithalia of endodermal origin thus correlates with

disturbance of the orderly structure of the system of intercellular junctions and junctions between cells and the basement membrane. Unfortunately, a scheme of this kind cannot yet explain all the observed facts. For example, the fact that single epithelial tumor cells invading the stroma do not contain vimentin [5]. This may probably be evidence of the existence of additional factors regulating vimentin expression. A new experimental system is evidently required to study these factors.

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