LIGHT-OPTICAL ULTRASTRUCTURAL IMMUNOHISTOCHEMISTRY OF ANTIGEN H4, A COMMON MARKER OF EPITHELIAL CELLS

S. V. Petrov, N. T. Raikhlin, and R. S. Nizamov

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Epithelial marker proteins include cytokeratins, which are proteins of intermediate filaments (CKR), epithelial membrane antigens (EMA), tissue-specific epithelial antigen, desmosomal peptides, Lu-5 antigen, etc. [4-7, 11-13]. The presence of these peptides in most types of epithelium means that they can be used to study epithelial tissue under normal and pathological conditions, including during tumor growth [1-3, 5-7, 12]. However, some epithelial antigens can appear sporadically in cells of nonepithelial origin: low-molecular-weight CKR in muscle cells, Lu-5 and EMA in leukocytes [5-7, 12]. Accordingly, the search continues for universal markers which may be specific for epithelial tissues only [5-7, 12, 13]. Such proteins can be used in oncomorphology to solve a number of diagnostic problems, such as the immunohistochemical analysis of undifferentiated tumors, and a diagnosis of metastases when the primary focus has not been identified.

S. M. Troyanovskii and co-workers at the Laboratory of Mechanisms of Carcinogenesis, Research Institute of Carcinogenesis, Oncologic Scientific Center, Russian Academy of Medical Sciences, using a hybridoma technique, obtained murine monoclonal H4 antibodies (McAb; IgM). The method of obtaining McAb H4 consisted of immunizing mice with fractions of the cytoskeleton of the epithelium of the rat large intestine, and it was described previously [9, 14]. According to unpublished data of these workers, McAb H4 react immunohistochemically and immunochemically with the majority of human epithelial cells. The concrete antigen of these antibodies has not been identified.

The aim of this investigation was to undertake the light-optical and ultrastructural localization of antigen H4 in cells of different types of epithelium.

EXPERIMENTAL METHOD

Material from the epidermis, taken at operation from an adult individual and a fetus at 9-26 weeks of development, the mucosa of the ectocervix and endocervix of adult women, squamous-cell skin carcinomas, and adenocarcinomas of the cervix uteri, as well as cell cultures of the epithelium of the guinea pig vas deferens were used in the experiments. Tumors of the skin and cervix uteri of nonepithelial genesis also were studied: fibro- and myosarcoma, nevus, etc. Immediately after the tissue was taken it was immersed in a 7% solution of gelatin in phosphate buffer (PBS) and frozen in liquid nitrogen, after which frozen sections were cut to a thickness of 7 and 20 μm, placed on coverslips, and fixed for 5 min in cold acetone. After washing in PBS the sections were used for immunohistochemical reactions with McAb H4. Seven- and 14-day cultures of epithelial cells from the vas deferens were fixed on a coverslip in three portions of methanol, washed in PBS, and used in the work. Sections and cell cultures were incubated with McAb H4 (dilution 1:2, protein concentration 10 μg/ml) for 1 h, and then washed 3 times in the course of 20 min in PBS, after which they were incubated for 1 h with antimouse antibodies labeled with peroxidase (TAGO, USA), and washed in PBS. Peroxidase was demonstrated with the aid of 3,3-diaminobenzidine (Sigma, USA)

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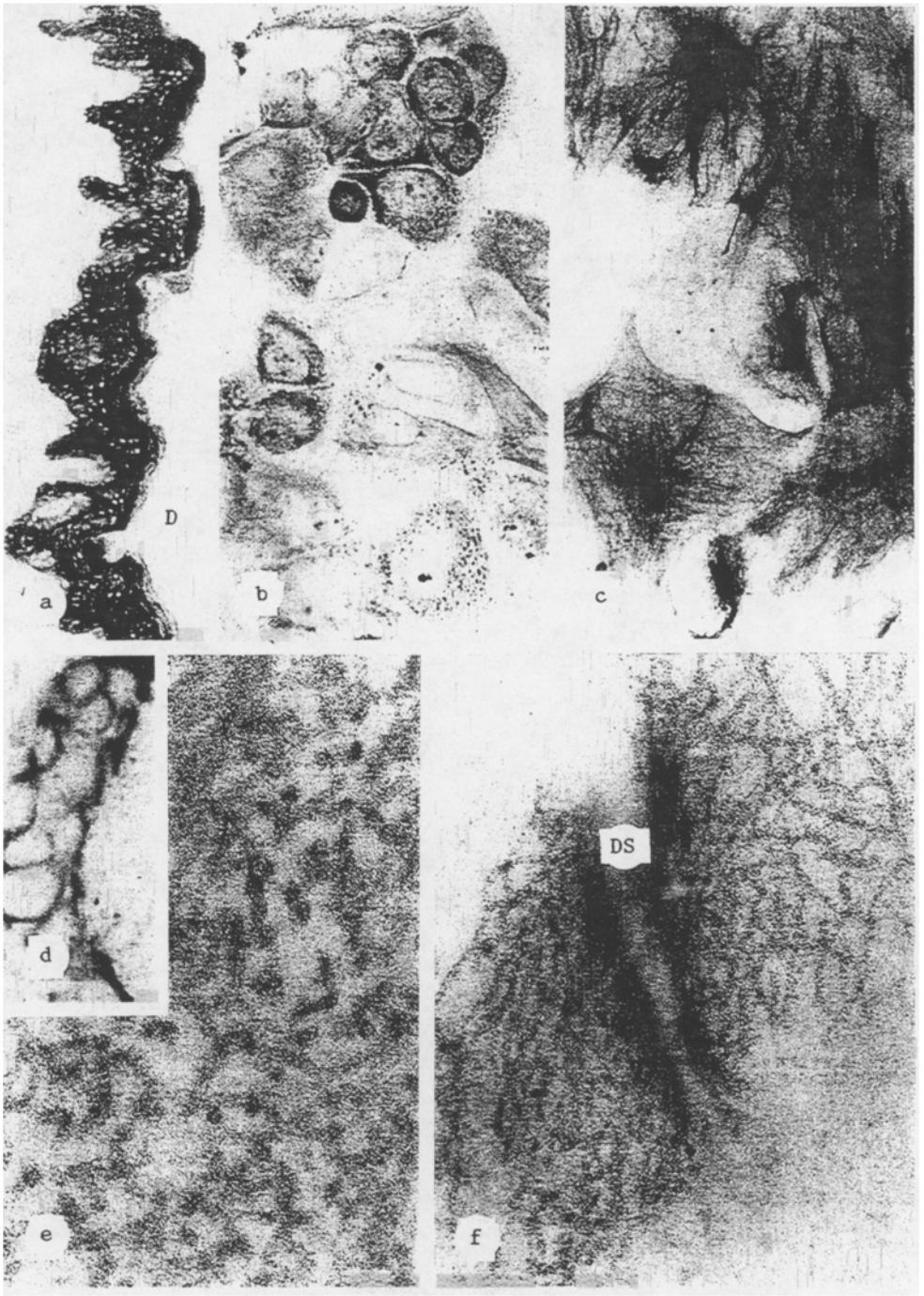

Fig. 1. Immunohistochemical reaction of McAb H4 in human tissues (a, d, e, f) and cultures of epithelial cells from the guinea pig vas deferens (b, c) at light-optical (a, b, c, d) and ultrastructural (e, f) levels: a) cytoplasm of cells of all layers of human epidermis gives a positive reaction. Basal layer stained somewhat less strongly (D – dermis); b) 7-day culture, reaction product located in cytoplasm in the form of grains; c) 14-day culture, staining as intracytoplasmic bands; d) in an Epon section 20 μm thick through tissue of a squamous-cell carcinoma of the cervix uteri the reaction product is located in the cytoplasm around the nuclei; e) ultrastructure of cytoplasm of cell of squamous-cell carcinoma of cervix uteri, immunohistochemical reaction product located on intermediate filaments; f) cell of squamous-cell carcinoma of cervix uteri, peroxidase label located on intermediate filaments, approaching a desmosome (DS). a-f) indirect immunoperoxidase reaction. Magnification: a) 240, b) 500, c) 700, d) 1600, e) 170,000, f) 100,000.

in Tris-HCl, pH 7.6. Sections for light microscopy were mounted in Canada balsam. Sections 20 μm thick for ultrastructural investigation were fixed for 1 h in a 1% solution of osmium tetroxide and embedded in Epon-812 in the usual way. After examination of semithin sections the block was trimmed to a pyramid in areas were staining of the cytoplasm was visible. Ultrathin sections were placed on copper grids with formvar backing and examined without contrast staining. Light-optical preparations were photographed in a "Polyvar" microscope (Reichert, Austria). Ultrathin sections were examined in the JEM-1200EX11 electron microscope (Japan) with accelerating voltage of 80 kV.

As the positive control, McAb CK-1 (Dakopatts, Denmark), which have specificity to a broad spectrum of cytokeratins [8], were used at the light-optical level.

EXPERIMENTAL RESULTS

A positive reaction with McAb H4 was given by cells of all epithelial structures of the embryonic and definitive skin (Fig. 1), its appendages, but with the exception of the myoepithelial cells of the acini and ducts of the sweat glands. A reaction also was absent in the basal layer of the definitive ectocervix uteri, whereas all layers of the ectocervix stained in the 24-week fetus. Cells of the simple epithelium of the cervical canal, body of the uterus, and also metaplastic squamous epithelium gave an intense reaction. Cells of the squamous-cell carcinoma of the skin and cervix uteri stained unevenly. The cell structure of epithelium from the vas defarens gave a bright stain in the form of grains or intracytoplasmic bands. In unstained semithin sections the residue of diaminobenzidine was identified in the cytoplasm of the cells. On ultrastructural investigation of the cells of different types of epithelium, including cancer cells, the peroxidase label was found in the cytoplasm on intermediate filaments 10 mm in diameter, including on filaments close to desmosomes. The structure of the desmosomes took no part in the reaction with McAb H4.

It must be pointed out that the McAb H4 gave no reaction with cellular and fibrous structures of the stroma. Cells of nonepithelial tumors, such as nevus, melanoma, lymphoma, fibrosarcoma of the skin, and leiomyosarcoma of the cervix uteri, likewise were unstained.

In their immunohistochemical characteristics McAb H4 are similar to McAb Lu-5 [13], KL-1 [15], and CK-1, which are specific for the broad spectrum of cytokeratin polypeptides. It can therefore be postulated that H4 antibodies recognize either a common epitope, characteristic of several cytokeratin proteins, or cytokeratin-associated peptide. The possibility of binding of McAb H4 with lipoproteins, which have recently been found on the surface of the intermediate filaments of epithelial cells, likewise cannot be ruled out [10].

These results may serve as the basis for the use of McAb H4 as a tissue-specific immunohistochemical marker of the cells of different types of epithelium, and-also as cells of malignant tumors. It must also be pointed out that identification of the epitope which reacts with McAb H4 requires further investigation.

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