DETECTION OF A CROSS-REACTING ANTIGEN COMMON TO STRATIFIED EPITHELIUM AND GROUP A STREPTOCOCCUS IN HUMAN EPIDERMAL TUMOR CELLS

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The study of the tissue and organ specificity of antigens of malignant tumor cells can help to solve a number of problems connected with the basic principles of carcinogenesis and can also create a basis for the development of tests for the differential diagnosis of tumors of different origin. Much research has already taken place in this direction [1, 4-6, 12]. The results of some of it have found practical application. The present writers showed previously [2, 8-10] that cells of the basal layer of stratified squamous epithelium contain a cross-reacting (CR) antigen with the polysaccharide of group A streptococcus (polysaccharide A). These investigations were done by the indirect immunofluorescence method. This CR antigen possesses tissue specificity and is found in the epithelium of ectodermal origin in animals and man [2, 7, 11]. This antigen is also found in cells of transplantable strains of tumors of the mouse esophagus and uterine cervix, histogenetically linked with the tissues that carry this CR antigen [3]. This last observation provided a basis for the investigation of human neoplasms.

The object of this study was to look for CR antigen in human epidermal tumors.

EXPERIMENTAL METHOD

Material for study was obtained during operations for removal of tumors on 32 patients with basal-cell and three patients with squamous-cell carcinoma of the skin in different situations: the skin of the face in seven cases, the neck in 11, the hairy part of the scalp in five, the trunk (back, chest, abdomen) in seven, the limbs in five. Skin from areas adjacent to the tumor and also from areas some distance away (up to 2 cm) also was studied. Skin from clinically healthy subjects dying from acute trauma and tumors histogenetically connected with epithelium of entodermal origin – adenocarcinoma of the stomach (two cases) and of the intestine (two cases) – were used as the control.

Sections 5 μ thick were cut in a cryostat (from -15 to -20°C) from tissue frozen at -86°C (with a mixture of dry ice and acetone), and were used unfixed. CR-antigen was revealed by the indirect immunofluorescence method, using a preparation of pure antibodies containing 0.8 mg/protein ml. Antibodies against CR antigen were isolated from the serum of rabbits immunized with a streptococcal culture by means of a Sepharose immunosorbent containing polysaccharide A [7]. Antibodies against rabbit IgG were isolated with the aid of an immunosorbent from donkey serum against rabbit immunoglobulins. The method of obtaining antibodies against IgG was described previously [10]. Rabbit antibodies against mouse immunoglobulins containing 0.75 mg/protein ml were used as the control.*

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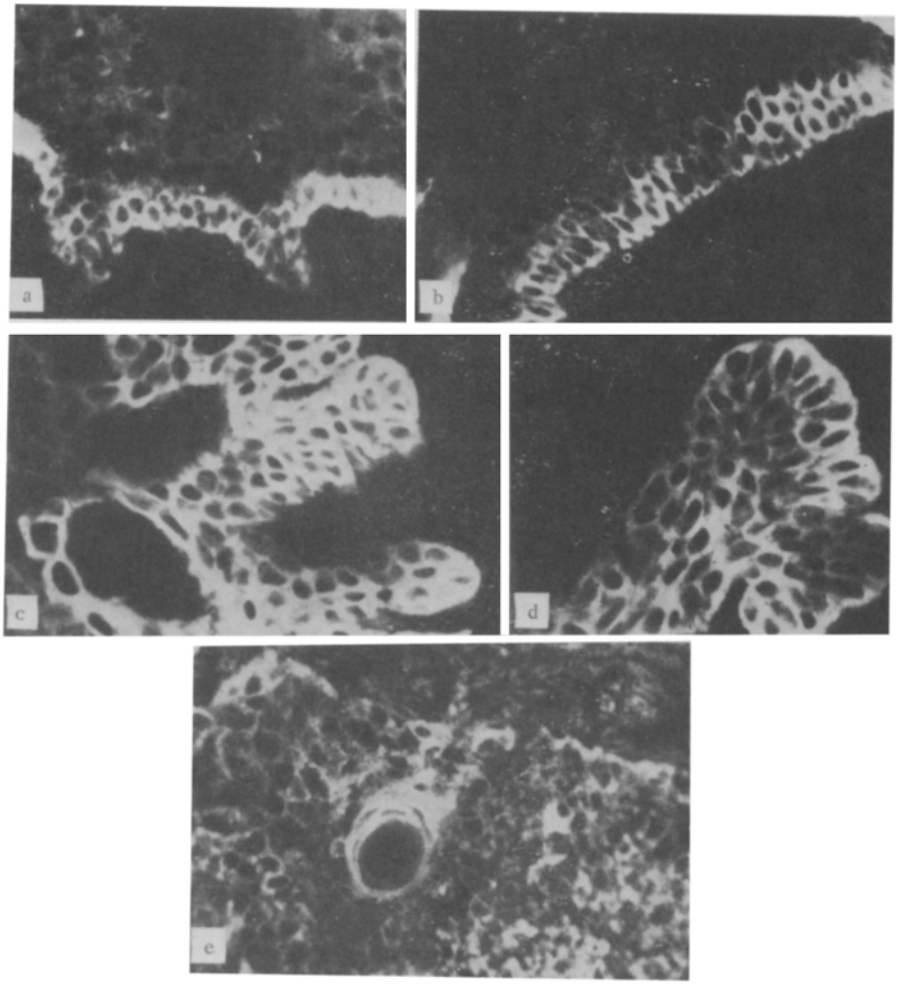

Fig. 1. Sections through skin of healthy subjects and patients, treated with antibodies against CR antigen common to basal cells of stratified squamous epithelium and polysaccharide of group A streptococcus. a) Section through healthy human skin, reaction in outer zone of cytoplasm of cells of basal layer of epidermis; b) section through skin of patient with basal-cell carcinoma, increase in number of layers of cells containing CR antigen; c, d) sections through tissues of basal-cell carcinoma, reaction in outer zone of cytoplasm of all tumor cells; e) sections through squamous-cell carcinoma, reaction in zone of separate cells and of cells surrounding pearl. Indirect immunofluorescence method, objective 40× (water immersion), ocular homal 3×.

The tissue sections, after drying for 30 min at room temperature, were rinsed for 5 min in phosphate buffer solution (PBS), pH 7.2, after which antibodies against CR antigens were applied to the wet sections for 18 h (at 4°C). After rinsing (10 min) in PBS the sections were treated with labeled antibodies against rabbit IgG for 35 min at room temperature, rinsed again in PBS, and mounted under a coverslip in neutral 60% glycerol. The tissues were examined in the ML-2 luminescence microscope with 40× (water immersion) and 90× (oil immersion) objectives. A homal 3 ocular and RF3 film were used for photography. Some sections were fixed for 30 min in 96° alcohol and stained with hematoxylin and eosin.

EXPERIMENTAL RESULTS

During consecutive treatment of sections of healthy human skin with antibodies against polysaccharide A and labeled antibodies against rabbit immunoglobulins a reaction was observed in the outer zone of the cytoplasm of cells in the basal layer of stratified squamous epithelium of the skin (Fig. 1a). On investigation of sections of a basal-cell carcinoma and in the skin adjacent to the tumor and in unchanged areas of epidermis

at a considerable distance from the tumor, the reaction was found to be localized principally in cells of the basal layer. In areas where thickening of the epidermis was observed on account of proliferation of tumor cells in situ, without disturbance of the integrity of the basement membrane of the epithelium, as confirmed by examination of histological sections, a reaction was found in the cells of two or three layers or more (Fig. 1b). The use of oil immersion revealed a reaction in many small processes of cytoplasm penetrating through pores in the basement membrane of the epithelium. If the integrity of the basement membrane was disturbed and tumor nodules penetrated into the dermis, a reaction was observed in the outer zone of cytoplasm of all cells of the basal-cell carcinoma (Fig. 1c, d). Investigation of a squamous-cell carcinoma of the skin showed a reaction of antibodies against CR antigen to be localized in the cytoplasm of only seven cells. Among cells forming "pearls" of squamous-cell carcinoma, only those of the outer layers of the pearl contained CR antigen (Fig. 1e). Antibodies against CR antigen of the tested series did not react with antigens of structures of the dermis from clinically healthy subjects and patients. When rabbit antibodies against mouse immunoglobulins were used as the control in the indirect immunofluorescence test, no reaction was observed with antigens of normal and tumor epithelium. When sections of the epidermis and skin tumors were treated with labeled antibodies against rabbit IgG only, no reaction was found with epithelial structures. In sections of adenocarcinoma of the stomach and intestine, antibodies against CR antigen characteristic of stratified squamous epithelium did not react with components of the tumor cells.

The results described above are evidence that a tissue-specific basal-cell antigen of stratified epithelium, cross-reacting with the group polysaccharide of group A streptococcus, is present in the cytoplasm of tumor cells of human basal-cell and squamous-cell carcinomas, histogenetically linked with the epidermis, and is not found in tumor cells of entodermal origin (carcinoma of the stomach and intestine). The fact that in a basal-cell carcinoma the reaction is observed in the cytoplasm of all tumor cells, whereas in squamous-cell carcinoma it is found in only some cells, can evidently be explained by differences in the degree of differentiation of the cells in these two forms of tumors. Whereas the CR antigen in the healthy human epidermis is contained only in the youngest cells (basal layer) in tumor cells of the basal-cell carcinoma, in the absence of any marked differentiation, all cells carry the CR antigen. In cells of the squamous-cell carcinoma, in which signs of cell differentiation are present even to the extent of keratinization, CR antigen is found only in the least differentiated cells. For a final solution to the problem of specificity of this CR antigen not only for basal-cell, but also for squamous-cell carcinoma, further studies of tumors histogenetically connected with stratified squamous epithelium of other organs (esophagus, larynx, cervix uteri) are necessary.

Together with other results obtained previously and indicating the presence of CR antigen in animal tumors of ectodermal genesis, the results of the present investigation can serve as the basis for research aimed at developing an additional method of differential diagnosis of human tumors derived from external epithelium of ectodermal origin.

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