MONOCLONAL ANTIBODIES TO GROUP A STREPTOCOCCAL POLYSACCHARIDE, REACTING WITH ANTIGEN OF BASAL CELL TUMORS HISTOGENETICALLY RELATED TO EPIDERMAL TISSUES

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Antibodies to antigens characteristic of cells of the stratum basale of the skin epithelium have been described in human and animal blood serum by several workers [2, 5, 11, 12]. One of the basal-cell antigens (BCAG) is cross-reacting and is common to epithelium and to group A streptococcal polysaccharide (A-polysaccharide) [5, 6, 14].

Histogenetic studies have revealed cross-reacting BCAG in epithelial cells of the skin, the skin appendages, conjunctiva, mucous membrane of the mouth, larynx, esophagus, anal region of the rectum, and urinary bladder and the epithelium of the thymus in man and animals [1, 4, 6, 14]. The above-mentioned tissues, according to the Soviet classification, are tissues of epidermal type and are derivatives of the embryonic epidermal anlage, formed from the ectoderm and the cranial and anal segments of the entoderm. This cross-reaction BCAG now under study appears in cells of the simple fetal epidermis at the beginning of the second half of pregnancy in the rat and at the 8th-9th week in man [3, 9]. Antibodies to cross-reacting BCAG, isolated from rabbit serum after immunization of the animal with a pepsin-treated streptococcal culture [5, 6, 14], have been used to create an experimental basis for the differential diagnosis of tumors histogenetically related to the tissues carrying this BCAG [2, 4, 10]. The antigen has been found in cells of a basal-cell skin carcinoma, squamous-cell carcinomas of the human larynx and cervix uteri [4, 10], and squamous-cell carcinomas of the mouse esophagus and cervix uteri [2, 4]. Monoclonal antibodies (MCAB) to BCAG, giving a cross reaction with A-polysaccharide, have recently been studied [7]. We know that synthetic polyelectrolytes (PEL) can induce an immune response to nonimmunogenic haptens [8], including to A-polysaccharide [7]. In connection with this, MCAB A6/1-D against cross-reacting antigen of epithelium and streptococcus have been obtained by immunizing mice with A-polysaccharide, conjugated with synthetic PEL [7].

The aim of the present investigation was to detect cross-reacting BCAG in a wider spectrum of human tumors by the use of MCAB A6/1-D. The aim of the investigation was to improve the experimental basis for the histogenetic diagnosis of neoplasms.

EXPERIMENTAL METHOD

Tumor material obtained in operations on 43 patients was investigated (basal-cell carcinoma — five cases, squamous-cell carcinoma: of the skin — six cases, mouth — three, larynx — seven, cervic uteri — five, lung — four, and esophagus — four; adenocarcinoma of the breast — five, stomach — two, and intestine — two cases). Tissues of organs from persons dying from acute trauma or of a human fetus (16-26 weeks) were studied as the control. The tissues (fragments measuring 5 × 5 × 7 mm) were frozen at —20°C or in a mixture of dry ice and acetone (—96°C). Sections 5-6 μ thick were cut in a cryostat (—20°C) and were used unfixed. The meth-

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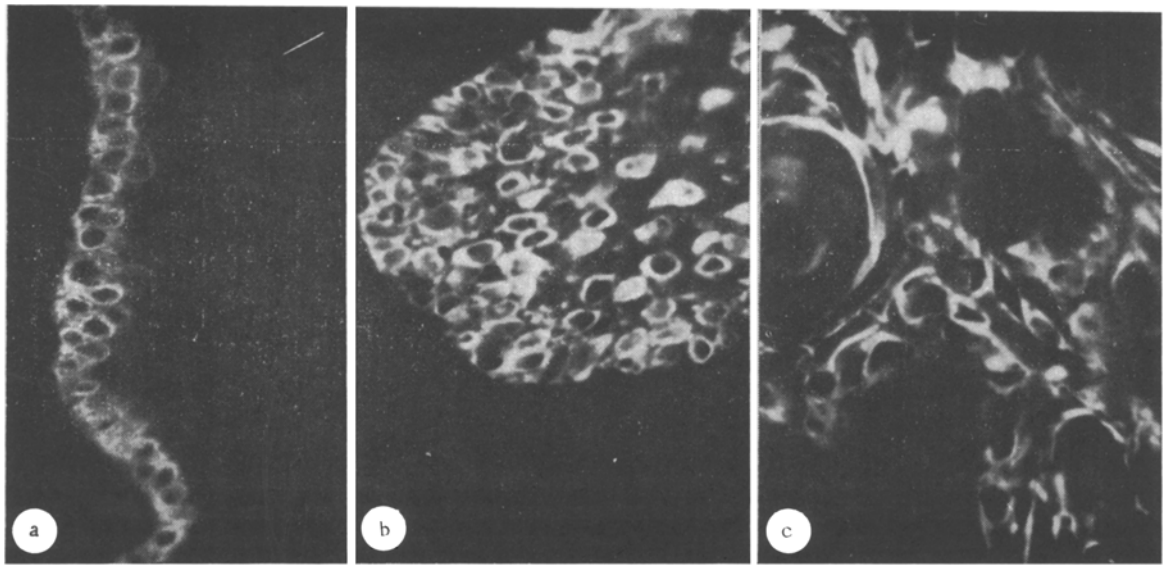

Fig. 1. Sections through normal and malignant human tissues, treated with MCAB A6/1-D to cross-reacting BCAG. a) Section through human skin. Reaction in outer zone of cell cytoplasm of stratum basale of epidermis; b) section through skin of cheek from region of growth of squamous-cell carcinoma. Reaction in outer zone of cytoplasm of tumor cells. No reaction present in structures of surrounding connective tissue; c) section through mucosa of tongue from region of growth of squamous-cell keratinizing carcinoma. Reaction in cytoplasm of tumor cells. No reaction present in keratinizing material from center of keratinization. Indirect immunofluorescence method. Objective ×40 (water immersion), ocular homal ×3.

od of processing the sections was described previously [3, 10]. MCAB A6/1-D to BCAG common with A-polysaccharide, obtained by immunizing BALB/c mice with the purified polysaccharide, conjugated with synthetic PEL [7], were used. The MCAB were prepared by the hybridoma technique in the usual way [13]. The globulin fraction to fluorescein-labeled mouse immunoglobulins was used in the indirect immunofluorescence test. Control immunofluorescence tests were carried out as described previously [6, 14]. The reactions were read by means of the ML-2 or LYUMAM-2 luminescence microscope, and photographed on RF-3 film with a 40× objective (water immersion) and homal 3 ocular. Some sections were fixed in ethanol and stained with hematoxylin and eosin as the histological control.

EXPERIMENTAL RESULTS

On treatment of skin sections from a clinically healthy person with MCAB A6/1-D a reaction was observed in the outer zone of cytoplasm of the cells only of the stratum basale of the epidermis (Fig. 1). Antigens of other skin structures did not react with MCAB. In sections of the stratified epithelium of other organs (mouth, esophagus, urinary bladder, etc.) the reaction also was localized in the cell cytoplasm of the stratum basale. In the epithelium of the vagina and cervic uteri BCAG also spread to more highly differentiated layers. Morphological investigation of the tumor tissues in all cases confirmed the clinical diagnosis. During investigation of skin sections from patients with basal-cell carcinoma (ulcerative, superficial, and nodular forms), obtained from the zone of growth of the proliferating tumor, with MCAB A6/1-D, a reaction was observed in the cytoplasm of tumor cells proliferating in the epidermis and in the tumor nodules. In the epidermis BCAG was contained in the cells of three or more layers. On investigation of sections of the tissues of squamous-cell carcinomas of the skin, tongue, esophagus, cervic uteri, and other organs with the aid of MCAB A6/1-D, reactions were found in the cytoplasm of most proliferating tumor cells (Fig. 1b, c). In cells of the inner zones of the epithelial pearls of a squamous-cell keratinizing carcinoma — the most highly differentiated elements — and also in keratinizing material, no cross-reacting BCAG could be found. No BCAG likewise was found in tumor cells of glandular genesis (adenocarcinoma of the stomach, intestine, and breast). In control experiments, when tissue sections of tumors were treated with antibodies to mouse immunoglobulins, no reaction was found. Proliferation of tumor cells in the epithelium of the mucosa in carcinoma of the cervic uteri was accompanied by active desquamation of BCAG-containing tumor cells from the surface of the

mucosa. In squamous-cell lung carcinoma, cells of the large and smaller foci of tumor proliferation contained cross-reacting BCAG.

Data on differences in the antigenic composition of cells in the stratum basale of the skin epithelium and of its differentiated layers have been obtained recently by means of immunochemical and immunomorphological methods. It has been shown that proteins (prekeratins) with mol. wt. of 46 and 56 kilodaltons are characteristic of basal cells [15].

The results of this investigation thus show that MCAB A6/1-D against cross-reacting BCAG characteristic of cells of the stratum basale of stratified epithelium may be used to detect this particular antigen in cells of tumors related to epithelial tissues of epidermal type. The use of these MCAB is bound to lead to improvement of the methods of histogenetic diagnosis of neoplasms.

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