IMMUNOMORPHOLOGIC STUDY WITH MONOCLONAL ANTIBODIES AGAINST INTERMEDIATE FILAMENT PROTEINS OF 1,2-DIMETHYLHYDRAZINE INDUCED CARCINOMAS OF THE RAT LARGE INTESTINE

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Difficulties which cannot be solved by ordinary histological analysis arise during morphological investigations of tumors in some cases. In particular, it is difficult to determine the tissue origin of some neoplasms or to discover initial stages of invasion of a tumor, in the form of ingrowth of small groups of tumor cells into the stroma of the tumor. Solutions to these problems are extremely important to the planning of the best course of treatment and determination of the degree of malignancy of tumors. Recently staining histological sections with antibodies against intermediate filament proteins has begun to be used for this purpose [10]. Depending on the type of cells, different, although closely related, proteins will be found in the composition of their intermediate filaments [9]. For example, the family of prekeratin proteins is a component of filaments of epithelial cells, the protein vimentin is found in the filaments of most cells of the mesenchymal series. An advantage of intermediate filament proteins as markers of histogenesis is their stable expression, which is often independent of the level of differentiation or the degree of transformation of the cells [5, 6, 10]. One problem when working with intermediate filament proteins is that the high degree of homology of these proteins with one another makes it difficult to use ordinary polyclonal sera. The writers have shown that by the use of monoclonal antibodies specific against one of the prekeratins of the rat large intestine it is possible to improve demonstration of minimal features of invasion of tumors of this organ considerably. The high specificity of the monoclonal antibodies used in the work virtually rules out any likelihood of mistakes when determining cell types after staining of histological sections with these antibodies.

EXPERIMENTAL METHOD

Starting from the age of 2 months, 1,2-dimethylhydrazine dihydrochloride (DMH) was injected into noninbred male rats in a dose of 8 mg/kg body weight, calculated as base (altogether 25 weekly subcutaneous injections). After 40 weeks of the experiment the animals were killed, and tumors of the large intestine together with areas of surrounding mucosa, and also pieces of intact mucosa of the small intesting were removed, washed free from mucus and intestinal contents in physiological saline, embedded in blocks in 7% gelatin solution in isotonic phosphate buffer (PB), and then quickly frozen by immersion in liquid nitrogen Frozen blocks were kept at −70°C [5, 6]. Frozen sections 5 μ thick were thawed, fixed for 5 min in 10% formalin solution in PB, and then stained by the indirect immunofluorescence method, using antibodies of hybridoma clones C12 and 30, obtained and characterized previously [3]. Antibodies of clone C12 react with prekeratin with mol. wt. of 49 kilodaltons (PC49) and antibodies of clone 30 react with vimentin. Antibodies against mouse immoglobulins labeled with fluorescein isothiocyanate (from Miles, England) were used as the second antibodies. Some sections prepared in this way were counterstained either with Mayer's hematoxylin or with Evans' blue. The finished sections were examined in an Opton photographic microscope with fluorescence attachment.

EXPERIMENTAL RESULTS

Antibodies of clone C12, reacting with prekeratin protein CP49, revealed cells of the epithelial layer of the mucosa in frozen sections of the normal large intestine (Fig. 1). All the enterocytes of the crypt stained with about equal intensity; proliferating cells in the bottom part, goblet cells in the middle, and absorbing cells

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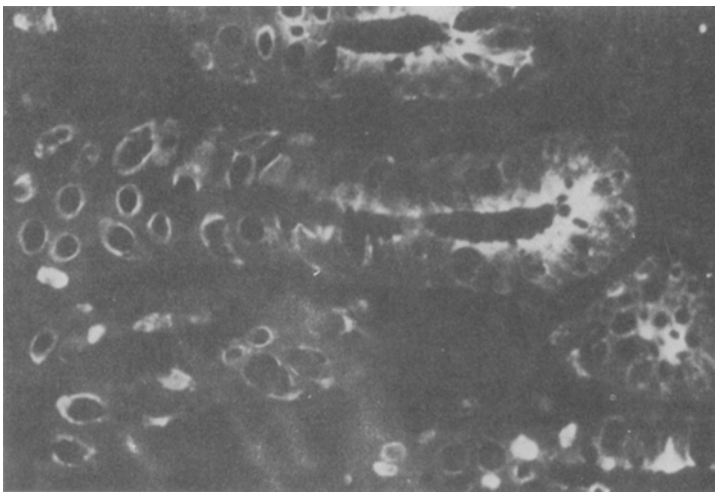

Fig. 1. Mucosa of intact rat large intestine. Indirect immunofluorescence microscopy with antibodies of clone C12 against PC49. Fluorescence of enterocytes uniform throughout depth of crypt. 500 ×.

in the topmost parts. In the submucous layer no cells stained with antibodies against PC49 could be found. Thus in direct immunofluorescence staining of frozen sections confirmed previous results indicating the presence of protein PC49 in all epithelial cells of the large intestinal crypts [3]. These cells remained unstained if antibodies of clone 30, reacting with vimentin, were used.

Histological analysis showed that the overwhelming majority of large intestinal tumors induced by DMH were exophytic adenocarcinomas with a well-marked glandular component, consisting of irregularly shaped tubular structures. The typical morphological picture of these neoplasms is illustrated in Fig. 2a, b. Staining with antibodies against PC49 showed even more clearly the morphological features of the epithelial glandular structures of carcinomas of the large intesting (Fig. 2b). These tumors consisted basically of cells with simpler morphology, in the form of small trabeculae and bands (Fig. 2c, d). These cells are considered to be foci far advanced in tumor progression, characterized by invasion by the tumor into the stroma and deeper [2]. By means of antibodies against PC49, yet another row of similar nodules, virtually indistinguishable by ordinary histological staining, could be found.

Several neoplasms exhibited endophytic growth, combined with marked structural and cellular anaplasia. In these cases also, however, virtually all of the epithelial component of the tumor stained with antibodies against PC49 and did not stain with antibodies against vimentin (Fig. 2e, f). Thus most epithelial cells of DMH-induced tumors of the large intestine in rats have the same phenotype as their normal analogs: they synthesize prekeratin protein PC 49 and do not contain vimentin.

Examination of sections stained with antibodies against PC49 showed that in some cases (in six of 32 tumors), besides trabeculae and bands, scattered atypical cells or small concentrations of cells (Fig. 2e, f), indistinguishable on ordinary histological analysis, appeared in the deep parts of the tumor stroma. No such cells were found, as had already been mentioned, in the submucosa of the normal large intestine. In some neoplasma they were found at a considerable distance from the main part of the tumor glands. Studies of cell cultures of epithelium of varied origin have shown that tumorigenic epithelial cells in some cases resemble mesenchymal cells [1, 4, 7, 8]. Cells possessing such properties are probably clearly visible after immunofluorescence staining for prekeratin, and are virtually indistinguishable from stromal cells surrounding them in sections stained in the ordinary way. Most probably these atypical single cells are cells which are far advanced in a certain direction of neoplastic progression and are capable of existing separately from the main tumor tissue. The presence of such cells in the tumor stroma may perhaps be an important diagnostic criterion of malignancy. The further study of the properties of these cells is extremely important, because they can perhaps play an important role in metastization and invasion.

The presence or absence of invasion into the submucosal layer or into deeper parts of the wall of the large intestine is usually the main criterion when evaluating the degree of malignancy of a tumor. The high resolution achievable after staining sections with antibodies against PC49 enables minimal features of invasion

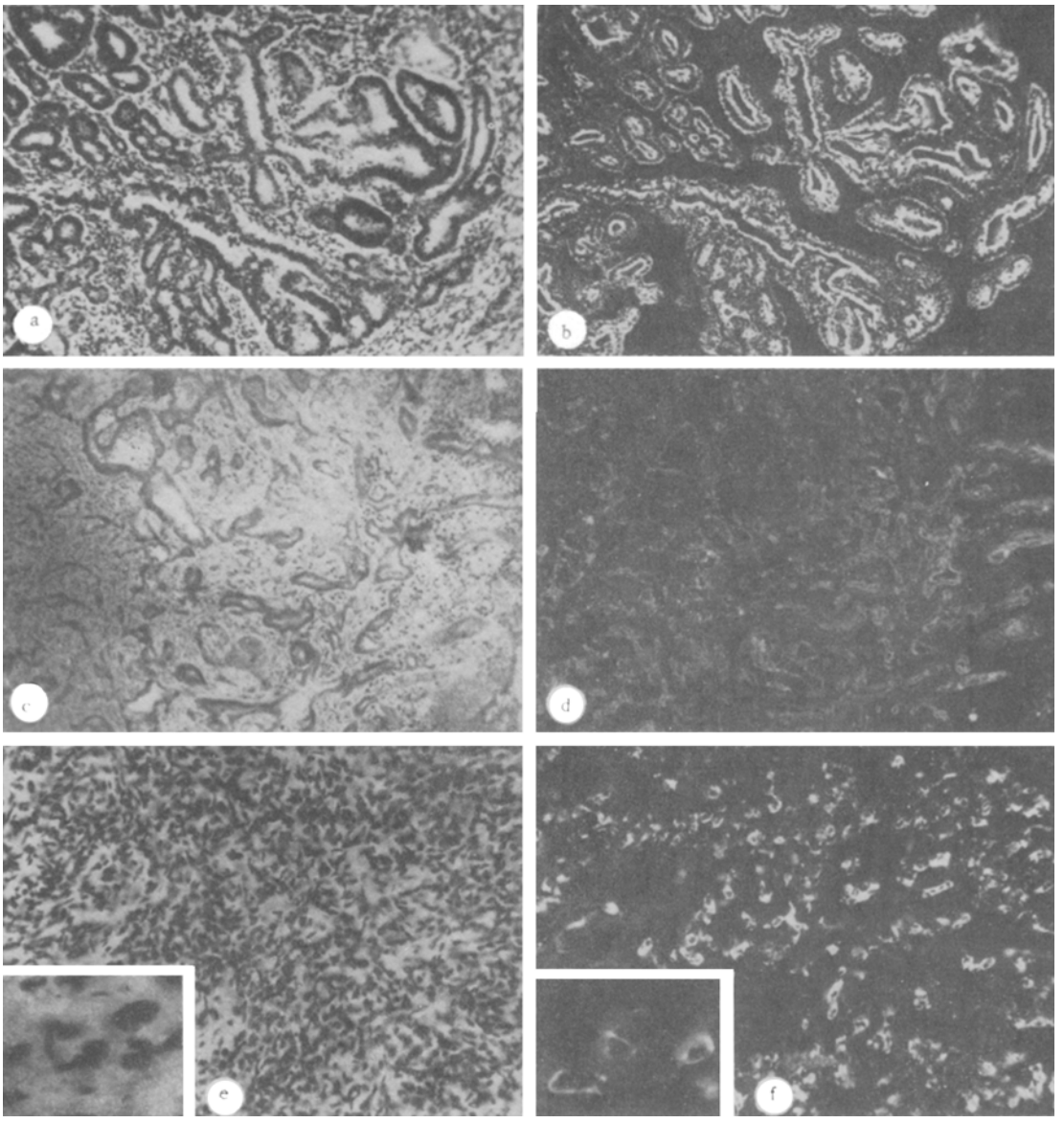

Fig. 2. Different morphological types of tumors of rat large intestine: a, b) adenoma with characteristic glandular structure (a – stained with Mayer's hematoxylin, b – indirect immnuofluorescence to PC49. 200 ×; c, d) invasive adenocarcinoma (c – stained with Evans' blue: besides glandular structures in topmost parts, concentrations of tumor cells in the form of trabecules are visible in the depth; d – indirect immunofluorescence to PC49; single tumor cells visible at base of tumor, indistinguishable in transmitted light. 100 ×); e, f) anaplastic carcinoma without glandular structures (e – stained with Mayer's hematoxylin; tumor cells indistinguishable from cells of loose connective tissue; f – indirect immunofluorescence to PC49: tumor cells clearly visible. 400 ×; inset – similar regions under higher power, 600 ×).

to be discovered more reliably and, in some cases, enables the histological diagnoses of the degree of malignancy of the neoplasm to be revised. Immunochemical staining of tumor sections with the aid of monoclonal antibodies, strictly specific for the various prekeratin proteins, can therefore be used with success for diagnostic and, probably, for prognostic purposes in practice.

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