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PREPARATION AND PROPERTIES OF MONOCLONAL ANTIBODIES TO INDIVIDUAL PREKERATINS OF SIMPLE RAT EPITHELIUM

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Intermediate filaments (IF) of epithelial cells consist of special proteins called prekeratins (PK). By now 19 PK have been identified by biochemical analysis of human tissues [11]. All are more or less homologous with one another and they evidently constitute an evolutionarily related group of proteins. This same group also includes proteins from IF of other types of cells: vimentin, desmin, and proteins of neurofilaments and glial filaments [5, 8].

Mainly biochemical methods have been used to study the distribution of individual PK in various kinds of cells, but these do not enable tissues with complex cellular composition to be investigated or fine heterogeneities of population to be detected. Nevertheless, even now it can be concluded that the IF of different types of epithelium are composed of different sets of PK. However, this unique multiplicity of the PK and the biological significance of specific sets relative to different types of epithelium await explanation. The complete pattern of distribution of individual PK in epithelium of adult animals has not been obtained. The principles governing the formation of this distribution in ontogeny have not been studied. The solution of these problems requires antibodies specifically recognizing individual PK. It is difficult to obtain such antibodies because of the close degree of affinity between members of the PK family. Polyclonal sera obtained by immunization with individual PK often give cross reactions with other proteins of this family [4, 9]. The preparation of monoclonal antibodies reacting with only one polypeptide also has proved to be a difficult task. Only a few such clones have been described in the liaterature [6, 10, 12].

The aim of this investigation was to obtain and study the properties of a series of hybridoma clones producing antibodies to individual PK from simple types of epithelium.

METHODS

Monoclonal antibodies were obtained by the method in [7] with minor modifications [1, 2]. BALB/c mice were immunized with a preparation of IF isolated from the mucosa of the rat large

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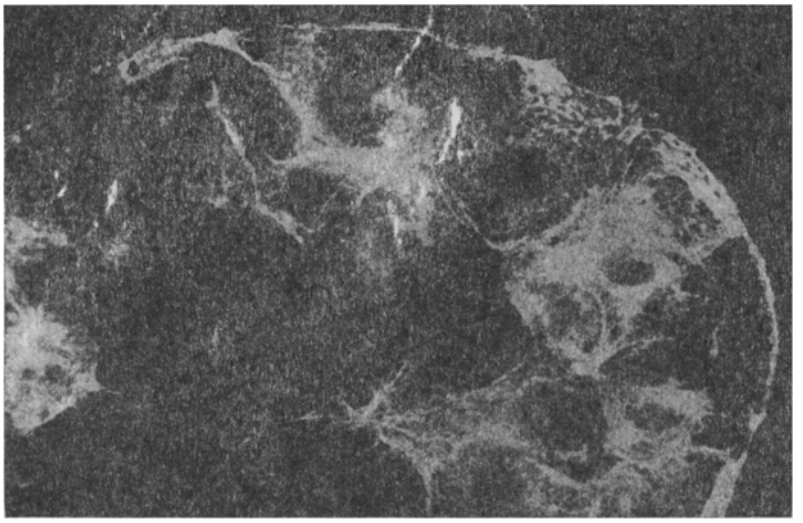

Fig. 1. Indirect immunofluorescence staining of hepatoma 27 cells by antibodies of clones. All antibodies revealed typical EF network. Magnification × 300.

intestine [2]. The culture medium of the hybridomas after primary seeding and after successive clonings was tested by the indirect immunofluorescence method. To stain rat hepatoma 27 cells, which have the same set of PK as intestinal enterocytes [2], the cells were seeded on 48-well (Terasaki) Polydishes (Flow Laboratories, England). After culture for 2 days under conditions described previously [2] the cells were fixed by the method in [3]. Each well of the Polydish was stained with medium of a particular clone of hybridomas by the standard indirect immunofluorescence technique. Antibodies labeled with fluorescein isothiocyanate against mouse immunoglobulins (Dako, Denmark) were used. The preparations were examined under the Diawert microscope (Leitz, West Germany) with fluorescence attachment. Only those clones whose antibodies revealed typical IF in hepatoma 27 cells (Fig. 1) on staining were used in the work. By this method, from about 800 initial clones examined, five, named E2, E3, E5, E6, and E7, were selected. Double immunodiffusion in agar with specific antibodies (Miles,* England) showed that clones E2 and E6 secrete immunoglobulins of the IgG1 type, and clone E3 secretes those of the IgG2b type. The specificity of the clones was studied by immunoautoradiography [2].

RESULTS

Antibodies of clones E2, E3, E5, E6, and E7 do not stain rat fibroblasts in primary culture by the indirect immunofluorescence method. In hepatoma 27 cells growing *in vitro* they revealed a network of fibrils (Fig. 1), with the characteristic distribution of IF. In 2-day hepatoma cultures all the antibodies revealed approximately the same network, although by no means all the cells were stained. The number of stained and unstained cells was about the same whatever antibody was used. Special double staining is necessary for a detailed study of the distribution of the corresponding antigens at both intracellular and population levels.

Staining the polyacrylamide gel for protein after electrophoretic fractionation of IF preparations isolated from a culture of hepatoma 27 cells in it in the presence of sodium dodecylsulfate (SDS), revealed four proteins in the region of molecular weights characteristic of PK (Fig. 2): three major proteins (55, 49, and 40 kilodaltons) and one minor (53 kilodaltons). These proteins, as was shown previously [2], according to their biochemical characteristics are PK: PK55, PK53, PK49, and PK40. Staining the nitrocellulose filters to which the electrophoretically separated IF preparations were first transferred, by antibodies produced by the clones thus obtained (immunoautoradiography) showed that antibodies of clones E3 and E6 reacted with PK40, and those of clones E2 and E7, with PK55. Antibodies of clone E5 which, like the rest, stained the IF network by the indirect immunofluorescence method, did not react with any protein of the IF preparations when the method of immunoautoradiography was used. Denaturation of the proteins probably destroyed the antigenic determinant recognized by this clone. Such cases have been described in the liaterature, for example for certain monoclonal antibodies to vimentin [6].

*Could be Ames; "Aules" in Russian original — Translator.

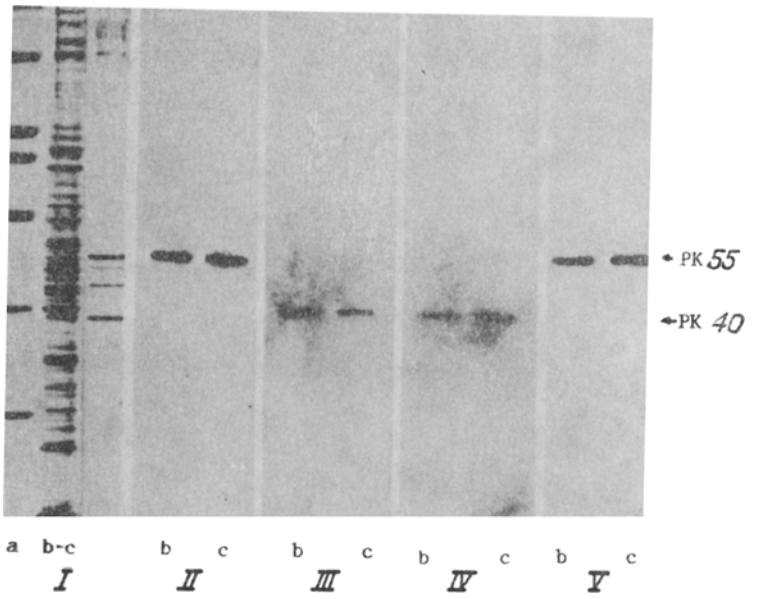

Fig. 2. Reactivity of monoclonal antibodies of experimentally obtained clones with PK of hepatoma 27. I) Staining for protein with Coumassie P-250; II-V) immunoautoradiography with antibodies of clones (same specimens as in I): E2 (II), E3 (III), E6 (IV), and E7 (V). a) Molecular weight markers, from top to bottom, 200, 97.4, 66, 45, and 29 kilodaltons; b) PK preparation from hepatoma 27 cells; c) total lysate of hepatoma 27.

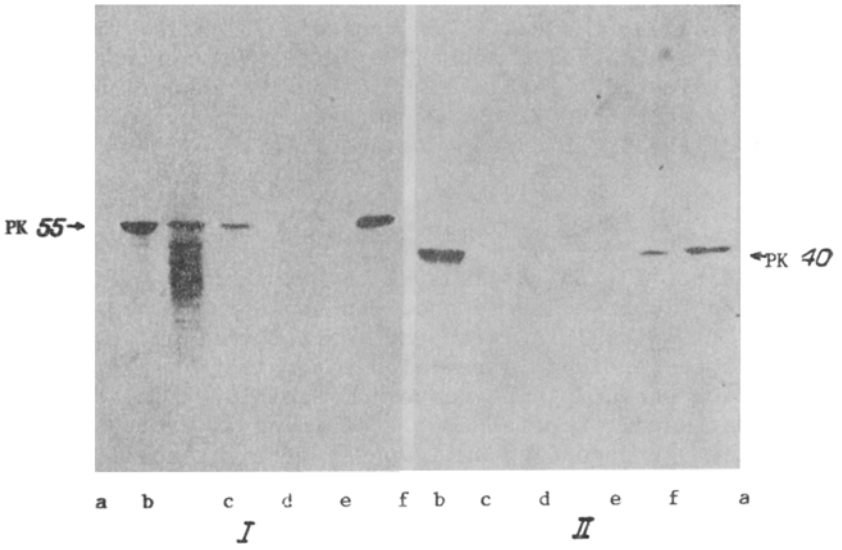

Fig. 3. Immunoautoradiography of proteins from lysate of various organs with antibodies: a) salivary gland, b) intestine, c) liver, d) esophagus, e) muscle, f) stomach.

The antibodies obtained revealed proteins of the same molecular weights in whole lysates of hepatoma 27 cells, i.e., antibodies of clones E3 and E6 revealed a protein with mol. wt. of 40 kilodaltons, and E2 and E7 a protein with mol. wt. of 55 kilodaltons (Fig. 2). The antibodies were thus strictly specific for individual PK of hepatoma 27.

For a more detailed study of the specificity of the experimentally obtained antibodies, we used the same immunoautoradiographic method to study their reaction with proteins of cells of other types. Immunoautoradiography with antibodies of clones E2 and E3 of lysates prepared from the mucous membrane of the rectum, salivary gland, stomach, liver, muscle, and esophagus

is shown in Fig. 3. Just as in the case of hepatoma 27, antibodies of clone E2 reacted in all cases with the 55 kilodalton protein. This protein was not present in lysates of esophagus and muscle. In lysates of salivary gland and, in particular, of intestine, several low-molecular-weight proteins were revealed by immunoautoradiography. The spectrum of these low-molecular-weight bands varied from one experiment to another, although in the same experiment both clones against PK55 (E2 and E7) recognized a very similar band spectrum. These data are evidence, first, that these low-molecular-weight proteins are probably degradation products of PK55 and, second, they show that both types of antibodies to PK55 recognize closely situated antigenic determinants. Antibodies of clones E3 and E6 recognize one protein with mol. wt. of 40 kilodaltons in the intestine, stomach, and salivary gland.

Previously the writers described monoclonal antibodies (clone C12) [1, 2] reacting with rat PK with mol. wt. of 49 kilodaltons. We have thus obtained monoclonal antibodies to three individual PK of simple types of rat epithelium: PK40 (clones E3 and E6), PK49 (clone C12), and PK55 (clones E2 and E7). The corresponding human PK have mol. wt. of 40, 44, and 52.5 kilodaltons. According to the catalogue compiled by Franke's group [11], they are designated PK Nos. 19, 18, and 8 respectively. By now several clones which, like our clones E2, E7, and C12, react with PK Nos. 8 and 18, have now been described in the literature [6, 10, 12]. Monoclonal antibodies to PK40 (No. 19), described in this paper, are so far unique. PK40 is found in by no means all simple types of epithelium, but it often appears in them, and even in stratified epithelium during neoplastic transformation [13]. The study of expression of this particular PK in ontogeny and in carcinogenesis is therefore of the greatest interest.

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