EXPERIMENTAL GENETICS

A GOBLET CELL ANTIGEN DETECTED BY D12 ANTIBODIES

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Previously the writers described several intestinal antigens isolated from human fetal meconiurn and characterized by monospecific rabbit antisera [2-4, 6]. This paper describes an antigen in meconium identified by monoclonal D12 antibodies (D12 MAB).

EXPERIMENTAL METHOD

The immunizing preparation was obtained by fractionating an extract of meconium on Sepharose 4B, and selecting the fractions with mol. wt. of 1000-500 kilodaltons. Mice were immunized with the preparation in a dose of 600-800 μg protein per animal during the whole cycle of immunization. Hybridomas producing MAB [10] were obtained by fusion of P3-X63-Ag8-653 mouse myeloma cells with spleen cells of BALB/c mice immunized with the meconium preparation [1]. The class of monoclonal antibodies was identified by Ouchterlony's method of immunodiffusion in gel with antisera to classes of mouse immunoglobulins (Meloy, England). To characterize the antigen detected by MAB, gradient (4-30%) PAG electrophoresis followed by immunodevelopment was used [13].

The tissue specificity of the test MAB was determined on sections of fetal and definitive normal tissues, and also in sections of pathologically changed tissues from the Archive of the Department of Pathomorphology of the P. A. Gertsen Institute. The indirect immunoperoxidase method [7] was used. To characterize the D12 antigen, sections were treated with neuraminidase from *V. cholerae* (7.7 U/ml) at pH 5.4 for 1 h at 37°C, with trypsin 1 mg/ml at pH 8.0, for 1 h at 37°C, with $NaIO_4$ in a concentration of 0.01, for 10 min, and with $NaBH_4$ in a concentration of 0.01% for 10 min, and also by consecutive treatment with 0.01% $NaIO_4$ and 0.01% $NaBH_4$.

EXPERIMENTAL RESULTS

MAB D12 have the properties of class M immunoglobulins, which do not lose their activity on lyophilization or on double-treble freezing–thawing. In meconium these MAB react with an antigen with mol. wt. (according to gradient electrophoresis) of 600-400 kilodaltons (Fig. 1). Ability to give immunoprecipitation is suppressed by heating to 90°C and with a 1% solution of SDS.

The D12 antigen was found in tissues both in frozen sections and in sections prepared by the routine method of fixation of the tissues with formaldehyde followed by embedding in paraffin wax. Treatment of sections of fetal intestine with neuraminidase led to total suppression of the reaction with MAB D12. Trypsin and $NaBH_4$ had no effect on the intensity of the reaction whereas $NaIO_4$ or $NaIO_4$ followed by $NaBH_4$ intensified the reaction of MAB D12 with the fetal intestine.

In the normal and fetal large intestine MAB D12 detected an antigen exclusively in a secretory vacuole of mucus in the apical zone of the cytoplasm of goblet cells (Fig. 2). Thus MAB D12 react with a carbohydrate determinant in which an important role is played by sialic acids, loss of which (after treatment with neuraminidase), leads to inhibition of the reaction. The important role of carbohydrates also is demonstrated by intensification of the reaction after oxidation of the

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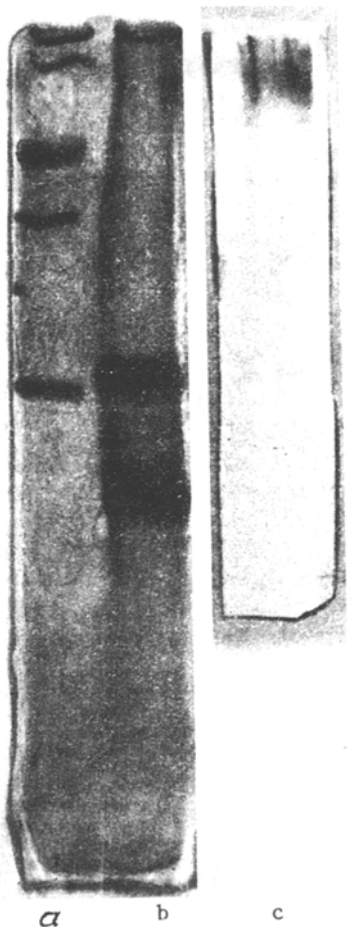
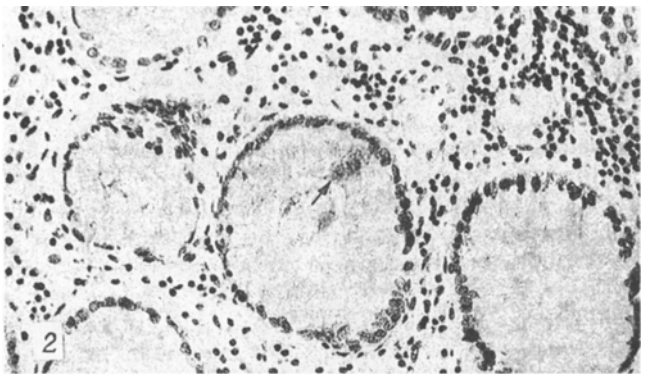


Fig. 1. Gradient electrophoresis of meconium extract in 4-30% PAG followed by immunodevelopment, using MAB D12. a) Marker proteins (Pharmacia, Sweden): thyroglobulin (669000), ferritin (440000), catalase (232000), lactate dehydrogenase (140000), and albumin (67000); b) meconium extract; c) immunoblotting. MAB D12 react with high-molecular-weight antigen. a and b) stained with Coomassie R-250, c) development of immunoprecipitate with 4-chloro-1-naphthol.

antigen by $NaIO_4$. Most probably D12 antigen is a secretory mucin, which corresponds also to its localization in the goblet cells. At the same time, disturbance of binding of MAB D12 with meconium after heating or after treatment with 1% SDS may be indirect evidence of the conformational nature of the D12 antigen, but it likewise does not contradict ideas of the possible complex character of the antigenic determinant.

Particular features of the distribution of D12 antigen in the tissues are given in Table 1. This antigen was found in fetal tissues predominantly in the distal parts of the intestine, and also in mucous cells of the trachea and bronchi. In definitive tissues, the D12 antigen was found only in the colon and rectum, and also in half of the preparations of the trachea and bronchi, where it is located in mucous cells. It must be pointed out that the mucous cells of the esophagus. stomach. and canal of the cervix uteri do not react with MAB D12. In the other epithelial and nonepithelial tissues, the antigen likewise was not present. A study of pathologically changed tissues showed that D12 antigen was detected in 32% of cases of carcinoma of the large intestine (highly differentiated adenocarcinomas in 40%, moderately differentiated in 22%, and undifferentiated in 16%), in the majority of cases of mucous carcinoma, and also in 43% of adenomas of the large intestine; additionally it was found in 30% of carcinomas of the stomach, in about 30% of foci of intestinal metaplasia, and was not found in other neoplasms except one case of weak focal reaction in carcinoma of the mammary gland and 2 of 3 cases of adenocarcinomas of the canal of the cervix uteri (Table 2). In carcinoma of the colon antigen D12 was found



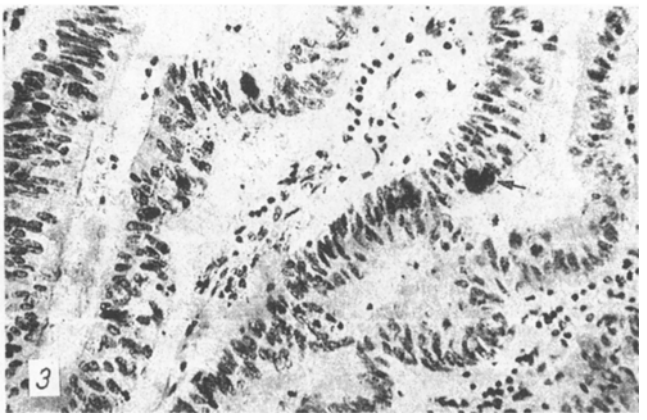


Fig. 2 Fig. 3

Fig. 2. Mucosa of human colon. Clear immunoperoxidase reaction with MAB D12 in one of the goblet cells of the crypts (arrow) and very weak reactions with other goblet cells. Counterstained with hematoxylin, 360×.

Fig. 3. Highly differentiated adenocarcinoma of the colon. Clear immunoperoxidase reaction in several goblet cells of glandular structures (arrow), weak reaction or no reaction in other cancer cells. Counterstained with hematoxylin, 360×.

TABLE 1. Reaction of MAB D12 with Normal Fetal and Definitive Tissues

Tissues (n = 180)	No. of positive	Location of antigen	No. of negative
Fetal (16-28 week fetus)			
Rectum and large intestine	16	Goblet cells and meconium	0
Appendix	4	» » » »	0
Distal part of small intestine	12	» » » »	4
Proximal part of small intestine	2	» » » »	14
Trachea and bronchi	3	Individual goblet cells of epithelium and mucous cells of glands	1
Other tissues	0		34
Definitive: rectum (aquamous epithelium of anus)	0		2
Colon and rectum	24	Goblet cells	9
Small intestine	0		5
Duodenum and pylorus	0		3
Trachea and bronchi	3	Goblet cells of epithelium and mucous cells of glands	6
Other tiisues	0		38

Legend. Other tissues: placenta,* thymus,* mammary gland,** skin, brain, salivary gland, larynx, esophagus, stomach, liver, gall bladder, pancreas, heart, lungs, spleen, kidneys, adrenals, ureters, urinary bladder, ovaries, uterus (phase of secretion,** phase of proliferation**), cervix uteri and canal, ovaries. *) tissues tested only in the fetus; **) only adult human tissues tested.

TABLE 2. Reaction of MAB D12 with Pathologically Changed Human Tissues

Tissues (n = 185)	No. of positive	No. of negative
Carcinoma of stomach	6	20
Sarcoma of stomach	0	1
Mucosa near focus of carcinoma of stomach	4*	13
Adenoma of stomach	0	2
Adenocarcinoma of intestine		
Highly differentiated	6	9
Moderately differentiated	3	19**
Undifferentiated	1***	5
Mucous carcinoma of intestine	3	1
Dimorphic carcinoma (adenocarcinoma + carcinoid)	1****	2
Carcinoid of intestine	0	1
Squamous-cell carcinoma ofintestine	0	1
Adenoma of intestine	3	4
Nonepithelial tumors of intestine	0	3
Ulcerative colitis	0	4
Adenocarcinoma of canal of cervix uteri	2	1
Other neoplasma*****	1******	66

Legend: *) focal reaction observed in foci of intestinal metaplasia of large intestinal type, **) in two of nineteen observations, negative reaction in primary focus, positive reaction in metastases of carcinoma in lymph nodes, ***) positive reaction in narrow zone of carcinoma adjacent to area of nonmalignant gastric mucosa, ****) positive reaction in area of moderately differentiated adenocarcinoma, carcinoid—negative; *****) carcinoma of mammary gland, lungs (in two cases a positive reaction observed in cells of mucous glands of bronchi outside focus of carcinoma), esophagus, uterus, ovaries, thyroid gland, adenoma of salivary glands, melanoma, sarcoma; ******) carcinoma of mammary gland – weak focal reaction.

in the apical part of the cytoplasm of the mucus-producing cells (Fig. 3). It must be pointed out that in two cases we saw no reaction in the primary focus of carcinoma of the colon, but we did observe a reaction in a metastatic focus in a lymph node. This fact, from our point of view, is not associated with the metastatic phenotype, but may be classed a manifestation of tissue-specific features, probably determined in these observations by an increase in the degree of differentiation of the carcinoma cells in the metastasis.

MAB reacting with a high-molecular-weight antigen, of which sialic acids are an important component, were thus obtained by immunization with a fraction of meconium. The D12 antigen described above may evidently fit into a series with known glycoprotein antigens described previously [5, 8, 9, 11, 12]. Under these circumstances the combination of qualitative characteristics of the D12 antigen, namely source from which obtained, thermoability, relative molecular mass, sensitivity to SDS, relations toward treatment with enzymes and redox reagents, suggests that it is an original meconium antigen.

D12 antigen is a marker of highly differentiated mucus-forming cells, and it can accordingly be used for additional characterization of tumors and of pretumor states of the human gastrointestinal tract.

LITERATURE CITED

1. S. G. Egorova, I. D. Kokhanova, T. D. Rudinskaya, and E. E. Sorkin, Ontogenez, **20**, 209 (1989).
2. K. K. Pugachev, R. I. Yakubovskaya, I. B. Shimbirev, and G. I. Avdeev, Éksp. Onkol., **8**, No. 6, 32 (1986).

3. K. K. Pugachev and G. I. Avdeev, Vopr. Onkol., No. 4, 41 (1987).
4. K. K. Pugachev, I. B. Shimbireva, and G. I. Avdeev, Éksp. Onkol., **11**, No. 4, 47 (1989).
5. G. A. Tkacheva, Med. Radiol., No. 5, 79 (1988).
6. G. A. Frank and K. K. Pugachev, Arkh. Patol., No. 3, 72 (1990).
7. G. A. Frank, K. K. Pugachev, I. P. Shabalova, and I. B. Shimbireva, Arkh. Patol., No. 8, 70 (1990).
8. M. Adachi, T. Sekine, K. Imai, et al., Jpn. J. Cancer Res. (Gann), **78**, No. 12, 1370 (1987).
9. D. V. Gold, G. Ishizaki, P. Keller, and K. Lew, Cancer Res., **49**, No. 22, 6412 (1989).
10. G. Kohler and C. Milstein, Nature, **256**, 495 (1975).
11. A. Quaroni, M. M. Weiser, L. Herrera, and D. Fay, Immunol. Invest., **18**, Nos. 1-4, 391 (1989).
12. J. Sakurai, S. Hirohashi, J. Shimosato, et al., Cancer Res., **48**, No. 14, 4053 (1988).
13. H. Towbin and J. Gordon, J. Immunol. Meth., **72**, No. 2, 313 (1984).