Patterns of Antigen Distribution in Human Carcinomas

M. Jules Mattes, Pierre P. Major, David M. Goldenberg, Arnold S. Dion, Robert V. P. Hutter, and Kenneth M. Klein

Center for Molecular Medicine and Immunology [M. J. M., D. M. G., A. S. D.] and Department of Pathology [K. M. K.] University of Medicine and Dentistry of New Jersey, Newark, New Jersey 07103; McGill Cancer Center, McGill University, Montreal, Quebec, Canada [P. P. M.]; and Department of Pathology, St. Barnabas Medical Center, Livingston, New Jersey 07039 [R. V. P. H.]

Abstract

Ten epithelial-specific monoclonal antibodies, including monoclonal antibodies to antigens that have been used extensively in immunodiagnosis and immunotherapy experiments, were tested for reactivity with 20 human carcinomas each of the colon, lung, and breast. The antibodies tested included B72.3, OC125, and antibodies to carcinoembryonic antigen, the 17-1A antigen, and the milk fat globule mucin antigen (epithelial membrane antigen). Striking differences in the pattern of antigen distribution were seen, with each antibody having a fairly consistent staining pattern, which was dependent on the tumor type. Two antibodies reacted with most or all tumor specimens and, when positive, reacted homogeneously with apparently every cell in the specimen. Other antibodies consistently produced a variegated staining pattern, typically with areas of positive cells surrounded by areas of negative tumor cells. A third pattern was strong localization to the luminal edge and/or secretions of glandular tumors; this pattern was seen primarily in colon carcinomas which have more well-developed glandular structures than breast or lung carcinomas. A correlation with biochemical properties of the antigens was evident, in that mucins or mucin-related antigens generally produced variegated staining of lung and breast carcinomas and luminal edge/secretion staining of colon carcinomas. Such differences in antigen distribution are likely to be a major factor in developing methods for immunodiagnosis and immunotherapy.

Introduction

Selection of the optimal mAbs for immunotherapy of cancer depends on multiple factors. Specificity of antibodies for tumor cells, relative to normal cells, has been most emphasized, yet other factors which may be equally important include homogeneity of expression on tumor cells and accessibility of antigen to injected antibody. The relative importance of various factors is strongly affected by the mode of therapy envisioned. For example, if the natural effector functions of antibodies (complement activation or antibody-dependent cell-mediated cytotoxicity) are to be utilized, it is essential to use a homogeneously expressed, accessible cell surface antigen as a target. Also, if localized therapy is possible, such as i.p. therapy of ovarian cancer, then antigen expression by normal cells outside the peritoneal cavity may be less of a problem, particularly if galactose-conjugated antibodies are used to cause rapid clearance from blood (1).

In this study we have tested 10 mAbs for reactivity with frozen sections of carcinomas of the colon, lung, and breast, the three leading causes of cancer deaths. The antibodies tested were selected on the basis of epithelial-specific reactivity and included antibodies generated in our own laboratory and elsewhere. They include 3 antibodies that have been extensively used in clinical and experimental studies, namely NP4 which reacts with carcinoembryonic antigen (2), B72.3 which reacts with the mucin core region carbohydrate structure sialyl-Tn (3–5), and OC125 which reacts with a mucin-like antigen most strongly associated with ovarian carcinoma (6), but present also in other carcinomas as well as in normal lung (7) and breast milk (8). Three other antibodies react with antigens that have also been extensively investigated, although primarily with other mAbs to the same antigens. MH99 (9) reacts with the same antigen as mAb 17-1A (10), a glycoprotein with molecular weight subunits of 38,000 and 29,000; the identity of antigens recognized by 17-1A antibody and MH99 was demonstrated by sequential immunoprecipitation (11). MA5 (12) and MH94 (9) react with mucin-like antigens of human milk fat globules, in immunoblotting experiments.3 By competitive inhibition experiments,4 MA5 has been found to recognize the same epitope as F36/22 (13) but to be distinct from MH94 and anti-epithelial membrane antigen. F36/22, in turn, was previously demonstrated to recognize the same epitope as DF3 (14) and all are similar in specificity to a larger group of mAbs including HMFG2 (15). Also tested were four more recently described mAbs which are epithelial specific and which were found to react with a considerable number of carcinomas of the colon, lung, and/or breast, namely, MT179, MW207, MX35 (16), and MOV-18 (17). MT179 reacts with a glycoprotein with molecular weight subunits of 130,000 and 90,000 (18), MW207 with a M, 37,000 protein, MOV-18 with a M, 40,000 protein, and the antigen recognized by MX35 has not been characterized.

Different mAbs produced distinct and relatively consistent staining patterns, which were partially dependent on the tumor type. In particular, some antigens were localized either: (a) in secretions and on luminal surfaces of glandular tumors and, therefore, likely to be inaccessible to injected mAb; or (b) in patches of positive cells surrounded by negative tumor cells, producing a variegated staining pattern.

Materials and Methods

Tumor Specimens. Fresh tumor specimens were obtained from University Hospital, University of Medicine and Dentistry of New Jersey, Overlook Hospital, Summit, NJ; St. Barnabas Medical Center, Livingston, NJ, and the Veterans Administration Hospital, East Orange, NJ. The colon tumors examined included 2 well differentiated, 16 moderately differentiated, 1 poorly differentiated, and 1 colloid adenocarcinoma. The breast tumors included 18 infiltrating ductal, 1 invasive lobular, and 1 medullary carcinoma. The lung tumors included 9 squamous, 3 adenosquamous, 2 large cell, and 6 adenocarcinomas; small cell lung carcinomas were excluded from this study. All tumors examined were primaries except for 2 colon carcinomas that were metastatic to the spleen and abdominal wall.

Immunohistology. Seven-μm cryostat sections were cut, dried thoroughly, and stored moisture free at ~70°C before use. After fixation with 95% ethanol for 10 min at room temperature, sections were stained by the avidin-biotin peroxidase procedure using reagents from Vector

---

1 Presented at the "Second Conference on Radioimmunoassay and Radioimmunotherapy of Cancer," September 8–10, 1988, Princeton, NJ.
2The abbreviations used are: mAb, monoclonal antibody; CEA, carcinoembryonic antigen.
3 A. Dion and C. V. Williams, unpublished data.
4 M. J. Mattes, unpublished data.

880s
Laboratories (Burlingame, CA) as recommended by the supplier, with diaminobenzidine as the substrate. MoAbs were used at a 1/500 dilution of ascites fluid, except that purified MA5 was used at 5.0 µg/ml. Destruction of endogenous peroxidase activity, by incubation with 0.03% H_2O_2 in methanol, was performed just before incubation with the avidin-biotin-peroxidase complex, to avoid potential denaturation of antigens; this procedure eliminated the endogenous peroxidase activity of hemoglobin but was only partially effective on the enzymatic activity of isolated positive cells seen in many specimens, which are probably macrophages. Sections were routinely counterstained with hematoxylin although for photographs counterstaining was omitted.

Results and Discussion

In the process of testing moAbs on human carcinomas, it became evident that different moAbs produced distinct staining patterns. Four patterns were consistently observed: type 1, homogeneous staining of all tumor cells, without a preference for the luminal surface, type 2, staining of >50% of tumor cells, but with a definite negative subpopulation, with positive and negative cells interspersed; type 3, staining of secretions and/or the luminal edge of tumor cells forming glandular structures; type 4, variegated staining, in which positive and negative areas were adjacent and <50% of tumor cells were positive; the percentage of positive tumor cells ranged from 1 to 2% to 30%. Typically, in type 4 staining, small clusters of 5–25 cells were stained, surrounded by negative cells, although considerable variation on this general pattern was observed. Certain staining patterns were consistently obtained with particular moAbs, for a particular tumor type. For example, the 2 moAbs that produced type 1 staining most frequently, MH99 and MT179, always produced this staining pattern and never produced type 3 or 4 staining. As another example, MH94 and B72.3 stained approximately one-half of breast and lung tumors with a type 4 pattern, but very rarely with a type 1 pattern.

A summary of our results is presented in Fig. 1 and representative photographs are shown in Figs. 2–4. Tumors which had variegated staining with one antibody frequently had variegated staining with other antibodies also, although the precise distribution of positive cells was generally different with individual antibodies. Often one antibody, commonly MH94 or B72.3, stained 2–5% of tumor cells, while other antibodies stained 10–30%.

Most staining patterns were readily placed in one of the 4 categories described, which are exemplified in Figs. 2–4. A few borderline cases, between type 1/type 2 or type 2/type 4, were seen, but this uncertainty would not affect our general conclusions. There were also a few cases of variegated staining of glandular structures, in which <50% of tumor cells were stained in a luminal edge/secretion pattern; this pattern was considered type 4. In breast and lung carcinomas, there was sometimes an indication of luminal edge staining of poorly differentiated glandular structures. However, this was never as well defined as with colon tumors, since the glands themselves were much less clearly defined, and luminal edge staining was invariably mixed with whole membrane and/or cytoplasmic staining.

A marked difference was seen between colon carcinomas, on one hand, and breast or lung carcinomas, which is related to the frequent occurrence of well-differentiated glands in colon tumors, on the other hand. Several antibodies usually stained only secretions and/or the luminal edge of colon cancers, including NP4, B72.3, and MA5. These antibodies reacted differently with breast or lung tumors, in which glandular structures were not present or much less well developed, producing either variegated staining (B72.3 and, rarely, NP4) or homogeneous staining (MA5). Lung and breast carcinomas tended to have variegated staining with a number of moAbs, primarily MH94, B72.3, NP4, MX35, and OC125, and these antibodies very rarely produced homogeneous staining. For example, B72.3 stained 10 of 20 lung tumors and 7 of 20 breast tumors in a variegated pattern, while it stained only one lung tumor homogeneously. Variegated staining was much less frequent in carcinomas of the colon. Another distinction between colon and breast/lung carcinomas was that several moAbs (MX35, MOV-18, and OC125) were negative in most or all colon tumors examined, but positive on approximately one-half of the lung tumors and approximately one-fourth of the breast tumors.

The results presented suggest that antigen distribution will be a major factor in obtaining effective immunotherapy and also that some of the antigens most widely used as targets for immunotherapy and immunodiagnosis have distributions within tumors that appear distinctly disadvantageous. Our results are generally consistent with previous reports of immunohistology using the same antibodies on similar tumors specimens (2–4, 6, 9, 12, 16, 17). In using an antigen with a type 4 distribution as a target, the problem is that a large fraction of tumor cells are antigen negative. A possible solution is to use antibodies conjugated to agents that will lyse adjacent antigen-negative cells, such as radioisotopes emitting high energy β particles. The obstacle posed by a type 3 antigen distribution, which appears to be less widely recognized, is that glandular lumina are lined by epithelial cells with tight junctions, which prevent the passage of macromolecules (19). Therefore, antigen within or on the surface of glandular lumina would probably be largely inaccessible to injected antibody. The antigens having the most consistent type 1 distribution, MH99 and MT179, are present on a relatively large number of normal epithelial cells, and this also is an obstacle to therapy.
Fig. 2. An adenocarcinoma of the lung, stained with MH99 (A, B), B72.3 (C, D), OC125 (E, F), or a negative control antibody Ag8 (G) by the immunoperoxidase method, without counterstaining. A section stained with hematoxylin and eosin is also shown (H, I). Photographs were taken with either a ×4 (A, C, E, G, H) or ×10 (B, D, F, I) objective. A 0.1-mm bar is shown on one photograph at each magnification. The staining patterns demonstrated are type 1 (MH99), type 4 (B72.3), and type 2 (OC125). OC125 staining is partially concentrated at the luminal edge of poorly differentiated glandular structures.
The fact that the 2 antigens that appear, from published reports, to be the most tumor specific, namely CEA and B72.3, are both localized to luminal edges and/or secretions is probably significant. Since normal luminal contents are continually excreted while contents of tumorous glands are retained and accumulate, and since CEA is known to be present in normal adult colon, we suggest that the relatively high levels of CEA in colon tumors are partly due to accumulation of CEA rather than increased production and that it is the glandular structure of colon tumors that causes this accumulation. Reduced flow in the fetal intestine may similarly explain the high CEA levels found in the fetal colon. Recent determination of CEA mRNA levels is consistent with this speculation, since colon tumors had only approximately 3-fold more mRNA than normal colon (20). The situation with B72.3 could be similar, since this antibody recognizes sialyl-Tn, a core region structure of O-linked carbohydrates, and since this disaccharide is present at a significant level in normal colon (5). However, in this case it seems more likely that incomplete glycosylation, which has been described frequently in carcinomas (21), contributes to increased antigen expression.

All of the moAbs included in this study are epithelial specific; therefore, they are more specific than current forms of cancer therapy and should not be excluded from consideration as potential therapeutic agents on the basis of their reactivity with many normal epithelial cells. The observations reported here cannot be used confidently to select a particular antibody for therapeutic studies or to exclude a particular antibody, but we suggest that the pattern of antigen distribution must be taken into consideration in any systematic attempt to utilize moAbs as therapeutic agents.

Acknowledgments

We are grateful to Dr. Joel Roth, Overlook Hospital, Summit, NJ, Dr. Raymond Dise, Morristown Memorial Hospital, Morristown, NJ, and Dr. Rhona Stein, Center for Molecular Medicine and Immunology, Newark, NJ, for providing tumor specimens; to Marisol Hernandez for technical assistance; and to Robbie Holden for preparation of the manuscript.
References


