Breast Tumor Radioimmunodetection with a $^{111}$In-labeled Monoclonal Antibody (MA5) against a Mucin-like Antigen

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Abstract

Monoclonal antibody MA5 recognizes a determinant displayed on high molecular weight antigens associated with secretory and malignant breast epithelial cells. MA5 reactivity with >95% of primary and metastatic breast tumors, surface expression of the antigen, as well as its ability to localize within breast tumor xenografts prompted this initial study to determine the efficacy of MA5 to localize breast tumors by radioimmunoscanning. A total of 17 patients was monitored, each receiving 2 mg of purified MA5 labeled with 5 mCi of $^{111}$In. Some patients also received 3 or 18 mg of unlabeled carrier antibody (MA5); no serious allergic reactions were noted. Primary tumors, bone lesions, soft tissue recurrences, and lung metastases >3 cm in diameter were detectable, whereas only one lesion ( hilar node) <3 cm was localized. Significant antibody accumulation was noted in the liver and less significant uptake in the spleen and bone. The extensive fibrosis and poor vascularization of breast tumors may partly explain the limited sensitivity obtained thus far. The imaging results obtained with MA5 are compared with other antibodies which we show recognize the same antigens.

Introduction

Membrane preparations from both breast carcinomas and secretory epithelial cells, as expressed on apical surfaces during lactation and termed milk fat globule membranes, contain high molecular weight glycoproteins. By virtue of the immunogenicity of these preparations and/or hybridoma selection bias, numerous monoclonal antibodies have been generated against these mucin-like components (1, 2). Of particular interest are various reports documenting that these antigens, which are associated with a highly differentiated function of mammary epithelial cells, are also expressed by breast carcinoma cells. Monoclonal antibody MA5, generated against breast tumor membranes (3, 4), also recognizes this family of antigens. Noteworthy as a common feature of these antigens are the often observed differences regarding the cellular site of expression, i.e., highly polarized on the apical surface of secretory cells, whereas these antigens are expressed peripherally on tumor cells with greater cytoplasmic localization (3).

For radioimaging studies in breast cancer patients, MA5 was selected based on characteristics established from previous studies, i.e., reactivity with >95% of primary and metastatic breast tumor lesions (3) with cell surface localization (5). Additionally, separate studies demonstrated excellent radiolabeled MA5-mediated tumor detection in nude mice bearing human breast carcinoma xenografts. Our findings with reference to 17 patients are presented and discussed vis-à-vis other reports of antibodies which recognize the same, or similar, antigens.

Materials and Methods

Monoclonal antibodies E29 (hybridoma supernatant) and HMF-1 (purified from ascites) were purchased from Dakopatts (Santa Barbara, CA) and Amac, Inc. (Westbrook, ME), respectively. F36/22 (hybridoma supernatant) was obtained from Dr. Chu, Roswell Park Memorial Institute (Buffalo, NY). The generation and tissue reactivity characteristics of MA5 were described previously (3).

Polycrylamide gel electrophoresis, following sodium dodecyl sulfate denaturation, was performed on 3.3% slab gels using a modified Laemmli buffer (6) and electrophotographically transferred (7) to nitrocellulose. For immunodetection, blots were blocked for 1 h with 1% (w/v) bovine serum albumin in 10 mM Tris buffer (pH 7.4) containing 0.9% (w/v) NaCl, washed, and reacted for 1.5 h with MA5 diluted in the same blocking solution. Following extensive washing, the blots were reacted for 1.5 h with peroxidase-conjugated goat antibody against mouse immunoglobulin diluted 1:1000 in blocking solution and washed, and antibody binding sites were visualized with 3,3'-diaminobenzidine [0.05% (w/v) in Tris-saline buffer, as above, containing 50 μl 30% hydrogen peroxide/100 ml]. All steps were carried out at room temperature.

For the clinical studies, the MA5 (IgG1 subclass)-producing hybridoma was supplied to Hybritech, Inc. (San Diego, CA), for mass production, antibody purification, and diethylene-triaminepentaacetic acid chelation. Chelated antibody was then supplied in kit form for $^{111}$In labeling; >80% of the radioactivity was associated with radiolabeled MA5. Fourteen patients with metastatic breast cancer and 3 with primary disease were entered into this study. By the criterion of immunohistochemical reactions with MA5 (3), both primary and metastatic lesions from these patients (when available) were noted to express MA5-reactive antigen, as demonstrated by moderate to intense staining of tissue sections. Imaging studies were conducted according to a protocol approved by the ethics and clinical pharmacology committees of the Montreal General Hospital. All patients received 2 mg of diethylene-triaminepentaacetic acid-chelated antibody combined with 5 mCi $^{111}$In to which 0, 3, or 18 mg of unlabeled MA5 were added for total injected doses of 2, 5, or 20 mg of MA5, respectively. Labeled antibody was infused over a 1-h period in a total volume of 100 ml of physiological saline on the side contralateral to the primary tumor. For selected patients, 3–6 blood samples were taken up to 3 h postinfusion for determination of circulating levels of radiolabeled antibody. Three days following infusion, anterior and posterior radiograms of the thorax and abdomen were obtained with an Elscint Apex 410 Gamma Camera. Lateral views were also obtained in some instances, and imaging was repeated 6 days postinfusion when abnormal radioscans were seen on day 3. An average of 750,000 counts/view were collected using the 174-keV energy peak and a 20% window. Metastatic tumor sites were documented within 2 weeks of radioimaging by chest X-rays, radiophosphate bone scans, abdominal ultrasonograms, and computerized tomography. Tumor sizes were estimated by the longest tumor dimension and the longest perpendicular diameter.

Statistical analyses were performed on a VAX 780 computer using the SAS 5.16 statistical analysis program (SAS Institute, Cary, NC) for cluster analyses and Fisher's exact P test.

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4 The abbreviations used are: MAb, monoclonal antibody; EMA, epithelial membrane antigen.
Results

Epithelial Membrane Antigen-specific MAbs. Clinical applications of radioimmunodetection for the localization of tumors have primarily utilized antibodies specific for the oncofetal antigens (8). The application of this procedure for the detection of breast carcinomas, however, is more recent and has been focused on the use of MAbs which recognize antigens associated with the epithelial membrane antigen complex. In this context, we wanted to establish the relationship between MA5 antigen recognition, as compared to other MAbs generated against breast tumor cells or membranes, with reference to antigenic molecular weight species, as well as the nature of the recognized epitopes. The immunoblot results shown in Fig. 1 demonstrate that MAbs HMFG-1 (9), F36/22 (10), and E29 (11) react with the same molecular weight antigens as recognized by MA5. These results, combined with data obtained from three previous comparative studies (1, 2, 12), demonstrating commonality of detected antigens on a molecular weight basis, are displayed in Fig. 2 as a connectivity diagram. The use of the same MAb in more than one survey established horizontal relationships and allows the conclusion that the reactivities of all the MAbs listed in Fig. 2 are isoantigenic.

The results presented in Fig. 2 also suggest that these mucin-like antigens are highly immunogenic in mouse strains used to generate MAbs. The multiplicity of antigenic sites carried by these molecules and differentiation of these MAbs on the basis of their identified epitopes are summarized in Table 1. As indicated, the lack of MA5 binding to milk oligosaccharides or glycoplipids, as well as the maintenance of antigenicity following various carbohydrate modifications, strongly suggests the recognition of a peptidyl determinant by MA5 (4). In distinct contrast, the LICR LON MAb series (M8, M18, and M24) bind to milk glycolipids and/or oligosaccharides and have been classified as carbohydrate specific (12). Although peptide specificity has been claimed for MAb F36/22 (13), sialidase sensitivity has cast doubt on this conclusion (2). Similarly, loss of Cal (14) and DF3 (2) binding by prior digestion of test antigens with sialidase indicates that sialic acid is an epitopic constituent recognized by these MAbs; however, a more recent observation suggests that the protein core is immunoprecipitable with DF3 (15). MAbs HMFG-1 and -2, as well, were originally described as carbohydrate specific but were later noted to react with vector-expressed antigen (16). To reconcile these disparate findings, DF3 and HMFG-1 and -2 have been claimed to recognize glycopeptide determinants (15, 16).

As of this time, three EMA-specific MAbs (HMFG-1, HMFG-2, and LICR LON M8) have been utilized in clinical studies aimed at determining the efficacy of tumor radioimmunodetection with these reagents. From the results which follow for MA5, our data are compared and discussed relative to these previous efforts.

Breast Tumor Radioimmunodetection. Regardless of total infused antibody dose, no serious allergic reactions resulted from the administration of 111In-labeled MA5. Side effects, consisting of chills and pruritis, were more frequently observed in patients receiving the highest dose (20 mg), but were not significantly different (P = 0.2) from patients infused with lower doses. In addition, no significant changes from preinjection values were found for blood chemistries and counts performed 3 days post-infusion.

Immunodetection of malignant lesions was attempted in three patients presenting with primary breast cancers. For two of these patients, significant radiolabeled antibody accumulation in the tumor sites was observed, i.e., within a 4- 8-cm tumor located in the upper outer quadrant of the right breast and in a large (4 x 5 cm) inflammatory cancer of the right breast.
IMMUNODETECTION OF BREAST TUMORS

Table 1 Summary of studies designed to identify the nature of the epitopes recognized by MAbs reacting with the same high molecular weight antigens (Fig. 1)

<table>
<thead>
<tr>
<th>Mab</th>
<th>Loss of MAb binding by*</th>
<th>MAb binding to*</th>
<th>Epitope</th>
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<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
</tr>
<tr>
<td>MA5</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>E29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LICR LON M24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DF3</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
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<td>No</td>
<td></td>
</tr>
<tr>
<td>Ca2</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca3</td>
<td>Dec.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCRC-11</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>LICR LON M8</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LICR LON M18</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>115 F5</td>
<td>No</td>
<td></td>
<td></td>
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<tr>
<td>115 D8</td>
<td>No</td>
<td></td>
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</tr>
</tbody>
</table>

* Loss of MAb binding following various treatments: sialic acid removal (T1); periodate oxidation (T2); lectin binding (T3); or deglycosylation (T4).
* MAb binding to carbohydrate-containing antigens: milk oligosaccharides (A1); milk glycolipids (A2); or glycopeptides derived by Pronase digestion of antigen (A3).
* Dec., decreased binding.

Fig. 3. Right lateral view showing radiolabeled antibody uptake in a large inflammatory breast tumor (arrow). Normal accumulation of radiotracer in the liver, which is disease free, is also shown.

For patients with metastatic disease, no subcutaneous lesions (<2 cm) were detectable in 4 patients. Similarly, extensive chest wall metastases in two additional patients, one with multiple skin nodules (1–2 cm in diameter) which were confluent in some areas, and the other with extensive recurrences on the right hemipect, could not be localized by radioimaging. In contrast, a large primary tumor mass in the right breast (8 x 10 cm) and a metastatic lesion in the left breast (4 x 5 cm) in a third patient were found to accumulate sufficient label to be detectable (Fig. 4).

Radioimaging of breast tumors which had metastasized to the lung was attempted in three patients. Only the largest metastasis was imaged for one patient having 3 pulmonary lesions with dimensions of 4 x 3.5, 3 x 2, and 2.5 x 2 cm. For another patient, a single lung lesion (4 x 3.5 cm), as well as accompanying hilar nodes (2 x 3 cm), were imaged, whereas in a third patient multiple lung nodules <1 cm in diameter could not be visualized.

Cumulatively, these data indicated that tumor size was a significant factor for imaging primary breast tumors and metastases to skin, the chest wall, and lungs. To assess the statistical significance of this relationship, a cluster analysis of the tumor sizes was performed and tumors were stratified by size and by tumor site (Table 2). With the use of Fisher’s exact P test, significant differences in imaging frequencies of larger versus smaller lesions were found for lung (P = 0.025), soft tissue (P <0.0001), and all lesions grouped (P <0.0001).

Radioimmunodetection of breast tumors metastatic to other sites was also attempted. For example, multiple metastatic lesions were visualized in 8 patients by radiophosphate bone scans; however, only one of these, a large (3- x 6-cm) eroding bone lesion in the upper sternum, was localized by accumulation of radiolabeled MA5. 111In-labeled MA5 was also localized in the inguinal and pelvic areas of a patient with documented metastases to the right inguinal nodes and ovaries, as well as in the supraclavicular nodes of another patient having a metastasis in the corresponding area.

Antibody uptake was monitored in three patients presenting with, or in remission from, pulmonary lymphangitic carcinomatosis. Intense uptake in both lungs was found for one patient with severe disease at the time of imaging, whereas significantly less uptake was noted for another patient in disease remission. As documented by a computerized tomographic scan of the chest, the third patient was in complete remission. For the latter, no pulmonary localization of MA5 was found and she continued to be in complete remission 1 year following immunoscanning.

Discussion

The results given in Figs. 1 and 2 provide additional evidence that numerous MAbs, including MA5, have been generated against the same mucin-like antigens of the epithelial membrane complex. This phenomenon likely results from the immunogenicity of various structural components of these high molecular weight molecules which, in turn, accounts for various identified epitopes consisting of peptide, glycopeptide, and oligosaccharide determinants (Table 1). For MA5, its recogni-
tion of a peptide sequence potentially circumvents the heterogeneity inherent in carbohydrate structures. This characteristic, in addition to MA5 antigen expression by the majority of both primary and metastatic breast tumors, as well as surface localization, are important criteria for the selection of candidate MAbs for the design of antibody-based protocols for tumor diagnosis, detection, and therapy.

The clinical studies performed with $^{111}$In-labeled MA5 demonstrated the radiocalocalization of at least one documented tumor mass in 9 of the 17 patients monitored, following infusion with 2, 5, or 20 mg of total antibody. All of these doses were well tolerated, although allergic reactions were more frequently noted for the highest dose. In this regard, our present data do not indicate a correlation between total administered MA5 and successful imaging. In contrast, there was a significant relationship between tumor size and positive imaging of primary breast tumors, lung metastases, and soft tissue recurrences (Table 2); i.e., tumors $>$3 cm were primarily detectable.

The results presented in this report are generally comparable to the experiences of others who have utilized radiolabeled MAbs specific for EMA. Thus, the glycopeptide-specific MAbs, HMFG-1 and HMFG-2, accumulated in tumor sites in four of six breast cancer patients having widely disseminated disease; however, the sizes of the metastatic lesions were not specified (17). In a subsequent study (18), radioiodinated HMFG-2 was found in tumor tissue 24 h postadministration, but in amounts insufficient for imaging by external scintigraphy, even of a primary tumor with an 8-cm diameter. From these results, Griffiths et al. (18) suggested that the lack of significant MAb accumulation was possibly a consequence of insufficient amounts of administered antibody (0.45 mg), as well as a relative lack of MAb accessibility within the primary and metastatic tumor sites.

MAb LICR LON M8 recognizes a carbohydrate, or possibly a glycopeptide determinant associated with EMA (12). Radioautographic analyses of tissue biopsies following systemic administration of this radioiodinated MAb demonstrated that primary tumors were preferentially labeled in comparison to adjacent breast tissues (19, 20). Even so, primary tumors had low tumor: blood ratios and could not be radioimaged. Soft tissue metastases as well, were not detectable. As an explanation for these results, higher levels of antigen expression and better vascularization for small bone lesions, as compared to other tumor sites, were postulated as possible factors affecting detection sensitivity. In contrast to these results, only one large destructive sternal lesion could be imaged in our studies.

From theoretical considerations, as well as data derived from animal models, it is likely that numerous factors may be potential prerequisites for successful tumor imaging. Some of these factors are relevant to the antibody (e.g., isotype, dose, determinant specificity, species of origin, route of inoculation, intact versus immunoglobulin fragments, clearance, etc.) while others pertain to less amenable biological parameters, including heterogeneity and density of antigen expression, tumor size, cellular site of expression, degree of vascularization, presence of circulating antigen, etc. (8). Presently, our studies and those of others have addressed only a few of these points. For the MA5 antigen, its expression on the surface membranes (5) of a higher percentage of breast tumors as compared to other antibodies (3, 21), its peptide specificity (4)5 as well as the higher MAb doses administered probably account for our better ability to image primary breast tumor and pulmonary and soft tissue metastases, as compared to the other studies cited. It should also be noted in this regard that the MA5 antigen is systematically accessible (3) and may be of prognostic value as a circulating marker for determining disease course and treatment efficacy (22). Approaches utilizing MA5 antibody fragments, alternate labeling and imaging techniques (23), as well as biological strategies to enhance antigen expression will be necessary to realize the full potential of this procedure.

Acknowledgments

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Note Added in Proof

Extensive clinical data relevant to these studies have recently been reported: Major, P., Wang, T., Ishida, M., Unger, M., and Rosenthall,

References


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