Radiolabeled Monoclonal Anti-Tumor Antibodies in Diagnosis and Therapy*

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Clinical work with radiolabeled anti-tumor antibodies has made remarkable progress in the past few years. Still, there is much to be done before these new reagents can have a substantial impact on the practical management of patients. In this discussion, the properties of an "ideal" radiolabeled antibody and important factors for in vivo localization in tumors are reviewed. Potential approaches to improving the localization of currently available "tumor specific" monoclonal antibodies are discussed and examples of patients examined and treated with this method are presented. Experience to date suggests that within the foreseeable future, radiolabeled antibody techniques will become a "genuinely decisive technology."


"(He continued) the sluggish motion that carried them forward step by step. Looking ahead, one tended to lose courage. But looking back it was impossible to deny the length of road already traveled." (1)

One reason for a review of this kind is to remind ourselves of the distance we have come towards our goal of greatly improving the accuracy of diagnosis and efficacy of treatment of malignant tumors using monoclonal antibodies (Fig. 1). A second reason is to acknowledge that we have a long way to travel still, before we reach the full potential of the radiolabeled monoclonal antibody technique.

We are far enough on our journey to be quite specific about one objective—the "ideal" radiolabeled antibody preparation for detecting and treating common human tumors. The properties of such a preparation are listed in Table 1. Some of these properties are achievable now; others must be the product of future research.

Even the perfect preparation will not be enough to assure success however; we must use radiolabeled antibodies with greater skill, and we now find ourselves at a point where much learning must occur before we are skillful enough to enjoy complete success in the clinic.

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In optimizing this use, we must take into account the biologic factors determining the uptake of radiolabeled antibody by tumor. Some of the important factors are listed in Table 2. Future discoveries will make this list longer.

My colleagues and I have been concerned both with achieving more nearly perfect radiolabeled monoclonal antibodies and determining how to best use these unique reagents. In all of our experimental work, whether in the laboratory or clinic, we have used a common end point: improvement in the targeting tumor, measured both as absolute concentration and uptake relative to other tissues. (For a discussion of factors important to target to background ratios, see Ref. 2). The potential for therapy, has intrigued us from the beginning; all the more so because the principle can be extended to many tumors for which conventional therapies are ineffective (Table 3).

We have worked extensively with melanoma, a tumor of the pigmented cells in the body. Once this tumor has spread beyond the confines of a reasonable surgical resection, there are no established therapies that are effective and patients succumb with widespread metastases of brain, liver, lung. Often during the course of this illness, patients have multiple subcutaneous lesions or involvement of regional lymph nodes. Once multiple organs are involved, the usual life expectancy is 1–2 mo (3).

Several tumor specific monoclonal antibodies have been raised against melanoma, and we have used monoclonal antibodies that target two distinct antigens:
Antibodies as Carriers of Radioactive Tracers for Diagnosis and Therapy

Antibody to p97
Melanoma Tumor Therapy

Label With Small Amount of Radioactivity

Attach Anti-Tumor Drugs or High Iodine Radiolabeled

Patient With Undisclosed Tumor
Patient With Tumor

Nuclear Image to Localize Tumor
Tumor Disappearing

FIGURE 1
Strategy for using antitumor antibody-radioisotope conjugates for diagnosis and therapy of human tumors

p97(4,5) and a chondroitin sulfate proteoglycan, the “high molecular weight antigen” (6).

Using these reagents, we have performed a variety of studies in animals and man—for anti-p97, whole immunoglobulin (7) and Fab fragments (8). With the regimens described, we obtained sensitivity of about 85%, and specificity of 100%. In some patients with particularly intense localization, sufficient radiation could be delivered to the tumor, to permit radiotherapy (9,10, Table 4).

These studies taught us that the uptake in vivo, was proportional to the antigen content of the tumor; that each targeting antibody had its own characteristic organ biodistribution in vivo; that with great care, it was possible to iodinate monoclonal antibodies without loss of immunoreactivity (11); that there was considerable variation from patient to patient with respect to uptake, but that each patient could be reproducibly imaged multiple times, with the same antibody preparation, that Fab’s were less immunogenic than IgG, and that non-specific Fab or IgG did not concentrate appreciably in comparison to specific immune preparations (For a review of this work see Refs. 12–16).

In a very preliminary study, two of three patients with metastatic melanoma who were treated with more than 400 mCi’s iodine-131 (131I) (anti-melanoma Fab) had an anti-tumor response (17). Toxicity was mild, with the marrow being the target organ, as expected from the initial biodistribution estimates. Studies are currently underway at the National Institutes of Health to confirm and extend these findings.

The present status of our ability to selectively target radiolabeled antibody to melanoma tumor is shown in Fig. 2. The 50-yr-old man presented with malignant melanoma, widely metastatic to subcutaneous (s.c.) tissue, and R. adrenal gland. The patient received an initial diagnostic dose of 10 mCi (anti-p97)-Fab, and three separate therapy doses of 100 mCi’s each at weekly intervals. All doses were delivered with 50 mg of anti-p97 Fab. He tolerated the procedure well, except that he was unable to take his perchlorate dose, because of GI intolerance to the drug. On all four occasions, images were

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**TABLE 1**

Properties of “Ideal” Radiolabeled Anti-Tumor Antibody/Fragment

| Targets abundant tumor specific antigen (>10^6 sites per cell)* |
| 100% immunoreactive at high affinity (K_a > 10^11 l/M) |
| Complete bioavailability for antigen sites. |
| Readily available in gram quantities |
| Radionuclide with optimal decay properties for Dx/Rx |

* Now available for a few tumors.

**TABLE 2**

Factors Important to In Vivo Localization of Radiolabeled Antibody

| Mass amount of antibody administered (AB dose) |
| Type of antibody fragment (ie., whole IgG, Fab, Fab', (Fab')_2) |
| Heterogeneity/concentration of antigen expression on tumor sites |
| Presence of nontumor reservoir of antigen |
| Metabolism/excretion of radiolabeled antibody |
| Route of antibody administration |

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obtained at 48 hr after infusion of the radiolabeled antibody, and the s.c. nodules and adrenal metastasis were reproducibly imaged, with considerably greater uptake and retention than nontumor tissues. There was no evident toxicity, in terms of changes in hepatic or renal function, or hematopoietic profile. The patients' subcutaneous lesions changed color and stopped growing, and whereas new lesions had begun to appear every 1-2 wk prior to treatment, there were no new lesions in the s.c. tissue until 3 mo after ending therapy. At 21/2 mo posttreatment, the patient developed a new brain lesion, and died 4 mo after the end of therapy.

If Fig. 2 is where we are now, Fig. 3 represents from where we have come. The patient was a 45-yr-old man with metastatic melanoma to s.c. tissue over the back, and to the right hilum and left lung near the left cardiac border. The patient received 2 mCi of $^{131}$I-(anti-p97) IgG (total protein injected, 100 μ), i.v., and images were obtained at 24 hr postinjection. Subtraction images, using the technique of DeLand et al. {18), were required to identify the hilar lesion, and a right axillary node. The lesion at the left cardiac border was not well seen, neither did we see the s.c. lesions over the back with any certainty.

The contrast between Fig. 2 and 3 is striking, with much higher target to background ratios being seen for Fig. 2. Figure 3 was one of our early images, and we were proud to be able to detect disease, such as axillary node and hilar mass. Nonetheless, we now see in retrospect that in comparison to our more recent studies (Fig. 2), these images had very low contrast between tumor and surrounding tissue, and that we missed many subcutaneous nodules altogether. The image subtraction technique helped considerably in identifying disease, but also gave rise to potential artifacts, such as those over the cardiac blood pool, which can be a problem for specificity of the technique.

It is important to point out that both of these studies were based on the identical hybridoma produced monoclonal antibody, 96.5. The improvements in the images came about because of several innovations, particularly in the use of Fab fragments instead of whole immuno-

| TABLE 3 |
| "Tumor Specific" Monoclonal Antibodies |
| Melanoma | Colon |
| Ovarian | Lung |
| Breast | Glioblastoma |
| T,B-cell malignancies | Sarcomas |
| Prostate |

TABLE 4
Dosimetry $^{131}$I-(anti-p97) Fab (100 mCi i.v.)

<table>
<thead>
<tr>
<th>Target organ (tissue)</th>
<th>Dose (rad)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>1040</td>
</tr>
<tr>
<td>Liver</td>
<td>325</td>
</tr>
<tr>
<td>Bladder</td>
<td>254* 160†</td>
</tr>
<tr>
<td>Kidney</td>
<td>71</td>
</tr>
<tr>
<td>Whole body</td>
<td>39</td>
</tr>
<tr>
<td>Marrow</td>
<td>30</td>
</tr>
<tr>
<td>Ovary</td>
<td>26</td>
</tr>
<tr>
<td>Testes</td>
<td>20</td>
</tr>
</tbody>
</table>

* Every 4 hr emptying.
† Every 2 hr emptying.
FIGURE 3
MM, patient with melanoma metastatic to right hilum, s.c. tissue over back, and border of left heart. In upper panels, anterior (left) and posterior (right) projection of chest are seen. There is focal uptake at right hilum, and some activity suggestive of metastases to right axilla, are observed (arrows).

FIGURE 4
Comparison of tumor localization [(anti-p97): IgG_{2a} vs. FAB (96.5) \pm s.e.m. (n = s)] in nude mouse bearing melanoma xenografts. Labeling index (L.I.) was calculated according to Moushakis (19). Basically, this reflects ratio of activities in tumor of specific over nonspecific Fab or IgG of same subclass, corrected for blood activity at same time. There was much more rapid development of specific uptake with Fab fragment, as compared to the IgG, of anti-melanoma antibody, 96.5.

globulin, and by increasing the mass amount of antibody.

The value of the Fab fragment is illustrated in Fig. 4, which shows a comparison between the anti-p97 IgG and the Fab fragment of the same antibody. The results are expressed as a specificity index, which is a measure of the antigen specific uptake seen in tumor. The specificity index for IgG is considerably lower than for the Fab preparation, and the ratios develop much more slowly for IgG (19).

Excellent targeting can also be obtained with other types of Fab also, as shown in Fig. 5. Avid uptake is seen in melanoma tumor metastatic to liver after intravenous injection of $^{131}$I(anti-chondroitin sulfate proteoglycan) Fab (20).

In regard to the dose of antibody being important, we noted very early in our experience that for both the IgG and Fab preparations, that low mass amounts of antibody injected into patients with metastatic melanoma formed complexes with the small amount of circulating antigen, (15) and that increasing the mass amount of antibody significantly reduced the amount of antibody localizing in nontumor reservoirs of antigen, such as liver in comparison to tumor (10). In some cases, the improvement of targeting at a higher dose was very marked, occurring at a very sharp threshold of dose (for example, see Fig. 6 of Ref. 2).

Despite these improvements, the targeting which we observe in Fig. 2 is not perfect, and we would hope for an even higher tumor to tissue ratio, with no uptake in the
FIGURE 5
Localization of $^{131}$I-(anti-chondroitin sulfate proteoglycan)-Fab in malignant melanoma metastatic to the liver. In left panel, a $^{99m}$Tc-sulfur colloid liver spleen scan is shown (anterior projection). Dark area shows where functioning hepatic parenchyma is present. There are many space occupying lesions throughout liver, and left lobe, (arrow), is virtually replaced by tumor. In right panel, there is avid localization in invading tumor, in same location (arrow), after i.v. injection of $^{131}$I-Fab

Liver, and much less retention in soft tissue. Also, note that there is metabolism of the radioiodinated antibody, so that free iodine is seen in thyroid, bladder, and stomach. Another very important consideration is that there is considerable variation in the uptake from subject to subject, even when closely similar protocols are used for study. How can we improve the targeting of antibody still further? As a starting point, it appears that by proper attention to the factors listed in Table 2, considerable improvements are possible, perhaps approaching the limits for target to background ratios that can be achieved in the test tube for antigen antibody reactions, namely, 1,000 to 1, in some cases. Table 6 shows the rationale for this ambitious goal.

That this may not be an idle dream, has been brought home to us by recent findings in an animal tumor model (21). An antibody was raised against the zona granulosa proteins of the rat ovary. After intraperitoneal injection, the localization to the rat ovary was very large, with concentration of 3,500% dose per gram, and ratios to surrounding tissues in excess of 100 to 1! One key to the exquisite localization observed, may have been the relatively large amount of antigen present in the ovary, or the route of injection. The intraperitoneal route may have permitted the antibody to come into more rapid and complete contact with the antigen. Studies are underway to explore this interesting possibility.

One great attraction of the monoclonal antibody technique is the ability to target a wide variety of tumor types. Recently, we have found that the anti-T cell antibody, T-101, is very effective for targeting the lymph nodes of patients with cutaneous T-cell lymphoma, or mycoses fungoides, after i.v. injection (22). Figure 6A shows the excellent targeting to lymph nodes after i.v. administration of the radiolabeled antibody. The percent injected dose to the inguinal nodes has been measured at biopsy in a series of patients, and is in the range of 0.1–0.5% dose per gram, for the tumor bearing nodes. The antibody in question, is a pan T cell antibody that reacts with a 65,000 kilodalton protein on the cell surface of both normal and malignant T cells (23). However, the malignant cells have more than ten times the concentration of antigen on their surface, and this may explain why visible uptake on the scan after i.v. administration, has so far been found only in areas that contain malignant T-cells.

Altering the route of injection of the $^{111}$In T-101 from the i.v. to the intralymphatic route has a profound effect on the degree of concentration in lymph nodes, so that even nodes not involved with the lymphoma can be readily imaged on the scan. The absolute concentration is about ten fold higher by way of the intralymphatic
FIGURE 6
Imaging with $^{111}$In T101, in patients with mycosis fungoides (cutaneous T-cell lymphoma). Gamma camera images of pelvis, anterior chest, and abdomen, and cervical region, in patient with diffuse adenopathy and erythroderma. After i.v. administration, by 72 hr, there is intense uptake in nodes of pelvis, cervical, and axillary area. Rim of uptake noted on lateral body (arrows) is in skin that is involved with disease.

route and uptakes as high as 5% of the dose per gram have been seen in inguinal nodes removed at 24 hr postinjection (A. Keenan, J. Carrasquillo, personal communication).

Colcher and associates have described an antigen-antibody system that seems very promising, based on early laboratory and clinical studies. This is the antibody B72.3, which reacts with a variety of human mammary, ovarian, and colon tumors in man (24). We have performed imaging studies in nude mice using a $^{131}$I B72.3 IgG, which showed progressive accumulation in tumor for 14 days after injection, with essentially no accumulation in other organs. The percent dose per gram in a human colon tumor xenograft in nude mice reached a plateau of greater than 20% by 5 days postinjection, which was a factor of 8–10 greater than the corresponding localization in a control human melanoma xenograft (25). One intriguing feature of this system, is the prolonged retention of the $^{131}$I B72.3 antibody in the tumor, indicating that the antibody is very resistant to deiodination in vivo (26). An example of the targeting which we have seen clinically, in patients with metastatic colon cancer, is shown in Figure 7. Tissues which have been removed surgically, have consistently shown greater uptake in tumor than in normal colon. Our initial impression, is that this uptake is highly antigen dependent, and some of the samples have shown ratios of more than 36:1, tumor to colon (Colcher D., personal communication). These ratios are among the highest reported from biopsy studies of solid tumors after i.v. injection of radiolabeled antibody.

In summary, the future looks bright for the successful application of radiolabelled antibodies as agents for diagnosis and therapy of many common human tumors. Thus far, nuclear medicine applications have grown progressively more satisfactory, based in large part on preceding developments in immunology, such as the hybridoma technique (27). From the standpoint of nuclear medicine as a discipline, it is my belief that within

<p>| TABLE 5 |</p>
<table>
<thead>
<tr>
<th>Potential Approaches to Improving Tumor-to-Tissue Ratios Using Radiolabeled Anti-Tumor Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Use immune fragments</td>
</tr>
<tr>
<td>Dose (mass amount) of antibody preparation</td>
</tr>
<tr>
<td>Route of injection</td>
</tr>
<tr>
<td>Multiple antibodies against several antigens</td>
</tr>
</tbody>
</table>

<p>| TABLE 6 |</p>
<table>
<thead>
<tr>
<th>Radioimmunodetection of Melanoma Theoretical Tumor/Nontumor Ratios (B/F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. $K(AG) = (AG-AB^<em>)/(AB^</em>)$ and $(AG-AB^<em>)/(AB^</em>) = B/F$ therefore, $K(AG) = B/F$</td>
</tr>
<tr>
<td>2. For antibody to p97 (mol wt = 100,000), melanoma membrane antigen, $K = 10^{10}$ $M^-$ and $A_g = 0.001$, of total membrane protein. From 1 g of tumor, 10 mg of membrane therefore: $(0.001 \times 10 \ mg) = 10 \ \mu g$ or $10^4 \ \mu M/g M$ tumor or $10^{-7} \ M/I = (AG)$</td>
</tr>
<tr>
<td>3. Therefore, at equilibrium, for $AB^*$, $B/F = (10^{10} \ I/M) (10^{-7} \ M/I) = 1,000$</td>
</tr>
</tbody>
</table>
the next five years, that radiolabeled monoclonal antibody techniques will become a "genuinely decisive technology."

The genuinely decisive technology of modern medicine, . . . comes as a result of a genuine understanding of disease mechanisms, and when it becomes available, it is relatively inexpensive, relatively simple, and relatively easy to deliver (28).

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