Experimental Studies of Radioimmunodetection of Cancer: An Overview

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Abstract

During the past 8 years numerous patients have been treated by injection of radiolabeled monoclonal antibodies for both diagnosis and treatment of cancer. It has become common to refer to this approach by such terms as "antibody guided delivery" or "antibody guided targeting." In general the results, while somewhat promising, have failed to fulfill our initial expectations. It is now clear that there are many physiological barriers that antibodies face in their travel toward their tumor associated antigen. The papers in the "Experimental Studies of Radioimmunodetection" section of the symposium describe in detail these barriers. We must remember that antibody conjugates are no more "guided" than classical drugs and hormones and are subject to the same physiological principals.

Introduction

Pioneering work in the tumor localization starting with Pressman in the 1950s (1, 2) and continued by Goldenberg and others in the 1970s (3, 4) was directed at the development of immunologically based radiolabeled proteins for the detection of cancer. Early work on the use of polyclonal antibodies utilized either transplantable or induced syngeneic animal tumors (1, 2, 4-6). The development of the immunoincompetent, athymic mouse model (7, 8) has complimented the development of human "tumor associated" monoclonal antibodies (9-11), allowing the testing of the abilities of these antibodies to "target" in vivo to human tumor. Because of the antihuman specificity of the antibodies, the availability of the athymic mouse human xenograft model was critical to the future development of radiolabeled monoclonal antibodies for both the diagnosis and treatment of cancer. This model has been used in the vast majority of experiments during the preclinical phase of radiolabeled antibody development (12).

While this model has proven to be very useful it has its limitations. These include: (a) work is limited only to human tumors that can be adapted either to growth in tissue culture or as transplantable xenografts; (b) even though numerous mice carrying a xenograft can be used in an experiment, in reality only a single tumor is being studied; (c) tumor growth rates for most xenografts are far faster than that found in the clinical setting; (d) the size of the tumor relative to the animal is greater in the mouse model than in the clinic; (e) the lack of immunocompetence, while allowing xenograft growth, may alter tumor physiology; (f) xenografts may not be similar to either naturally occurring primary tumors or metastases with respect to their physiological functions. These and other differences make difficult the extrapolation of the results obtained in the laboratory to those expected in the clinic. Despite these restrictions we are limited, in most cases, to seeking explanations for clinical results by examining data obtained using the murine model.

In spite of early promising results in animals (13) clinical studies have rarely fulfilled this promise. In most clinical trials very little of the injected dose, usually between 0.001 and 0.01% injected dose/g, accumulates in the tumor (13). In immunoscintigraphic studies approximately 80% of known (and many occult) lesions have been detected. The limit of resolution appears to be about 1 cm (13). A clear positive impact on the management of the cancer patient has yet to be demonstrated. The papers in the "Experimental Studies of Radioimmunodetection" section of the symposium detail attempts to utilize preclinical models to help understand and manipulate the mechanisms which control uptake and metabolism of radiolabeled antibodies and thus to maximize the uptake of radiolabeled antibodies by tumors.

Role of the Vascular System

Radiolabeled monoclonal antibodies, be they IgMs, IgGs, or their F(ab')2 or Fab fragments, are large molecules compared with classical drugs and hormones. While the 50,000 Da of an F(ab')2 or Fab fragments, are large molecules compared with classical drugs and hormones. While the 50,000 Da of an Fab seems small in comparison to the 140,000 Da of an IgG or the 900,000 Da of an IgM, it is very large compared to the several hundred Da of classical drugs and several thousand Da of most hormones. One of the largest hormones, follicle stimulating hormone, has a molecular weight of only 34,000 (14). The immune functions of these large molecules require them to remain in the vascular compartment. The classical work by Waldmann and Strober (15) demonstrated that the majority of immunoglobulins are cleared relatively slowly and remain predominantly within the vascular compartment. For these immunoproteins to bind specifically to a solid tumor cell they must pass out of the vascular compartment and into the interstitial space. While the vasculature of tumors has been shown to be more permeable than that of normal organs (16-19) it still represents a significant barrier through which large immunoproteins must pass. Passage through this barrier will be determined by both the size and shape of the penetrating molecule and the conformation and number of the vascular pores. In addition, such factors as the rate of diffusion, rate of convection and existence of specific active transport mechanisms will affect the ultimate rate of transport across this barrier.

The contribution of vascular permeability to the amount of antibody accretion by tumors is shown in Fig. 1. In this experiment the vascular permeability of a renal cell carcinoma to radioiodinate bovine serum albumin (60,000 Da) and to non-tumor specific IgG was found to be quite different than the breast carcinoma vascular permeability to the same proteins. The ability of radioiodinated tumor "specific" IgG1s (A6H for the renal cell carcinoma and B6.2 for the breast carcinoma) to localize to the tumor was roughly in proportion to the differences in permeability. This relationship also held when an anti-human HLA antibody was studied. Since the only "human" component found in the mouse was the xenografts this IgG1 also served as a tumor "specific" antibody (19).

The clinical treatment of hepatocarcinoma using low dose external beam X-rays to enhance the accretion and thus the therapeutic effects of radiolabeled antibody has been reported by Msirikale et al. (20). The proposed mechanism of action of the external beam radiation was an increase in the permeability of the tumor vasculature. This treatment may not always result in a significant increase in radiolabeled antibody uptake by tumor. The preclinical data reviewed by Schlom et al. (21) suggest that low dose external beam radiation does not always result in an increased antibody accretion. Increased therapeutic

1 Presented at the "Second Conference on Radioimmunodetection and Radioimmunotherapy of Cancer," September 8-10, 1988, Princeton, NJ.
responses after a combination of low dose external beam therapy and radiolabeled monoclonal antibodies also can be explained by either a direct effect of the radiation on tumor growth or a collapsing of the vasculature and subsequent tumor kill.

As elegantly explained by Jain and Baxter (22, 23) the location of the tumor vasculature can influence interstitial pressure and direction and degrees of convection forces to which an antibody is exposed. Increases in interstitial pressure can markedly reduce the amount of antibody which leaves the vascular space. Due to the heterogeneity of vascular supply, antibody molecules may have to travel great distances (up to 1 cm) and since convective forces determine the direction and can reduce the rate of molecular transport, the rate of movement in the interstitium is very slow. This further increases the nonhomogeneity of the binding of antibody throughout the tumor.

The nonuniformity of antibody distribution has major implications for the use of toxin, drug, and radionuclide conjugates. In the case of drugs and toxins, since every cell must be exposed directly to the therapeutic agent, the nonuniform distribution suggests a low probability of success for delivery by monoclonal antibodies. On the other hand, if the heterogeneous binding pattern of antibody (due to antigenic heterogeneity as well as delivery factors) can be matched with a therapeutic radionuclide of proper emission energy, then even those cells which do not bind antibody will be exposed to sterilizing radiation. Therefore, radionuclide linked monoclonal antibodies may have a clear advantage over drug and toxin conjugates.

An example of the heterogeneity of the delivery of antibody within the tumor is illustrated in Fig. 2. $^{125}$I-Labeled antibodies to different tumor associated antigens were injected into athymic mice bearing xenografts of either a human mammary carcinoma or a renal cell carcinoma. Twenty-four h after antibody injection the tumors were removed, sectioned, and prepared for autoradiographic analysis. The in vivo binding pattern of the tumor associated antibody, $^{125}$I-A6H, to the renal cell carcinoma is shown in Fig. 2A. The binding was a streaking pattern, predominantly at the tumor periphery. In Fig. 2B is shown the in vivo binding pattern in the same xenograft of an antibody to a major human histocompatibility marker (anti-HLA). If delivery were uniform this antibody, which binds to human HLA (Fig. 2D), would have shown the in vivo binding pattern in the same xenograft of a renal cell carcinoma. Twenty-four h after antibody injection the tumors were removed, sectioned, and prepared for autoradiographic analysis. The in vivo binding pattern of the tumor associated antibody, I$^{125}$I-A6H, to the renal cell carcinoma was compared to the pattern obtained using the anti-human HLA (Fig. 2D). In this case both antibodies localized in discrete “nests” scattered throughout the tumor. The fact that the anti-HLA had binding patterns which were different in both tumors, yet similar to the tumor associated antibody, strongly suggests that the delivery of the antibody by the blood is a major factor in determining where the antibody binds.

Mechanism of Liver Uptake of Radiolabeled Antibodies

In comparison to human polyclonal antibody blood clearance, the clearance of radiolabeled murine monoclonal antibodies in humans has been shown to be considerably faster (13). Several possible reasons for this observation include: species differences; effects of radiolabeling; and possible difference in the amount, nature, and/or positioning of the carbohydrate. The exact mechanisms by which the murine antibody is cleared are not known. Uptake by the liver of IgGs and clearance through the kidney of the antibody fragments appear to be the major routes of elimination. Accumulation of radionuclides in these organs is a major detriment to the use of radiolabeled monoclonal antibodies for diagnosis and therapy. Two studies, detailed more completely in other papers in this supplement, increase our understanding of the liver mechanisms by which antibodies are cleared (24, 25).

Liver accumulation is probably related to the carbohydrate found on the antibody. The time course of rat liver uptake of a murine antibody radiolabeled with either $^{125}$I or $^{111}$In is shown in Fig. 3. Values at early time points indicated that antibody accretion was equal regardless of the radionuclide label. Differences occur with time; i.e., radioiodine accretion decreases with time while that of the radioindium increased with time. This result was most likely due to the trapping of metabolized radioindium in the liver and extrusion of the freed radioiodine out of the liver.

Data from studies to determine which of the two liver cell types take up antibodies are shown in Fig. 4. The results of these experiments show that the predominant cell type responsible for the uptake of radiolabeled monoclonal antibodies by the liver appeared to be the hepatocyte (major function, metabolism and protein synthesis) rather than the nonparenchymal cells (major function, phagocytosis). Experimental details can be found in the paper by Jones et al. (25). Rats were given injections of either $^{99m}$Tc-microaggregated albumin (a specific marker for Kupffer cells) or $^{99m}$Tc-Hepatolite (a specific marker for hepatocytes). In addition the animal received antibody radiolabeled with either $^{125}$I or $^{111}$In or $^{99m}$Tc. One h after injection of the radiolabels the animals were killed, livers were removed and perfused with collagenase, and the hepatocytes were separated from the nonparenchymal cells using a Percoll gradient. As expected, almost all of the $^{99m}$Tc-Hepatolite was found in nonparenchymal cells while almost all of the $^{99m}$Tc-Hepatolite was found in hepatocytes. The radionuclides from the radiolabeled antibodies were found to be predominantly in the hepatocytes. Since there are far more hepatocytes in the liver than nonparenchymal cells (roughly 5:2) these data indicate that the accretion of radionuclide per liver cell is approximately equal for both cell types. Most of the radionuclide could be found in hepatocytes due to their being present in larger numbers. Similar results have been found by Beatty et al. (24).

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<tr>
<th>Tumor Type</th>
<th>Specific Antibody</th>
<th>Anti-HLA</th>
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<tbody>
<tr>
<td>Breast Carcinoma</td>
<td>150</td>
<td>50</td>
</tr>
<tr>
<td>Renal Cell</td>
<td>125</td>
<td>30</td>
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**Fig. 1.** Correlation of the tumor accretion of radiolabeled monoclonal antibodies with vascular permeability. See Ref. 19 for experimental details. BSA, bovine serum albumin; I.D., injected dose; bars, SD.
OVERVIEW OF RADIOIMMUNODETECTION

Fig. 2. *In vivo* autoradiographs. (A) renal cell carcinoma xenografts obtained from mice given injections of 125I-A6H, 24 h previously. × 156. (B) Clouser xenografts obtained from mice given injections of 125I-B6.2 24 h previously. × 156. (C) renal cell carcinoma xenografts obtained from mice given injections of 125I-anti-HLA, 24 h previously. × 156. (D) Clouser xenografts obtained from mice given injections of 125I-anti-HLA, 24 h previously. × 156. Taken from Ref. 19.

Fig. 3. Time course of the uptake of radiolabeled antibody by the rat liver. See Ref. 25 for experimental details.

Fig. 4. Liver cell uptake of cellular markers and radiolabeled antibodies one hour after i.v. injection into rats. See Ref. 25 for experimental details. , parenchymal; , nonparenchymal.

Studies using the radiolabeled F(ab')2 have shown reduced accretion of radionuclide by the liver. One possible explanation for the reduced liver uptake is that the more rapid clearance from blood reduces the available F(ab')2 for liver accretion. Experiments in our laboratory have shown that this is not the case. Reduced uptake of both radioiodine and radioindium were seen both *in vivo* and in rat livers perfused *in vitro*. These data strongly suggest that liver cells have receptors for a component of the Fc portion of the antibody which is responsible for the non-antigen specific liver uptake of radiolabeled murine monoclonal antibodies. It is not known at present if these receptors are for the carbohydrate, which is found predominantly on the Fc, or for a protein component of the Fc.

Liver Metabolism of Radiolabeled Antibodies

An understanding of the mechanism of the liver metabolism of radiolabeled antibodies and identification of the metabolic products may lead to the generation of techniques for radiolabeling which overcome the high liver accretion seen for such radionuclides as 111In. While little is known about the mechanism of liver uptake of antibodies, even less is known about liver metabolism of these proteins. Two papers by Jones *et al.* (25) and Beatty *et al.* (24), dealing with this subject appear in this supplement. Both describe the rapid production of a low molecular weight 111In labeled metabolite found in the supernatant of livers after the i.v. injection of 111In-DPTA2 labeled monoclonal antibodies. Fig. 5 shows the low molecular weight peak found after size exclusion HPLC analysis of the liver supernatant. Molecular weight analysis suggests that the peak represents a metabolite of less than 1000. The data of Jones *et al.* indicated that this metabolite did not comigrate with ferritin.

The abbreviations used are: DTPA, diethylenetriaminepentaacetic acid; HPLC, high performance liquid chromatography.
and accounts for almost 60% of the $^{111}$In found in the liver 24 h after radiolabeled antibody injection into rats. Further analysis indicated that this peak did not coelute with $^{111}$In-DPTA on size exclusion HPLC analysis under certain conditions and that it could be further resolved by ion exchange HPLC into four components, one of which coeluted with $^{111}$In-DPTA. These data suggest that $^{111}$In-DPTA labeled monoclonal antibodies are rapidly metabolized to small metabolites, most probably $^{111}$In-DPTA attached to one or several amino acids of different charges. The mechanism for the retention of the metabolite by the liver is not known. A likely explanation is the inability of the liver cells to extrude a charged molecule.

**Intracavitary Administration**

Direct administration of radiolabeled antibody into a body cavity (e.g., peritoneal, pleural, cranial) rather than systemic injection has been proposed as a way to overcome both the physiological and metabolic barriers to antibody delivery described above. Numerous papers in this supplement report results of clinical trials conducted using the intercavitary route of administration. Several important preclinical studies have investigated the benefit of this approach using various animal models. Two studies indicated an enhancement of tumor accretion over i.v. administration (26, 27) while one study found no uptake advantage over systemic administration (28). The clinical advantage of i.p. administration over i.v. in the uptake of radiolabeled antibodies by colorectal peritoneal metastases was shown in elegant dual labeled antibody studies summarized by Schom et al. (21). In most cases the enhancement of tumor uptake after intercavitary administration has been found to be less than 10-fold greater than after systemic administration. It is yet to be determined if this increase is sufficient to have a positive impact on the use of radiolabeled monoclonal antibodies for either the diagnosis or therapy of cancer.

**Pharmacological Intervention**

One approach to increase the uptake of antibody by tumor is pharmacological intervention to either alter the delivery process or increase antigenic expression on the tumor. Several preclinical and clinical research groups have tried to increase radiolabeled antibody delivery by increasing the blood flow to tumors by pharmacological agents. To date results of these studies have not appeared in the published literature.

More successful preclinical studies are those that attempted to improve tumor accretion by increasing the expression of the targeted antigen on the tumor cell surface. The paper by Schom et al. (21) reviews the data generated by Greiner et al. (29, 30) who reported an increase in the accretion of radiolabeled antibody $[F(ab')_2]$ of B6.2 in a breast carcinoma xenograft by 83% following pretreatment of the mice with recombinant human interferon, an agent shown to increase the expression of the targeted antigen in vitro. It is interesting to note that not all cell lines increased antigen expression after recombinant human interferon treatment in vitro. These results suggest that recombinant human interferon treatment will not work to enhance antigen expression in all cases. *In vivo* results paralleled those found in vitro suggesting that the in vitro responsiveness may be used as a screen for those patients who will benefit by this type of treatment.

**Conclusion**

During the past 8 years numerous patients have been treated by injection of radiolabeled monoclonal antibodies for both the diagnosis and treatment of cancer. It has become common to refer to the approach by such terms as “antibody guided delivery” or “antibody guided targeting.” In general the results, while somewhat promising, have failed to fulfill our initial expectations. It is now clear that there are many physiological barriers that antibodies face in their travel toward their tumor associated antigen. They reach their binding site via the same delivery mechanisms, face the same barriers (e.g., leaving the vascular compartment, moving against interstitial pressures, and organs of metabolism) and accumulate in proportion to the density of their “receptors” (antigens) as do classical drugs and hormones. In planning further work in this field we must keep in mind the complexity of the task we are expecting from an antibody. We must remember that antibody conjugates are subject to the same physiological rules as drugs and hormones. Antibodies are no more “guided” than any drug or hormone.

Our knowledge and understanding of the physiological factors which have an impact on the uptake of tumor associated monoclonal antibodies are limited. To date very few studies have been reported which deal with this problem. For radiolabeled monoclonal antibodies to fulfill their promise, greater understanding of these physiological factors are needed in order to maximize the specific delivery of radionuclide, drug, or toxin via monoclonal antibodies. Several papers in this supplement report progress in the study of these nonimmunological factors. Additional data from these types of studies are essential if we are to see the development of radiolabeled monoclonal antibodies into useful diagnostic and therapeutic agents.

**References**

OVERVIEW OF RADIOIMMUNODETECTION


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