Quo Vadis Radioimmune Imaging

Radioimmune imaging has stirred excitement during the past decade with its promise to usher in a new era of disease detection, characterization and therapy. For all of its initial promise, however, the experience with this technique suggests radioimmune imaging may have a long adolescence before gaining a place in the daily diagnostic armamentarium.

Antibodies are the ultimate "magic bullet", combining the desirable properties of high affinity and specificity for tissue expressing the antigens under study. Technical progress in the field has been nearly miraculous. Over the short span of a decade, the radiolabeled antibodies have progressed from polyclonal anti-CEA labeled with iodine-131 (^{131}I) used in the late 1970s (*1*) as the initial agent to image tumors in human subjects, to chelate labeled monoclonal reagents (*2,3*) raised to precisely defined antigens (*4*) for the production of highly specific antibodies. Despite this technical progress, the technique has failed to provide a high sensitivity and high specificity imaging modality for localizing a vast array of lesions.

To understand the theoretical framework for radioimmune imaging, let us consider a model system consisting of 1.0 g of cardiac myosin suspended in saline and incubated with a radiolabeled antimyosin antibody ($K_a \simeq 10^9$ l/M). Under these circumstances, binding can be described entirely by mass action considerations.

$$Ag + Ab^* = AgAb^* \quad (1)$$

$$K_a = \frac{[AgAb^*]}{[Ag][Ab^*]} \quad (2)$$

Rearranging:

$$[AgAb^*]/[Ab^*] = [Ag] * K_a \quad (3)$$

Thus: Target to Background Ratio (T/B) = $[Ag] * K_a$

Assuming a monomeric molecular weight for cardiac myosin of 50 kD, the antigen concentration is 2×10^{-6} *M*/l. At equilibrium:

$$T/B = (2 \times 10^{-6}\ M/l)\,(10^9\ l/M) = 2.000.$$

Using similar reasoning, a nearly identical result was reported by Larson for an antibody directed against a melanoma membrane antigen ($K_a = 10^{10}$ l/*M*) (*5*).

Since the quantity of antigen is usually limited, antibody affinity becomes the critical factor defining the maximal theoretical T/B ratio. In general it is easier to produce high specificity than high affinity monoclonal antibodies and high specificity/high affinity antibodies are rare. Usually, high specificity antibodies have low affinity and vice versa. Monoclonal antibody production and screening are very labor intensive processes. The nature of the process often results in selecting high specificity antibodies of only moderate affinity. While antibodies with high affinity and specificity are difficult to obtain, the random nature of antibody production can clearly reward the persistent investigator. The premature selection of a high specificity/modest affinity antibody may explain many of the mediocre results seen to date with antibody imaging.

An additional factor, the absolute level of antibody accumulation, is equally important for lesion detection. Increasing the mass of antibody administered tends to improve localization (*5*). This could explain the spectacular imaging results obtained with xenografts of human tumors in nude mice (*6*) (up to 25% injected dose/gram tumor) compared to studies of the same tumors in patients (*7*) (0.01–0.001% injected dose/g tumor). If the same total amount of antibody is injected in both types of studies the relative antibody concentration in patients is ~3,500 fold lower than in the nude mouse (70 kg/20 g). The same argument holds if the concentration of antibody is scaled down, but the lesion size in the mouse is relatively large (e.g. 1 g tumor in a 20 g mouse–5% of body weight–equivalent to a 3.5 kg tumor in a 70 kg

human). These results can be explained if the lesion is assumed to be functioning like an affinity column and the blood flow and exposure time are the same in mouse and man. Under these circumstances, total uptake should be 3,500 fold lower in patients than nude mice i.e., ~0.007% vs. 25%. This relatively low level of antibody accumulation may partially explain why in general, <80% of known tumor foci have been effectively imaged with radiolabeled antibodies (*7,8*). The problem is compounded with targets that shed antigen into the circulation: a significant percentage of the radiolabeled antibody can be bound by circulating antigen and cleared by the liver before adequate target binding can occur. With high dose administration there should be enough antibody for specific target binding, even in the face of significant shed antigen. Thus both the dilution and shed antigen problems can be simultaneously addressed by administering higher doses of antibody. These concepts have been demonstrated in clinical practice in patients with malignant melanoma (*5*). Another problem in imaging with antibodies is the presence of partially cross reacting antigens or nonspecific Fc mediated localization. High doses of unlabeled antibodies may be necessary to override these effects. Thus high doses are a two edged sword: a larger dose of antibody can improve image quality; but it also increases the likelihood of a human antimouse antibody response (HAMA) (*9*).

Utilization of Fab fragments of murine monoclonal antibodies at ≤0.5 mg doses does not appear to elicit a HAMA response, even with repeated doses. The lack of a HAMA response to low dose murine Fab is different than that with $F(ab')_2$ or intact antibody, where the human anti-mouse antibody response reproducibly occurs with repeat administrations. Aside from the potential allergic responses associated with HAMA, the presence of human-anti-mouse antibody decreases the efficacy of murine antibody localization at the target site.

In general, antibody fragments have lower physiologic barriers to target localization than intact antibodies. Studies of multicellular tumor spheroids cultured in vitro, have revealed that tumor penetration is strongly dependant on molecular size, Fab > $F(ab')_2$ > intact Ab (*10*). Similarly, studies of xenografts of human melanoma in nude mice demonstrated that Fab fragments had much faster and greater specific tumor uptake than the corresponding intact antibody (*11*). These observations emphasize the importance of permeability barriers and nonspecific localization in designing antibody based imaging agents. Based on affinity criteria alone, the monovalent character of Fab fragments should reduce localization. The improved imaging characteristics of fragments suggest that unique molecules, combining the specific antigen binding site, (i.e., the Fv region of the antibody molecule (*12*)), to small polymers with increased target permeability could enhance both localization in the target and the target/background ratio. In addition, it is possible that by coupling multiple Fv's to a single carrier polyvalency and associated high avidity can be restored.

As antibody production has become more sophisticated, radiolabeling techniques have also evolved. The first generation of chelate labels, using the bicyclic or mixed anhydride of diethylenetriaminepentaacetic acid (DTPA) (*13,14*) enabled formulation of kits, which could be readily labeled with indium-111. Unfortunately, both of these methods used one carboxylic acid group of DTPA for binding the chelating agent to the protein. This decreases the binding constant for trivalent cations and allows transchelation to tranferrin in vivo, resulting ultimately in high bone-marrow and liver uptake. To overcome these problems, methods of covalently linking the chelate moieties to antibodies without tying up carboxylic groups have been described (*15,16*). Initial applications in animal studies showed diminished hepatic and nonspecific organ sequestration with maintained lesion specificity (*17,18*). However, in clinical trials of tumor imaging, these reagents have shown high levels of hepatic activity (*19*). Further improvements in chelate chemistry will likely be necessary to produce radioactive trivalent cation labeled reagents with a favorable in vivo biodistribution.

Like size, antibody charge can have profound effects on target localization (*20*). The effect of charge on localization was tested in a dog model of acute myocardial infarction. A highly negatively charged polymer was coupled to antimyosin Fab, resulting in a striking decrease in isoelectric point. This preparation produced diagnostically useful images as early as 30 minutes after injection (comparable to images acquired 24–48 hr after injection of unmodified antimyosin Fab). The proposed explanation for this improved localization is based on the change in zeta potential of damaged cells. Normal myocytes have a negative surface charge.

With injury, holes are produced in the plasma membrane and the surface charge is partially dissipated. A highly negatively charged antibody derivative should be able to bind to the exposed antigen of the damaged cells and be repelled from intact cells, resulting in earlier visualization of the lesion.

At present, it appears that full potential of radioimmune imaging may be best realized in imaging lesions expressing large quantities of antigen, such as in the detection of myocardial necrosis (*21–24*) deep vein thrombosis (*25*), pulmonary emboli (*26*), and focal sites of infection (*27–29*). Problems encountered with these applications each differ from those seen in tumor imaging. Antimyosin imaging to detect myocardial necrosis is limited by decreased perfusion to the abnormal myocytes and potentially confounding blood pool activity (but antigen expression is vast in the area of necrosis). Clot imaging is limited by the surface area of the lesion and spontaneous rate of thrombolysis, since only surface antigen is available for antibody binding. Infection imaging is limited by the intensity of the inflammatory response, a mechanism necessary to deliver increased blood flow and extracellular fluid to the site. While these problems are significant, they are easier to address than the difficulties inherent in tumor imaging. As a result, these non-neoplastic applications of radioimmune imaging are likely to advance more rapidly then oncologic applications.

In addition to antibodies, other molecules which do not directly involve the immune response have potential as imaging agents. These molecules could greatly expand the scope of radioimmune imaging. The recent identification of cell surface receptors for biologically active molecules, such as leukocyte attractant peptides, intercelluar adhesion molecules, and leukotreines has introduced many candidate molecules. These agents or their antagonists may be useful for imaging the cells to which they bind. The lower molecular weight and high binding affinity of these molecules appear to have three advantages over antibodies. First, they are smaller and hence more diffusible, resulting in faster equilibration with the extravascular space; second, the agents have faster blood clearance, resulting in lower concentration at nontarget sites; and third, the agents have well-defined receptor systems on known populations of cells or tissues, resulting in predictable distributions. When the proposed imaging agent is a small peptide, its imaging properties can be optimized by chemical synthesis of multiple analogs. The analogs may vary in molecular properties including: size, charge, polarity, and hydrophobicity (*30*). The study of these analogs permits a rational approach to the development of an optimal biological pharmaceutical.

As methods of antibody or peptide modification and radiolabeling techniques improve to allow early imaging of various types of lesions, application of shorter-lived positron emitting radionuclides will permit antibody imaging with positron emission tomography (PET) (*31–33*). PET imaging has two advantages over single photon techniques for radioimmune and receptor based imaging (*34*): (a) the short physical half lives of positron agents will allow serial injection and imaging to follow the rapidly changing course of a lesion in response to therapy (with single photon radionuclides such as ^{111}In or ^{131}I, residual activity from one administration can complicate the interpretation of images obtained after a second injection performed within 24 hr); and (b) the high sensitivity and improved spatial resolution of PET will allow early detection and quantification of changes in lesion size and avidity for the radiopharmaceutical.

While clinical experience with radioimmune imaging in neoplasia has indicated that current antibodies cannot yet deliver the high sensitivity results required for reliable early detection of tumor, success in non-neoplastic areas has been very encouraging (*21–29*). Recent developments in receptor based techniques, and potential improvements in resolution and quantification with PET, suggests that the field will continue to grow with a broad range of high affinity reagents.

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