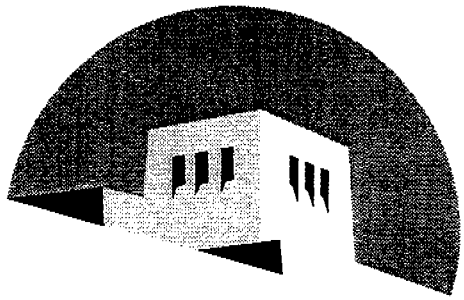

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THE UNIVERSITY OF NEW MEXICO
COLLEGE OF PHARMACY
ALBUQUERQUE, NEW MEXICO

Correspondence Continuing Education Courses for Nuclear Pharmacists

VOLUME I, NUMBER 6

Current Status of Radiolabeled Antibodies for Imaging and Therapy

by:

George H. Hinkle, M.S., R.Ph., B.C.N.P.

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CURRENT STATUS OF RADIOLABELED ANTIBODIES FOR IMAGING AND THERAPY

STATEMENT OF OBJECTIVES

The primary goal of this correspondence course is to increase the reader's knowledge and understanding of the potential clinical use of radiolabeled antibodies for diagnosis and/or therapy of disease. In order to complete this goal, the course discusses the basic science regarding the immune response, antigens associated with diseased tissue, antibody production, fragmentation and radiolabeling. Finally, the benign and malignant disorders that investigators have attempted to diagnose and/or treat using radiolabeled antibodies are discussed.

Upon successful completion of this material, the reader should be able to:

1. Describe the differences between radioimmunoassay, radioimmunodetection and radioimmunotherapy.
2. Describe the underlying principal associated with the immune response and its relation to the localization of radiolabeled antibodies at disease-associated antigens.
3. Describe the basic structure of an antibody indicating the variable and constant regions, the "business end" and the portion of antibody involved with human anti-mouse antibody (HAMA) production.
4. Describe the hybridoma method of producing antibodies.
5. Explain the differences between polyclonal antibodies and monoclonal antibodies (MAbs).
6. Describe the chemical cleavage of the IgG molecule during antibody fragmentation.
7. List the advantages and disadvantages of the clinical use of $F(ab')_2$ and Fab fragments over the intact IgG molecule.
8. List two tumor-associated antigens found on colon cancer cells that have played a major role in the development of radioimmunodetection.
9. Describe the method of preparation and the properties of "second generation" monoclonal antibodies.
10. List two "second generation" monoclonal antibodies of MAb B72.3.
11. Describe the "designer" molecules including chimeric antibodies, humanized antibodies, single chain antigen binding proteins (SCABPs) and molecular recognition units (MRUs).
12. Explain the primary clinical improvement associated with the use of "designer" molecules over the original MAb structure.
13. List the four clinical uses that have shown promise during investigations involving radioimmunoscintigraphy.
14. List the organ systems in which radioimmunoscintigraphy has been shown to localize solid tumors.
15. Describe the differences in biodistribution patterns, pharmacokinetics and routes of excretion for radioiodine-labeled MAbs and radiometal-labeled MAbs.
16. Explain why the use of Tc-99m for radioimmunoscintigraphy is more appropriate with MAb fragments.
17. Describe the underlying pathology associated with myocardial infarction which allows the radioimmunodetection of this disorder.
18. List the two different radioimmunologic agents that have been studied for their ability to localize infectious disorders or sites of abscess.
19. Describe the different combinations of antibody/compounds that have been investigated as potential therapeutic agents.
20. List the advantages and disadvantages associated with the use of radiolabeled MAbs as therapeutic agents for cancer treatment.
21. List three radionuclides undergoing clinical investigation for radioimmunoscintigraphy.
22. List three radionuclides that have been proposed for use in radioimmunotherapy.
23. Describe the placement of radioactive atoms on the MAb molecule during radioiodination and compare this with the site-specific radiolabeling with In-111 using a linker-chelate structure.
24. Describe the characteristic properties that should be tested during the quality assessment of radiolabeled MAbs.

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IX. SUMMARY

CURRENT STATUS OF RADIOLABELED ANTIBODIES FOR IMAGING AND THERAPY

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INTRODUCTION

Since the development of the hybridoma technique by Köhler and Milstein in 1975, a variety of monoclonal antibodies (MAbs) that specifically recognize and react with (bind) various antigens have been produced in laboratories throughout the world. The diversity of antigenic substances these MAbs target, the exquisite specificity of antigen recognition that occurs, and the biologic activity of the MAbs and their corresponding fragments makes them ideal agents for clinical use for *in vitro* testing (radioimmunoassay), nuclear medicine imaging of benign and malignant disorders (radioimmunodetection), and therapeutic intervention (radioimmunotherapy).

MAbs that localize and bind to specific tumor-associated antigens as well as antigens associated with benign disorders (such as infectious processes or abscesses, forming clots, and myocardial infarction) have reached clinical testing stages. Many of these MAbs or their fragments radiolabeled with radionuclides such as I-123, Tc-99m, In-111, or I-131 are near U.S. Food and Drug Administration (FDA) approval and, in fact, are currently approved and being used in a number of European countries. Genetic engineering methods are being developed to improve the antigen binding molecule in order to overcome the biologic localization difficulties. Sophistication of radiochemical techniques is leading toward an improvement of the *in vitro* and *in vivo* stability of the radiolabeled MAbs as well as broadening the number of useful radionuclides for imaging or therapy. Increasing our knowledge of the clinical utilization of these agents will help make radioimmunopharmaceuticals an integral part of the practice of nuclear medicine.

THE IMMUNE RESPONSE

Our immune system is responsible for one of the most basic biological responses that is vital to our survival. When a foreign substance enters the body, our

immune system responds by producing antibodies. Antibodies are large molecular weight structures called immunoglobulins (Ig) that recognize, identify, and bind to the foreign substance or antigen. The antibody -antigen complex starts a series of processes leading to the neutralization and eventual elimination of the foreign substance from the body (1,2). Although five different classes of human Ig have been identified, immunoglobulin-G (IgG) is the principle class of antibody found in blood and is the Ig involved in toxin neutralization, agglutination, opsonization, and lysis of bacteria (2).

An antigen is any substance capable of triggering an immune response. Viruses, bacteria, fungi, and other parasites or any portion of these organisms such as a protein, carbohydrate chain, or other complex structure can act as an antigen. An antigen capable of eliciting an immune response is called an immunogen and is recognized as foreign due to intricate and characteristic shapes called epitopes which are part of the antigen's membrane structure (3). Most antigens have several epitopes on their surface. Each epitope presents a slightly different structural shape which results in antibodies of different binding affinities during an immune response.

The most common types of immune responses are the allergic reactions seen during bouts with hay fever, asthma, hives, colds, and flu. However, the immune system also provides the body's main defense against cancer. Most tumors synthesize and release, in large quantities, a number of substances found in the normal cell of the same tissue or organ where the tumor is located. For instance, certain metabolites, hormones, enzymes, receptors, immunoglobulins, and antigens are elevated in tumor cells. Some of these substances are used as tumor markers since their concentration in blood or other fluids becomes elevated as the tumor grows. For example, the oncofetal carcinoembryonic antigen (CEA) is used to monitor the progression of gastro-intestinal cancers since it is found in elevated levels in the serum of patients with this disease. These antigens are said to be shed from the surface of the tumor cell which allows their identification and quantitation in blood.

TUMOR-ASSOCIATED ANTIGENS

The presence of these new or altered antigens on the surface of tumor cells (tumor-associated antigens) enables the body to elicit an immune response to the tumor as part of the defense mechanism. It is believed that tumors progress into life-threatening diseases when the body's defense system breaks down or is over-whelmed. In fact, one form of cancer therapy uses biological response modifiers to stimulate or replenish the cancer patient's immune system. The ability to isolate tumor-associated antigens and prepare antibodies that bind specifically to them has also enabled radio-immunodiagnosis and radioimmunotherapy to advance to the clinical trials ongoing today.

ANTIBODY STRUCTURE

The IgG molecule, shown in Figure 1, is composed of two identical light polypeptide chains and two identical heavy polypeptide chains held together by noncovalent interactions and covalent disulfide bonds. Within the antibody structure are variable regions (V_H and V_L) occurring at the amino end of both the light and heavy chains and constant regions (C_{H1}, C_{H2}, C_{H3} and C_L). The variable region is sometimes referred to as the "business end" of the antibody since the binding of antibody to the epitopes of the antigen occurs here. The polypeptide sections, which make up the tips of the variable regions, vary greatly from one antibody to another. This gives each antibody a unique shape in this area in order to provide specific antigen binding capability. It is important to preserve this end of the antibody intact during radiolabeling or any other type of modification since any changes near the end of the variable region may interfere with antibody binding. Following the variable region, the light chain has one constant region (C_L) while the heavy chain has three (C_{H1}, C_{H2}, C_{H3}). This part of the antibody is identical (constant) for all antibodies in the same class and serves to link the antibody to other molecules involved in the immune response. It is thought that this area plays a significant role in the production of human anti-mouse antibodies (HAMA) in those patients receiving injections of murine antibodies.

Antibody Production

Early attempts at radioimmunodetection of cancer, which only started thirty years ago, used polyclonal antisera produced by immunizing animals with tumor cells. The first attempts to image tumors in man in 1978 used purified goat antisera to the CEA radiolabeled with iodine-131. Due to poor specificity and lack of consistency in the antibodies that were being used, a method of producing homogeneous antibodies with one defined specificity for a particular antigen was a long-standing goal.

In 1975, Köhler and Milstein developed a technique that allowed for the growth of clonal cell populations that secreted virtually unlimited quantities of pure, identical antibodies with a defined, single specificity (4). Specificity is the ability of antibodies to distinguish between binding sites on an antigen. An antibody with single specificity, a monoclonal antibody (MAb), will bind to only one unique target site on an antigen (5).

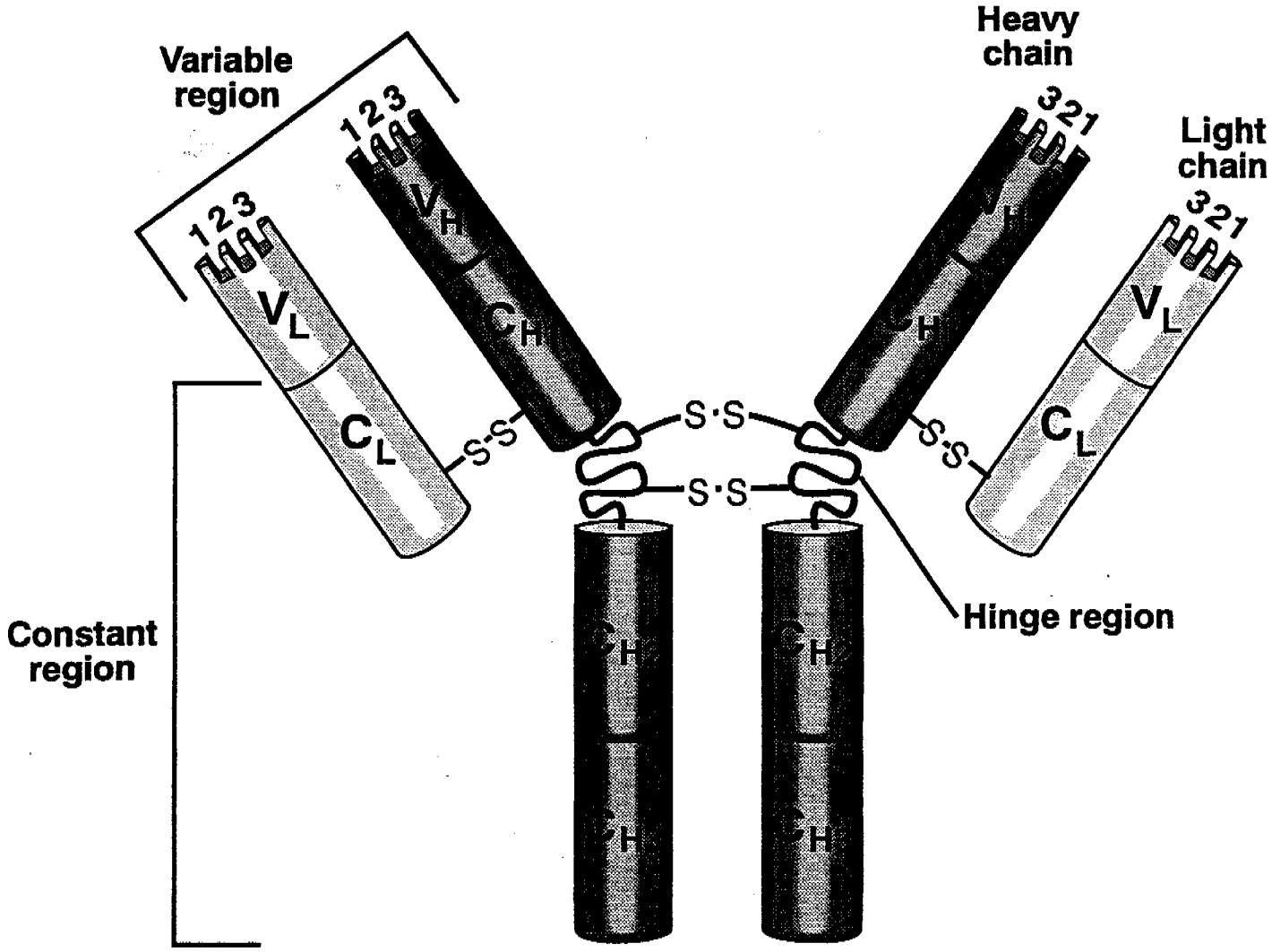

Figure 1. ***Schematic representation of antibody immunoglobulin structure showing light and heavy chains, variable and constant regions, the hinge region and the disulfide bonds between protein chains.***

To produce MAbs, mice are immunized with tumor cells or purified antigen. The spleen or lymph nodes of the immunized animal are removed and a cell suspension is made. Individual B-lymphocytes from this cell suspension are fused with myeloma cells which provides the cell fusion or hybridoma immortality. These hybrid cells can be maintained *in vitro* or in animals and will continuously secrete MAbs with a defined specificity. The hybrid cells are cloned, and single cell species producing one type of MAb are cultured to insure a virtually endless supply of the specific antibody (6). The hybridoma cells can then be placed into the peritoneal cavity of mice where the antibodies are produced in ascites fluid. The quantity produced using this method is limited and a number of purification steps are needed to produce pure MAb from ascitic fluid obtained from mice. The production of MAbs from ascitic fluid of mice is associated with a number of problems, including the presence of low levels of extraneous mouse proteins, difficulty in controlling the reproducibility and consistency of the product, possibility of contamination by viruses or other organisms, and the large numbers of mice needed to produce clinically-useful amounts of antibody. Despite these problems, the first FDA-approved MAb for therapy, OKT-3, was (and still is) purified from mouse ascites (7).

The need for large quantities of MAbs to complete clinical trials led to an improved method of production involving the use of large fermentation systems or "bioreactors" to keep the mammalian cell suspensions alive and producing. These fermentation systems provide all the nutrients and oxygen supply for the maintenance of the hybridoma cell line while the cells produce MAbs. The MAbs produced have less contamination of undesirable protein material reducing the number of purification steps. The hybridoma technique for the production of MAbs allows the production of large quantities of MAbs with identical characteristics from batch to batch. In addition, it allows for the selection of the best MAb with the greatest reactivity with a specific antigen which improves detection and/or therapeutic results.

MAb Fragmentation

The ability to chemically dissect the intact, whole IgG MAb molecule using specific enzymes led to the use of the portions of the IgG structure where antigen specificity resides while removing the nonspecific constant region. The two enzymes pepsin and papain are commonly used to prepare fragments of the IgG molecule, as shown in Figure 2, adding a new dimension to the use of MAbs by changing the biodistribution patterns, pharmacokinetics, and localizing properties of the MAb.

Cleavage of the IgG molecule with pepsin, a nonspecific protease, removes the Fc portion of the structure and produces a $F(ab')_2$ fragment. Papain, a nonspecific sulfhydryl protease, cleaves the IgG molecule slightly higher in the hinge region creating one Fc and two Fab fragments. The Fc portion is removed leaving the portion of the MAb with the ability to bind to antigen since the variable regions are left intact. However, these fragments have different blood clearance half-times (faster than whole IgG), different elimination routes and rates (faster and through the kidneys rather

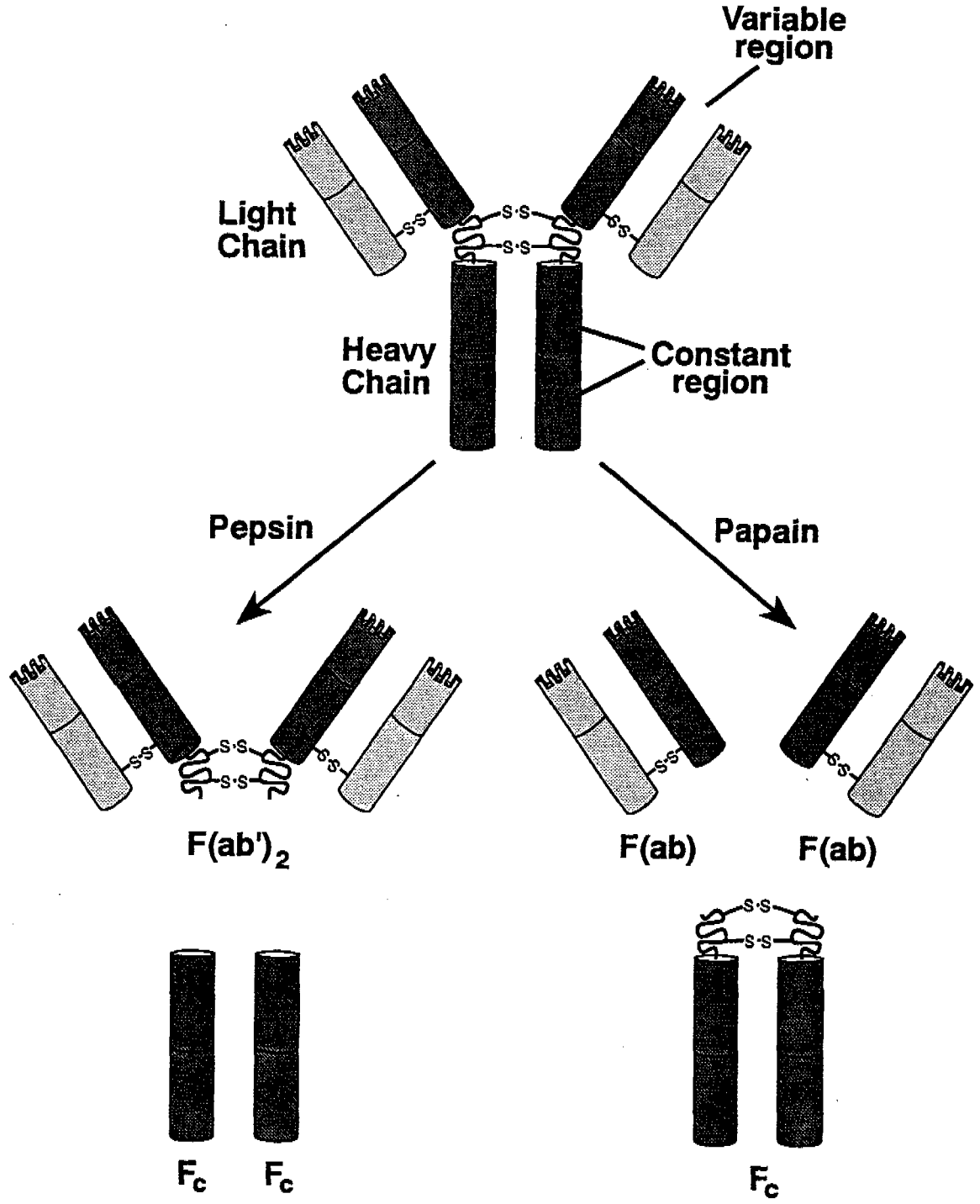

Figure 2. ***Schematic representation of antibody immunoglobulin structure showing various fragments formed by papain or pepsin enzyme digestion of the whole, intact IgG molecule.***

than the hepatobiliary system), and reduced nonspecific binding properties. Because metabolism and clearance from the circulation is faster than with whole IgG molecules, shorter half-life radionuclides such as Tc-99m can be used for radiolabeling fragments for radioimmunodetection.

Further Antibody Developments

In addition to antibody fragmentation, there are other parameters to consider when improvement of radiolabeled antibody uptake is the goal. Development of MAbs with higher specificity and greater affinity would improve the efforts of radioimmunodetection and radioimmunotherapy. An example of such a development has been the preparation of "second generation" MAbs which have higher affinity and improved ability to bind to target antigen. Second generation MAbs are made by first purifying the target antigen, and then using the purified antigen as the immunogen. A series of second generation MAbs to the pancarcinoma antigen, TAG-72, were prepared by first purifying TAG-72 using affinity chromatography with the first generation MAb, B72.3, as the binding agent (8). Using the purified TAG-72, a number of second generation MAbs were developed with two, CC49 and CC83, having 8 and 10 times the antigen binding constant, respectively, compared to the original B72.3 MAb. In theory, second generation, higher affinity MAbs should be more efficient for radiolocalization when compared with their first generation counterparts.

Further efforts to improve radioimmunodetection and radioimmunotherapy with reduced risk to the patient has led to a virtual redesign of MAbs. The specificity of an antibody is governed by the variable regions on the heavy and light chains while the constant region is thought, among other things, to illicit the HAMA response as well as serve as a point of attachment for various ligands used in radiolabeling with radioactive metals. Recombinant DNA and gene transfer techniques have made possible the development of "designer" molecules which have the ability to bind to target antigen while reducing the undesirable effects associated with the use of intact or whole IgG molecules (9).

In Greek mythology, a chimera was an animal with the head of a lion, the body of a goat, and the tail of a dragon. Analogously, a chimeric antibody is built, using recombinant DNA techniques, from a murine variable region and a human constant region. The primary goal is a reduction in the HAMA response seen when murine antibodies are given to human patients. This technique was further developed to produce "humanized" antibodies which have murine hypervariable regions where antigen binding occurs while the remainder of the protein structure is constructed of human amino acids. Again, the primary goal is a reduction in the HAMA response.

Single chain antigen binding proteins (SCABPs) are composed of the variable regions from one heavy chain and one light chain connected by a linker peptide bond. Since SCABPs are much smaller than the intact IgG molecule, they will have faster pharmacokinetics in addition to a decreased HAMA response.

Molecular recognition units (MRUs) are linear or cyclic molecules composed of amino acid residues. MRUs are synthetic peptides which mimic the portion of the hypervariable region where antigen binding occurs. Proposed advantages of MRUs include faster pharmacokinetics, less normal tissue background levels, and decreased HAMA response. Figure 3 provides a schematic representation of these "designer" molecules.

Not all cancer cells express a particular tumor-associated antigen, and heterogeneity among specific antigens is sometimes seen. An effort to overcome these difficulties and increase the chances of radiolocalization in a particular tumor involves the use of mixtures of MAbs or antibody "cocktails." For example, the TAG-72 antigen is only found in approximately 80% of colorectal carcinomas. It is predicted that the use of a MAb that binds to this antigen such as B72.3 along with an anti-CEA MAb may improve targeting. Further discussion of monoclonal antibody technology can be found in articles by Tami (5) and Keenan (10).

RADIOIMMUNODETECTION

The common radiologic studies, including conventional radiography, nuclear medicine, magnetic resonance imaging (MRI), computed tomography (CT), and ultrasound suffer from a lack of specificity for tumor cells. Radioimmunoscintigraphy, a nuclear medicine imaging study involving the use of radiolabeled antibodies that bind specifically to antigens associated with diseased tissues, has been shown to be useful in the diagnosis and staging of a variety of disorders, including cancers. This technique involves the infusion of an antigen-specific MAb that has been radiolabeled with a gamma-emitting radionuclide followed by external imaging with a gamma camera for the identification of the anatomic distribution of primary tumors and metastatic sites, cardiac muscle cell death, sites of abscesses or infections, and forming clots.

Several studies in the U.S. and Europe involving radiolabeled MAbs have demonstrated the clinical usefulness in the radioimmunodetection of both benign and malignant disorders. Using a variety of different MAbs that have been radiolabeled with one of several radionuclides compatible with current nuclear medicine instrumentation, the efficacy of radioimmunoscintigraphy to localize solid tumors of the colon and rectum, lung,

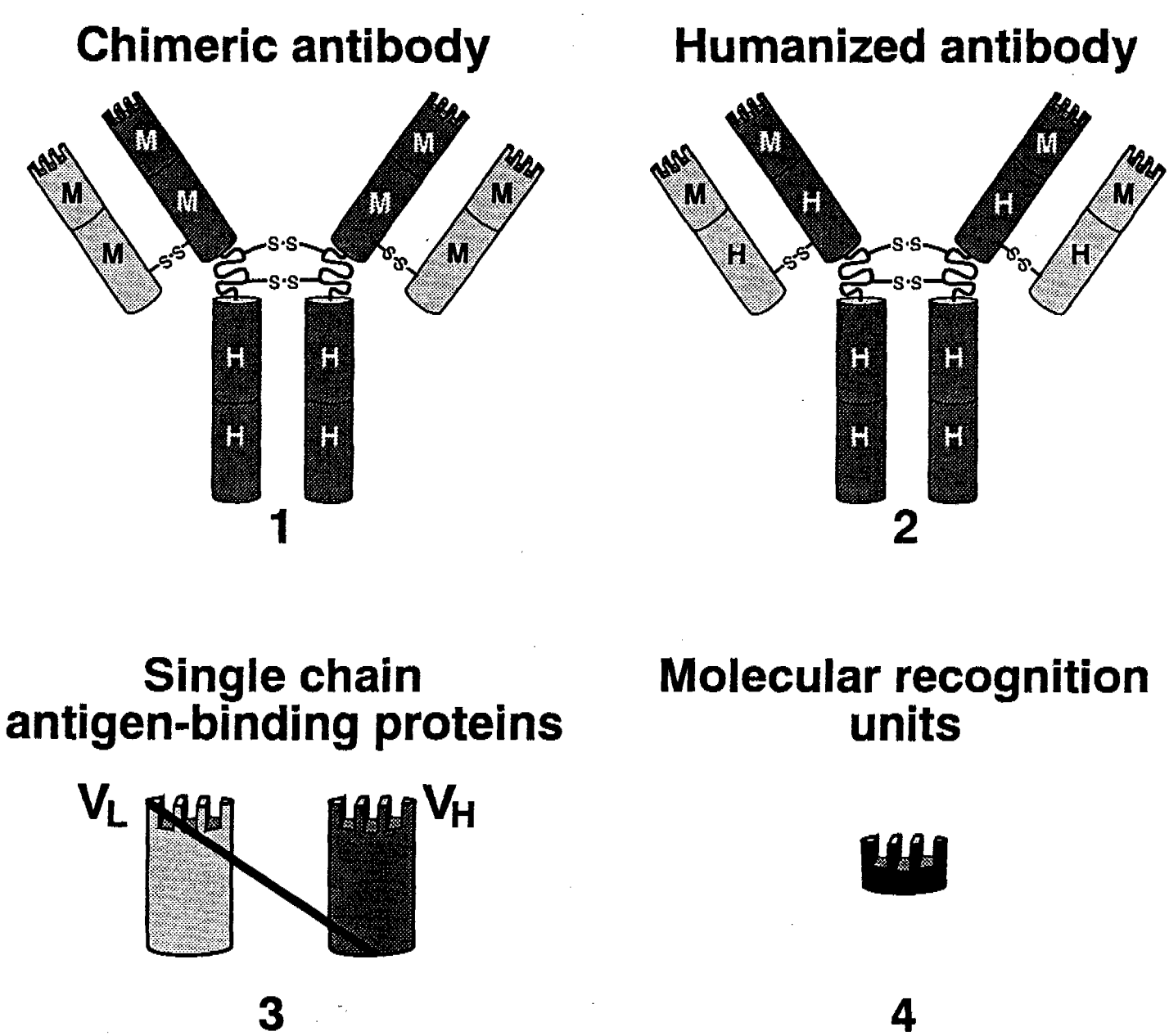

Figure 3. ***Schematic representation of "designer" molecules including (1) chimeric antibody with humanized constant region below the hinge region; (2) humanized antibody with only the variable region composed of mouse protein; (3) single chain antigen binding proteins composed of the variable regions of the light and heavy chains held together by an inter-amino acid bond and (4) molecular recognition units which mimic the antigen binding region of the variable region of the antibody.***

kidney, ovary, breast, prostate, and brain, as well as disseminated cancers such as melanoma, lymphoma and other hematologic cancers has been studied. In addition, pathological disorders of the heart, sites of abscess or infection, blood clots, and autoimmune diseases have been assessed with this modality. When used in the evaluation of cancer, indications include the detection of occult disease in those patients with high clinical suspicion, the staging of the extent and degree of disease pre- and post-therapy, the corroboration of results from other diagnostic tests that are indeterminate, the assessment of tissue viability, the assessment of the potential role of therapy using radiolabeled MAbs or immunotoxin MAb conjugates, and the follow-up of patients with known disease that present with new symptoms indicating increased metastatic involvement (11).

Radioimmunoscintigraphy was first attempted almost forty years ago using radiolabeled antisera to a rat sarcoma grown in rabbits. This early animal study proved that a protein which occurs in high concentrations in tumors could be used for localization (12). The development of the tumor model consisting of human tumor xenografts that could be implanted into athymic, nude mice led to a number of studies involving the use of radiolabeled polyclonal antibodies for imaging human tumors (13-15). The first attempts to image cancer in

patients were not successful with the authors concluding that circulating CEA bound the radiolabeled anti-CEA polyclonal antibodies after intravenous administration and did not allow sufficient localization at the tumor sites (16,17). Goldenberg and his colleagues used an affinity-purified goat anti-CEA polyclonal antibody radioiodinated with iodine-131 to demonstrate tumor lesions in patients (18).

Further attempts at improving clinical radioimmunoscintigraphy include the utilization of a computerized, dual-radionuclide subtraction technique to enhance the tumor images in the normal background surrounding tissues (19,20), the use of radiolabeled fragments of antibody (21-24), improvement of imaging techniques to include single photon emission computed tomography (SPECT) images as well as planar views (25), and the use of a mixture of antibodies, the so-called antibody "cocktail."

Improvements in the methodologies used to radiolabel proteins enabled different radionuclides to be used for a specific clinical need. When radioimmunodetection is the goal for localization of disease sites, staging of disease or monitoring response to therapy, radionuclides which decay with the emission of gamma photons without particulate radiation are preferred. For external imaging with nuclear medicine systems in use today, gamma emissions of 100-200 keV energy range are ideal.

Primarily because radioiodination chemistry for labeling large protein molecules with radionuclides of iodine was well established, I-131 was the first radionuclide used for radioimmunodetection studies. However, it is not the best choice for several reasons. The high energy of the detected gamma photon leads to suboptimal images, the beta emissions cause significant internal radiation dose limiting radiopharmaceutical dosage, and dehalogenation of the radiolabeled protein further degrades the quality of the images (26). Although I-123 provides excellent nuclear properties for imaging, the short physical half-life, limited availability, and relatively high cost of this radioiodine species has not enabled its potential to be fully realized for radioimmunodetection.

Along with improvements in the antibodies available for clinical investigations came the need for improved radionuclides for diagnostic imaging. Nuclear medicine practitioners turned to the familiar radionuclides, In-111 and Tc-99m, both of which have ideal nuclear properties for diagnostic imaging. Although the physical characteristics of both of these radionuclides were nearly ideal for radioimmunoscintigraphy, the chemistry associated with radiolabeling proteins was an obstacle to overcome. Unlike the radioiodine nuclides which have naturally occurring sites of incorporation on antibodies in the tyrosine and histidine amino acid residues, stable attachment of In-111 (as well as other radiometals) to antibodies needed development (27). Although In-111 has proven to be a valuable radiolabel for MAbs, it has a major disadvantage of high non-specific uptake in the liver (28). This makes it very difficult to detect liver metastases, a common site of spread for colorectal cancer. In the diagnosis of disease where the liver is not a potential site of occurrence such as myocardial infarction, thromboembolic disorders, and cancers with minimal hepatic involvement, In-111 will certainly prove valuable.

The gamma emissions of Tc-99m makes this radionuclide the most commonly used in diagnostic nuclear medicine. However, the short half-life may not be of sufficient length to allow adequate tumor-to-background ratios for imaging of cancers using whole, intact MAbs. Waiting three to five days after intravenous administration of the IgG molecule usually improves the images by reducing the circulating quantities of the protein. Studies recently completed using Tc-99m labeled to antibody fragments, which clear from the circulation must faster than the intact IgG structure, indicate this radionuclide is ideal for use with MAb fragments (29-31) and should also prove useful with the smaller molecules not yet at the clinical trials stage of development.

New chemicals and improved radiolabeling methods have made it possible to bind radionuclides for diagnosis and therapy to any MAb and/or its fragments. Table 1 provides a list of those radionuclides that have been investigated as a possible radiolabel for MAbs for use in radioimmunodetection. Because each MAb and its fragments are affected differently by radiolabeling techniques, careful *in vitro* and *in vivo* quality assessment procedures must be completed on each radionuclide-antibody combination to be certain the immunoreactivity, radiochemical purity, and pharmacokinetics are acceptable (32,33).

Table 1. Radionuclides For Radioimmunodetection

Radionuclide	*Half-life (hrs)*	*Decay emissions (energy in keV)*
Indium-111	67.9	gamma (173, 247)
Technetium-99m	6.02	gamma (140)
Iodine-131	192.9	gamma (364), beta (600)
Iodine-123	13.2	gamma (159)

Colorectal Cancer Imaging

A majority of the early development of disease detection using radiolabeled antibodies was directed towards the imaging of cancer of the gastrointestinal system, primarily the colon and rectum. A primary reason was the early pre-clinical work completed with CEA which led to an extensive knowledge base and good supply of pure antigen and antibodies. MAbs to this tumor-associated antigen, which is found in over 95% of colorectal carcinomas, continue to show promise in both diagnosis and treatment of a number of different cancers.

The first clinical studies involved I-131 anti-CEA polyclonal antibody for the localization of colorectal carcinomas (17,18). Although the overall success was not overwhelming, there were some positive results which encouraged continued investigations. In 1981, only three years after the early studies with polyclonal IgGs, the first report of the use of a MAb for imaging was made with CEA as the target antigen (21). The I-131 labeled MAb had a detection rate of 50% in a patient population with tumors of the colon and pancreas.

A number of investigators around the world working toward the improvement of radioimmunodetection used various techniques including different radionuclides (22,28,29), different imaging methodologies (19,20,25), different MAb fragments (22-24), and varying dosages (34,35). Finally, these encouraging results along with the further development of the knowledge base of tumor immunology and the resultant identification of many tumor-associated antigens, led to the study of a number of antibody-antigen combinations for the detection of gastrointestinal as well as other cancers. Several recent reviews of radioimmunodetection of colorectal cancer are recommended (33,36-38).

The clinical radioimmunodetection of colorectal carcinomas illustrates the systematic improvements still ongoing with this new imaging modality. The first planar images using purified goat polyclonal anti-CEA antibodies labeled with I-131 led to good visualization of some tumor deposits (18). Using SPECT imaging in 17 patients imaged with the same radiolabeled antibody, this same group reported an improvement to 90% detection rate (25). Using I-123 labeled Fab and $F(ab')_2$ fragments of this anti-CEA MAb led to detection of 86% of lesions in patients with colorectal cancer (22).

Improvements in chelate-protein chemistry led to the utilization of radioactive metal-MAb combinations for radioimmunodetection (39,40). The poor nuclear properties of I-131 and the expense and unavailability of I-123 spurred the investigations into alternative radionuclides. A large, multicenter trial of radioimmunodetection of colorectal cancer using an $F(ab')_2$ fragment of an anti-CEA MAb evaluated a number of variables including a comparison of I-131 to In-111 radiolabeling. This study indicated the In-111 labeled fragment was most useful for imaging lesions outside the liver (41). Most clinical trials involving In-111 labeled MAbs or their fragments show greater normal hepatic uptake compared to radioiodine studies (42). This phenomenon appears to be independent of MAb used, but depends on the method of chelation and perhaps the total dosage of MAb administered. This has led clinical investigators utilizing In-111 labeled MAbs to conclude that immunoscintigraphy of cancer is complimentary with CT. Radioimmunoscintigraphy provides a better evaluation of extra-hepatic disease while CT gives a more accurate determination of liver involvement.

The short half-life radionuclide Tc-99m has been relegated to use with fragments of MAbs to CEA as well as other tumor-associated antigens (29,30). Clinical trials involving Tc-99m anti-CEA MAb Fab' fragments have reported lesion detection rates of 80-90% (43,44). Most studies using Tc-99m labeled anti-CEA MAbs have shown less hepatic uptake when compared with their In-111 counterparts. In addition, radiolabeled MAb fragments clear more rapidly from the blood, primarily through renal excretion, resulting in earlier imaging times of 12 hours post-infusion compared with three to five days for In-111 labeled intact MAbs.

Although a number of anti-CEA MAbs have been characterized and studied clinically (43-47), two have been the subject of extensive clinical trials in the U.S. for imaging colorectal cancer and, in fact, are currently under review by the FDA. Table 2 lists those MAbs currently under clinical investigation in the U.S. In-111 labeled anti-CEA murine MAb ZCE-025 has been used over the past few years in a multi-institutional clinical trial involving pre-surgical patients with colorectal carcinoma. In that patient population, 80% of lesions were detected with a positive predictive value (percent of positive images which were surgically confirmed) of 94% and an accuracy of 76% (48). Figure 4 shows an image with this radiolabeled MAb in a patient with primary colorectal cancer.

Figure 4. Anterior view of lower abdomen and pelvis of patient with primary colorectal carcinoma 6 days post injection of In-111 anti-CEA MAb. Arrows indicate sites of uptake in the cecum, ascending colon on the patient's right side and midline below the inferior tip of the liver.

TABLE 2. RADIOLABELED MONOCLONAL ANTIBODIES UNDER REVIEW BY U.S. FDA

PRODUCT NAME	COMPANY	INDICATION	STATUS
Fibriscint anti-fibrin antibody	Centocor (Malvern, PA)	blood clot imaging agent	Phase III
ImmuRAID-CEA technetium 99m-FAb' fragment (colorectal)	Immunomedics (Warren, NJ)	extent of disease staging of colorectal cancer	application submitted
ImmuRAID-CEA technetium-99m-Fab' fragment (lung)	Immunomedics (Warren, NJ)	extent of disease staging of lung cancer	Phase I
ImmuRAID-CEA technetium-99m-Fab' fragment (breast)	Immunomedics (Warren, NJ)	extent of disease of breast cancer	Phase I
ImmuRAID-LL2 technetium-99m-Fab' fragment (lymphoma)	Immunomedics (Warren, NJ)	extent of disease staging of lymphoma	Phase I
ImmuRAID-MN3 technetium-99m-Fab' fragment (infectious disease)	Immunomedics (Warren, NJ)	diagnosis of fever of unknown origin	Phase I
ImmuRAIT-CEA iodine 131-intact IgG (colorectal)	Immunomedics (Warren, NJ)	treatment of colorectal cancer	Phase I
ImmunRAIT-LL2 iodine 131-intact IgG (lymphoma)	Immunomedics (Warren, NJ)	treatment of lymphoma	Phase I
Myoscint mifarmonab	Centocor (Malvern, PA)	cardiac imaging agent	application submitted
OncoRad® GI103 CYT-103-Y-90	CYTOGEN (Princeton, NJ) Sterling Drug (New York, NY)	targeted radiotherapy for gastrointestinal malignancies	Phase I/II
OncoRad® OV103 CYT-103-Y-90	CYTOGEN (Princeton, NJ) Sterling Drug (New York, NY)	targeted radiotherapy for ovarian cancer	Phase II
OncoScint® CR103 celocolab	CYTOGEN (Princeton, NJ)	detection, staging and follow-up of colorectal cancer	application submitted
OncoScint® CR372 CYT-372-In-111	CYTOGEN (Princeton, NJ)	detection, staging and follow-up of colorectal cancer	Phase I
OncoScint® OV103 celogovab	CYTOGEN (Princeton, NJ)	detection, staging and follow-up of ovarian adenocarcinoma	application submitted
OncoScint® PR356 CYT-356-In-111	CYTOGEN (Princeton, NJ)	detection, staging and follow-up of prostate adenocarcinoma	Phase II
pancarcinoma Re-186 MAb	NeoRx (Seattle, WA)	breast, colon, lung, ovarian, pancreatic and prostate cancers	Phase I

Another clinical trial studied a Tc-99m labeled Fab' fragment of an anti-CEA MAb (Immu-4) for the localization of colorectal cancer (49,50). In these studies, radioimmunodetection was positive in 85-95% of patients who had lesions detected by conventional diagnostic studies. A positive predictive value of 80-90% was found in those patients who went to surgery.

Radioimmunoscintigraphy is based on the principle that a specific, radioactively-labeled MAb recognizes and binds to a tumor-associated antigen in quantities that can be distinguished from normal tissue. In addition to the previously-mentioned clinical studies involving MAbs that bind to CEA, a large number of different radiolabeled MAbs have been shown to localize in colorectal cancer following intravenous administration in patients. Some of these studies include the use of MAb CR-086 (51), MAb CA 19-9 (52), MAb 17-1A (23,53), MAb to colon-specific antigen-p (CSA-p) (54), and MAb B72.3 (55). In general, all but MAb B72.3 provided poorer imaging results than the MAbs to CEA. Extensive studies have been completed with MAb B72.3 which binds to TAG-72, a tumor-associated glycoprotein antigen. Early imaging studies using I-131 or In-111 labeled B72.3 provided an overall detection rate of 80% in patients with recurrent colorectal cancer (56). This same MAb has been utilized in a multicenter clinical trial for the radioimmunodetection of colorectal carcinoma. In-111 CYT-103, MAb B72.3 site-specifically labeled on the Fc portion of the IgG structure, provided a 74% sensitivity for tumor localization in the pelvic region. In-111 CYT-103 imaging and CT were found to be complimentary in the detection of colorectal cancer with the MAb imaging detecting 69% of proven positives, CT detecting 68%, and MAb imaging and CT combined sensitivity of 88% (57,58).

Radioimmunodetection of colorectal carcinoma provides information on the disease status of the patient that is not obtainable with conventional diagnostic tests. CT and MRI are not very good for imaging extrahepatic metastases, especially in the differentiation of tumor from abscess or fibrosis, or for lymph node or mesenteric involvement. In addition, neither can detect metastases that reside out of the field-of-view. Radioimmunodetection can easily incorporate wholebody imaging where it routinely detects distant metastases in the skeleton, chest, and brain. Radioimmunodetection of colorectal carcinoma has provided new lesion detection information in 25% of patients imaged, thus helping the referring physician better plan therapy.

Ovarian Cancer Imaging

Radioimmunoscintigraphy is an exciting possibility for the noninvasive detection of ovarian cancer both in the intitial workup of the patient and for evaluation of the response to therapy. Ovarian cancer is the fourth leading cause of cancer deaths in women in the U.S., primarily because the disease is not symptomatic until the advanced stages. Although, as with all cancers, early diagnosis is key to treatment, it is the exception with the majority of patients presenting with metastatic involvement of the entire peritoneal cavity.

MAb OC-125, which targets the antigen CA-125 found on some ovarian carcinomas, has been investigated for radioimmunoscintigraphy of gynecologic carcinomas (59-61). Since the CA-125 antigen is a shed tumor marker (it is elevated in approximately 75% of ovarian cancer patients), this antibody-antigen combination has found great use as a serum assay to evaluate response to therapy. In the radioimmunodetection studies completed, low tumor-to-background ratios of 1.8 to 2.5 were found with tumor detection rates ranging from 70-90%. Although it has been shown that circulating levels of CA-125 do not interfere with radioimmunoscintigraphy (62), there is concern that the infusion of the radiolabeled OC-125 will interfere with the serum assay results. As in the case with radioimmunodetection of colorectal cancers with In-111 labeled MAbs, high background levels in normal liver prevented the discrimination of hepatic tumors from normal tissue using In-111 labeled OC-125.

A number of MAbs that bind to a membrane component of human milkfat globules (HMFG) have been developed. Anti-HMFG MAbs have been shown to bind to cell surface antigens found on ovarian tumors as well as other tumors of epithelial origin (63). Clinical investigators using radiolabeled anti-HMFG MAbs for imaging of ovarian cancer have reported sensitivities of 90-100% (64,65). Lesions as small as 0.8 cm were detected with tumor-to-background ratios of 1.4 to 2.8 achieved (66).

MAb B72.3, studied extensively because of its ability to detect colorectal cancer, has also proven valuable in radioimmunoscintigraphy of ovarian cancer. This antibody (produced from a breast cancer metastasis to the liver) has been shown to bind to a wide variety of mucin-producing adenocarcinomas, including colon, breast, ovary, pancreas, and stomach (67). MAbs which bind to a variety of different tumors due to the presence of a specific target antigen are referred to as pancarcinoma antibodies. The largest U.S. clinical trial of ovarian carcinoma imaging was completed with site-specifically radiolabeled In-111 B72.3 MAb, the same radiopharmaceutical used in the clinical trial for radioimmunoscintigraphy of colorectal cancer mentioned previously. Over 100 ovarian cancer patients were

entered in this multicenter trial which compared radioimmunoscintigraphy with CT for their abilities to identify sites of primary and recurrent tumors (68). CT provided an overall sensitivity of 44% compared to 66% for In-111 CYT-103 imaging with patient management positively affected by radioimmunoscintigraphy in 27% of the patients. Figure 5 shows a patient with ovarian carcinoma who was imaged with In-111 CYT-103.

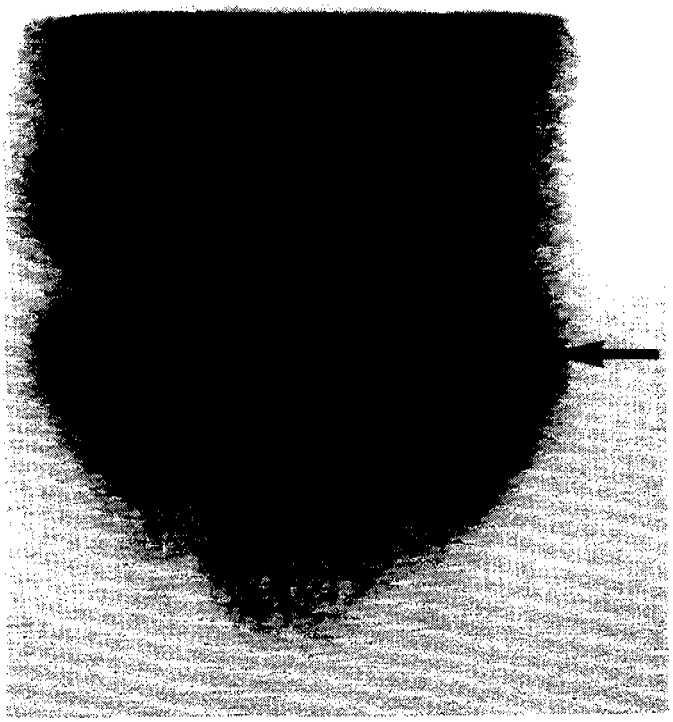

Figure 5. Anterior view of lower abdomen and pelvis of patient with ovarian carcinoma 3 days post injection of In-111 anti-TAG-72 MAb (CYT-103). Arrow points to "seeding" of tumor throughout the mesentary of the pelvis.

Although radioimmunoscintigraphy has proven useful in a number of well-controlled clinical studies, one can only speculate as to whether this new diagnostic modality will be routinely integrated into the management of ovarian cancer. Improvements in sensitivity and specificity should accompany development of second-generation MAbs (69).

Imaging Melanoma

Radioimmunoscintigraphy has been studied extensively for the detection of metastatic melanoma since several antigens expressed by melanoma cells have been identified. Two that have been investigated are the P-97 antigen (70) and the high molecular weight melanoma-associated antigen (HMWMAA) (71). Both antigens are expressed by most melanomas and several MAbs and their fragments have been employed for imaging.

Early imaging studies using I-131 labeled anti-P-97 antibody (MAb 96.5) demonstrated localization in 88% of metastatic lesions although high blood background and liver uptake degraded the images (72). A chelated form of MAb 96.5 labeled with In-111 was used in a multicenter dose-ranging clinical trial (73). Higher tumor-to-normal tissue ratios improved the images and provided localization of 81-83% of metastatic lesions. A Phase III clinical trial reported that the major difficulty with the In-111 MAb 96.5 was the poor detection rate of micrometastatic lesions (74).

Clinical studies involving radiolabeled antibody to the HMWMAA provided similar results (75) with I-123, Tc-99m, and In-111 labeled $F(ab')_2$ fragments of the MAb 225.28S having similar detection rates regardless of the radiolabel (76).

A multicenter study of the $F(ab')_2$ fragment of MAb 225.28S provided better detection rates with Tc-99m compared to In-111 (77). Fab fragments of this MAb radiolabeled with Tc-99m are under investigation to evaluate the effect of further fragmentation on lesion detection (78,79).

Imaging Other Cancers

A number of clinical studies have demonstrated the potential of radioimmunodiagnosis for detection of other cancers. Investigators reported that In-111 was the preferred radionuclide in the radioimmunoimaging of nodal as well as cutaneous sites of lymphomas (80). Further studies indicated that changing the route of administration to subcutaneous infusions could provide a more efficient localization into lymph node lesions (81). More recently, a Tc-99m labeled Fab' fragment of the LL-2 MAb has been successful in imaging 29 of 48 tumor sites in patients with non-Hodgkins lymphoma (82).

Because of the ease and unmatched exquisite detail in which CT and MRI detect brain lesions, there has been little activity in radioimmunodetection of lesions in this organ. However, the potential of radioimmunotherapy of brain tumors, which generally have a poor prognosis with conventional therapy, has led to some activity involving radiolabeled MAbs for lesion detection (83,84). Specifically, two studies have recently appeared that report promising results for radioimmunodetection of neuroblastomas (85) and gliomas (86).

Radiolabeled MAbs have also been used to detect cancer of the prostate (87-89), lung (90-92), and breast (93-96). From these early studies, a number of Tc-99m and In-111 labeled MAbs have been placed into clinical trials for further evaluation of their safety and efficacy.

Non-tumor Radioimmunodetection

The development of radiolabeled MAbs for the detection of noncancerous diseases is proving to be as exciting as the radioimmunodetection of malignant

disorders. An FDA Advisory Panel recently approved a radiolabeled MAb for detection of myocardial infarction after more than a decade of developmental work.

After myocardial injury and cell destruction, the protein substance cardiac myosin becomes extracellularly accessible because of the increased capillary permeability and rupturing of the cardiac muscle cell membrane. This allows access of an injected anti-myosin MAb to the myocardial protein (antigen) which normally is only found intracellularly where the MAb cannot bind to it (97). The cell damage associated with a myocardial infarction leads to the binding of a radiolabeled anti-myosin MAb to the infarcted area creating focal sites of increased activity ("hot spots") in the normal myocardial tissue. Figure 6 illustrates the localization of a myocardial infarction with In-111 anti-myosin MAb. The initial clinical studies completed with In-111 labeled anti-myosin MAbs demonstrated the safety, diagnostic accuracy, and clinical utility of this radioimmunopharmaceutical for identifying the site and extent of myocardial necrosis in patients suspected of having a myocardial infarction. However, other applications include the determination of the size of the infarct and the percentage of myocardium affected, differentiation of a new myocardial infarction from "standard" tissue, and detection of tissue viability or perfusion defects. Fragmentation of this MAb and development of a means for labeling with Tc-99m should enable the circulating radioactivity to be reduced quickly and improved images obtained sooner (98). Two recent publications contain excellent summaries of the abundance of published literature describing the development of radioimmunodetection of myocardial disorders (99,100).

The development of anti-fibrin MAbs that do not bind with fibrinogen has brought new hope to the nuclear imaging of blood clots (101). Specificity for fibrin alone is extremely important since fibrinogen circulates in large quantities and would quickly bind a cross-reacting antibody and degrade the images. Clinical trials involving imaging of both fresh or forming clots as well as aged venous thrombi indicate that In-111 anti-fibrin MAb is highly sensitive for localization of clots even in patients on anticoagulant therapy (102). In-111 labeled anti-fibrin MAbs have also been used successfully to detect large pulmonary emboli (103). Preliminary results from a multicenter clinical trial with a Tc-99m labeled anti-fibrin Fab' fragment in 153 patients with clinical signs and symptoms of deep vein thrombosis indicated an overall diagnostic sensitivity of 80% (104). The sensitivity improved to 94% in patients with proximal (thigh) deep vein thrombosis, a group of patients at greatest risk for development of pulmonary embolism. Over 90% of the patients' images were diagnostic as early as 90 minutes post infusion (105).

The anatomic localization of focal collections of inflammatory cells in the setting of infections or inflammatory states is currently achieved by imaging with Ga-67 gallium citrate, In-111 oxine-labeled autologous leukocytes, or Tc-99m labeled autologous leukocytes, using one of several methods. Direct

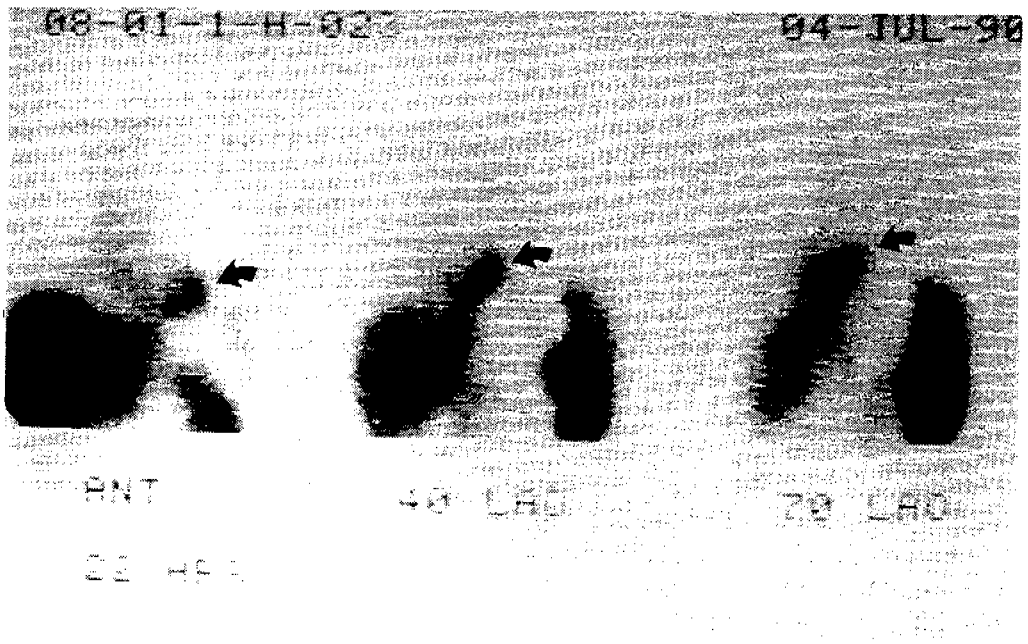

Figure 6. ***Anterior and two left anterior oblique views of large inferior wall myocardial infarction 22 hours after infusion of In-111 anti-myosin MAb. Images provided by McNeil Pharmaceutical.***

administration of radioimmunodetection agents such as anti-granulocyte MAbs or non-specific IgGs which localize at inflammatory sites may prove to be a widely available diagnostic test for the localization of sites of infections or abscesses (106).

MAbs that strongly bind to leukocytes have been shown to be reliable in the detection of sites of inflammation (107). The immunologic labeling of cells with In-111 will bring a fresh approach to the studies which currently are conducted using In-111 oxine and involve lengthy, complicated cell-labeling techniques. The oxine indiscriminately radiolabels all blood cells as well as transfers the radionuclide to plasma proteins. This creates the need for potentially cell-damaging separation procedures prior to the addition of radioactivity to the cells. MAbs which bind exclusively to surface receptors on leukocytes would allow the intravenous administration of the Tc-99m or In-111 leukocyte-specific MAb with earlier imaging times following administration (108).

Nonspecific polyclonal IgG, prepared from pooled human serum gamma globulin and labeled with In-111, has been reported to be equivalent to radiolabeled antigen-specific MAb in the localization of focal infection or inflammation during the first 24 hours after injection. Early clinical studies have been completed using a common kit form for the preparation of the In-111 IgG which is then administered as a single intravenous infusion. No patient blood withdrawals, handling of hazardous biological specimens, or complex, time-consuming laboratory procedures are needed. Pre-clinical work (109,110) and early clinical results (111,112) indicate In-111 IgG may be superior to the currently-used agents, Ga-67 gallium citrate and In-111 oxine-labeled white blood cells. Sensitivities as high as 92% and specificities up to 100% were reported with whole body images similar to those seen with In-111 oxine-labeled autologous leukocytes (113). Because many lesions were detected as early as six hours after infusion, infectious imaging using Tc-99m IgG rather than the longer half-life In-111 IgG may be possible. Initial studies of Tc-99m labeled polyclonal human IgG in mice have shown good localization in a thigh infection induced by *S. aureus* (114).

RADIOIMMUNOTHERAPY

Life-saving advances in the treatment of cancers have been made over the past five decades. Many investigators from a variety of medical disciplines contributed to improvements in the traditional three-prong approach to cancer treatment including surgery, radiation oncology, and chemotherapy.

Theoretically, solitary cancers are "curable" by surgical removal. Assuming the tumor is in an anatomical location that renders it resectable, the surgical procedure is complete and leaves no residual disease, and there are no "unknown" lesions or occult disease, the patient should do well after a recovery period.

For patients with certain tumors that cannot be removed surgically, radiation oncology (with carefully-designed treatment plans involving external beam and/or intra-tumor implants) has greatly improved survival. Radiation therapy is also being used both pre- and post-surgery to help improve survival in those patients with local spread of the cancer. Irradiation is used to reduce the size of colon and rectal masses prior to surgical removal and exploration. After surgery, local irradiation of metastatic sites or distant single metastatic lesions not in the surgical field is common.

Patients with disseminated metastatic cancers or carcinomatosis require systemic treatment with chemical agents. Although oncologic agents are toxic to normal cells as well as cancer cells, chemotherapy is currently the only systemically effective treatment for cancer.

The biotechnology explosion occurring in medicine has lead to benefits for each of these treatment regimens. MAbs directed towards tumor-associated antigens has allowed the medical practitioner to use a characteristic of the tumor cell to improve the specificity and sensitivity of therapy. MAbs may be used directly in immuno-therapy of cancer, but they show much promise as carriers of radionuclides, toxins and oncologic pharmaceuticals to desired diseased tissue.

An area in which radiolabeled MAbs have already proven to significantly improve cancer therapy is in radioimmunoguided surgery (RIGS™) (115). In this procedure, the patient receives an injection of an I-125 labeled MAb which binds to the tumor and creates a signal generator. At the time of surgery, an intraoperative gamma-detecting probe is used to help the surgeon do a more complete resection. The instrument is used to help localize diseased tissue for removal, evaluate the surrounding organs for metastatic involvement, define the margins for resection, and scan the resected area for residual cancer tissue. In some instances, the detector is used by the surgical pathologist to direct the dissection of tissues during the histopathologic diagnostic process.

Generally speaking, chemotherapy agents have a low therapeutic index. The many adverse effects suffered by cancer patients due to the system-wide toxic effect on normal cells often leads to therapeutic failure. By using a drug-MAb combination to achieve a direct delivery of the oncologic agent specifically to the cancer cell, the amount of drug being delivered to the tumor cells increases while that going to normal cells is lowered. In this way, the therapeutic index may be improved if more drug reaches the tumor mass while at the same time sparing normal tissues associated with the dose-limiting toxicity problems.

Investigators have covalently linked cytotoxins (such as ricin) or chemotherapy agents (such as methotrexate) to the MAb, creating compounds called immunoconjugates. This drug-MAb immunoconjugate method of drug delivery is being investigated as a therapeutic approach for cancer treatment (116-121).

The selective delivery of drug-MAb immunoconjugate to tumors relies on the binding of the hypervariable region of the MAb to tumor-associated antigens formed on the surface of tumor cells but not on normal cells. For successful delivery of the cytotoxic agent, the chemical reaction associated with production of a drug-MAb immunoconjugate must not lead to a loss of cytotoxic action or loss of immunoreactivity of the MAb. In addition, studies have shown that internalization of immunoconjugates, or at least the drug or toxin, is necessary for efficient cell killing.

Early investigations of the use of antibodies to treat disease involved the administration of antibody alone, and demonstrated a therapeutic effect in both animal tumor models and in humans (122). Currently, there are a number of unmodified antibodies available or under review by the FDA that are used to treat life-threatening non-malignant disorders such as septic shock (123).

In an effort to increase the cytotoxic effects of MAbs, the idea of using antibodies as carriers of therapeutic dosages of radioactivity was introduced (124). The use of radiolabeled MAbs, as in the case of immunoconjugates, was seen as a method of improving the potency of MAbs in eliminating tumor cells. In theory, the coupling of a radiation emitter to the MAb would make the radionuclide-MAb complex intrinsically toxic to tumor cells while protecting normal organs and tissues which did not have the target antigens on their cell surface.

Radionuclides are available which emit different forms of radiation with different ranges of action. This allows the selection of a radionuclide for treatment of a tumor with specific size, type, and anatomic location. The goal is to use a radionuclide-MAb combination that delivers cytotoxic dosages of radiation to the tumor while minimizing the irradiation of normal tissue surrounding the tumor (40,125,126).

The radionuclide-MAb complex can lead to destruction of tumor cells by simply binding to the cell or even getting nearby the cell. This is in contrast to the use of immunoconjugates of chemotherapy agents or cytotoxins which is dependent upon internalization by each individual target cell for its killing effect (127).

The most common use of a radionuclide for therapeutic procedures involves the use of I-131 for treatment of benign and malignant disorders of the thyroid. It is interesting to note that the majority of radioimmunotherapeutic trials in animals and humans have also used this radionuclide, presumably due to the availability and well-developed chemistry of radioiodination of proteins. I-131 may prove adequate for some radioimmunotherapy applications. However, because of the problems associated with the nuclear properties of this radionuclide, including its relatively long half-life, gamma photon emissions, and low energy beta emission, other radionuclides may prove more useful for therapeutic applications of antibodies. In his review of radionuclide therapy, Karesh provides several characteristics of ideal therapeutic radiopharmaceuticals which also apply to the newer radioimmunotherapeutic agents currently under development (128). Table 3 summarizes these characteristics.

Table 3. Ideal Characteristics of Therapeutic Radiopharmaceuticals

1.	A pure negatron emitter - provides high linear energy transfer (LET) - decreases radiation dose to adjacent normal tissue
2.	Medium/high energy particle - energies greater than 1 MeV
3.	Moderately long effective half-life - typically in days - easy to control dosimetry
4.	High target:nontarget ratio - decreases exposure of normal tissues - provides selective delivery of radiation
5.	Readily available and inexpensive - increases utilization at many institutions
6.	Minimal radiation dose to personnel - easy to control with negatron emitters

The first clinical trials of radioimmunotherapy were reported over a decade ago and used I-131 labeled anti-ferritin and anti-CEA IgGs along with brachytherapy and chemotherapy to obtain encouraging results in a group of patients with primary hepatic carcinomas (129). This group initially reported a response in six of nine patients who received from 37 to 157 mCi of I-131 labeled polyclonal anti-ferritin or anti-CEA IgG. A follow-up report showed even more encouraging numbers with 11 of 28 hepatoma patients in remission, four of six biliary cancer patients in remission and four of five non-oat cell lung cancer patients with tumor regression (130). Currently, this group is reporting a response rate of 48% (50/105) in hepatoma patients using I-131 anti-ferritin antibody (131).

A clinical trial of the treatment of melanoma using cumulative dosages from 137 to 870 mCi of I-131 labeled anti-p-97 MAb was reported by Carrasquillo et al. (132). Only transient, partial tumor regression was

seen in this disseminated cancer with bone marrow toxicity causing hematological effects limiting the administered dosage. Myelosuppression and hematologic toxicity have been correlated with the dosage of radioactivity administered. Irradiation of the patient's bone marrow by high levels of circulating I-131 radioactivity is the major obstacle to overcome with this form of radioimmunotherapy (133).

Epenetos et al. entered over 100 ovarian cancer patients into an I-131 labeled anti-HMFG MAb therapy trial where the administration of the radiolabeled MAb was by intraperitoneal intracavitary instillation rather than intravenous injection (134). Administration of up to 200 mCi of I-131 anti-HMFG MAb has led to partial and complete remission in 20 of 80 patients with an overall reduction in bone marrow toxicity side effect in the group as a whole.

I-131 labeled antibodies have undergone clinical trials for the treatment of hematological cancers such as T-cell lymphoma (135) and B-cell lymphoma (136) with both studies reporting 100% response rates in six and 28 patients, respectively. Radiolabeled antibodies should prove to be a significant advancement in the therapy of these disseminated cancers which have no solid tumors amenable to surgical excision or brachytherapy.

Treatment of brain gliomas has also been attempted using I-131 labeled MAbs which recognize and bind to epidermal growth factor receptor (EGFr) (86,137). The investigators used dosages from 40 to 140 mCi either intravenously or intraarterially, and reported partial responses in six patients and a complete remission in one patient out of a total of ten treated.

Clinical investigations of radioimmunotherapy have been limited to the use of negatron-emitting radionuclides. Early trials used I-131 which, due to its gamma emissions, allowed whole body imaging after administration to determine biodistribution patterns, clearance modes and rates, and estimates of dosimetry. I-131 was a good radionuclide to begin clinical radioimmunotherapy studies, providing encouraging results in some cancers, and it still may find a place in clinical practice. However, it is clear that for some applications, a higher therapeutic index is possible by simply radiolabeling the antibody with a different radionuclide (138). The choice of radionuclide and radiolabeling technique used to form the radionuclide-MAb complex drastically alters a number of properties of the labeled MAb for therapy. Both *in vitro* and *in vivo* drug stability can be altered as well as the complex's pharmacokinetics. The chemical properties of the radionuclide must allow the synthesis of a product that is stable *in vivo* and affords good biodistribution properties. Ongoing investigations are studying the radiolabeling of whole, intact MAbs and MAb fragments with potent negatron emitters as well as alpha emitters (139). These efforts are directed toward increasing the success rate of radionuclide-MAb complexes while reducing the radiation risk to the patient.

Table 4 provides a list of a number of radionuclides that have been proposed for use in radioimmunotherapy. Of those listed, yttrium-90 (140-142), iodine-125 (137), iodine-131 (135), and rhenium-186 (143) have provided encouraging results in clinical investigations.

Table 4. Radionuclides For Radioimmunotherapy

Radionuclide	*Half-life (hrs)*	*Decay emissions (energy in keV)*
Iodine-131	192.9	beta (600), gamma (364)
Yttrium-90	64	beta (2779)
Rhenium-186	84	beta (1100), gamma (123-774)
Rhenium-188	17	beta (2116), gamma (155-2021)
Iodine-125	1445	gamma (27,35), Auger electrons

Although the introduction of new radionuclides promises to improve the therapeutic index currently obtained with radiolabeled MAbs, other factors which deal with the pharmacological properties of these agents could be exploited to help improve the delivery of cytotoxic dosages to tumor cells. A number of these factors are identical to those under consideration for radioimmunodetection. An approach that would greatly increase the clearance of labeled antibodies from the normal tissue is the use of MAb fragments instead of intact MAbs. Recombinant DNA technology that allows further modification of MAbs, and the use of chimeric molecules, human MAbs, small peptides of the variable regions of MAbs, and even molecular recognition units holds much promise for improving radioimmunotherapy. Utilization of a different route of administration such as intracavitary, intraarterial, or intralymphatic injection may lead to faster and higher tumor localization.

A number of excellent reviews which discuss the use of radiolabeled MAbs for therapy are available for further study (122,126,131,144-149).

RADIOLABELING OF ANTIBODIES

Methods for the preparation of radiolabeled MAbs for both diagnostic and therapeutic use must produce an

in vitro and *in vivo* stable bond between the radionuclide and the protein molecule at a site that does not interfere with antigen binding. The radiolabeling procedure should be easily adaptable to a reagent kit method, require few manipulations, and provide a high specific activity radiolabeled MAb with identical immunologic and pharmacokinetic properties as the unlabeled ("native") MAb. The goal of the developers of radiopharmaceutical kits for the preparation of radiolabeled MAbs for use in nuclear medicine has been the manufacture of simple reagent kits. These kits require little hands-on preparation time, involving those activities which the nuclear pharmacist (who is responsible for the daily preparation, quality assessment, and end use of radiopharmaceuticals in the clinical setting) conducts routinely.

An additional consideration for the radiolabeling of MAbs is the suitability of the radionuclide for the clinical use (therapy or diagnosis), with physical properties, availability, and cost of the radionuclide being very important. New chemical linking agents and improved radiolabeling methods have enabled several stable radionuclide-MAb combinations to be developed. Because each MAb and its fragments are affected differently by radiolabeling techniques, careful *in vitro* and *in vivo* quality assessment procedures must be completed on each radionuclide-MAb combination to be certain the immunoreactivity, radiochemical purity, and pharmacokinetics are acceptable.

Radionuclides for Diagnosis

Selection of a radionuclide for labeling MAbs depends on the clinical use. When the radiolabeled MAb is to be used for detection, staging of disease, or monitoring response to therapy, a radionuclide with different characteristics will be selected from one that would be used for radioimmunotherapy. Radionuclides which decay with the emission of gamma photons without particulate radiation emissions are preferred in radioimmunodetection. For external imaging with instrumentation commonly found in most nuclear medicine departments, gamma emissions of 100-200 keV energy range are ideal. Along with the types and energies of emissions, another important consideration is the half-life of the radionuclide. Whole, intact IgG MAbs (which may require longer clearance times prior to imaging) should be radiolabeled with long half-life radionuclides such as In-111 or I-131 (150). MAb fragments which have faster clearance times from the blood may be radiolabeled with short half-life radionuclides such as Tc-99m or I-123. Presently, there appears to be no optimal form of the MAb, whole or fragment, for all diagnostic applications. The goal is to choose the most appropriate form of the MAb and the radionuclide for the disease state being evaluated.

Radionuclides for Therapy

Radionuclides decay by one or more of five different modes. Those radionuclides that decay with the emission of alpha or beta particles are useful for radioimmunotherapy because of the high radiation absorbed dose which they impart to the tissue of localization during decay. Although nuclear particle decay is detrimental in radioimmunodetection and provides no diagnostic information, it is useful for radioimmunotherapy. In order to be useful for radioimmunotherapy with radiolabeled MAbs, a radionuclide should have a physical half-life of one to three days, energy deposition in tissue of 0.5-30 cell diameters, and a simple decay scheme to ground state (151).

Radioiodination Labeling

Early animal testing and clinical studies involved MAbs labeled with radionuclides of iodine. The primary reasons for this include the ready availability and low cost of I-125 and I-131, along with the simple methods for radioiodination of proteins developed through the years. Several useful and effective radiochemical procedures, which involve a simple oxidation of the radioiodine from its chemically stable, reduced form, to the reactive oxidized form which directly substitutes onto tyrosyl residues of the protein structure, have been developed (152). The IODO-GEN™ method (153), which mediates rapid iodination in the solid phase with aqueous solutions of proteins, has become popular for radioiodination of MAbs due to its high yields and minimal protein damage (154).

However, new radioiodinating agents have been synthesized that radioiodinate proteins under mild conditions and form stable bonds that resist *in vivo* deiodination which can lead to high accumulation of radioiodine in the thyroid and other tissues (155). Any radionuclide of iodine can be attached to MAbs and their fragments using these methods.

Radiometal Labeling

Unlike radioiodine, the radioactive metals commonly used in nuclear medicine imaging do not bind directly to any complex organic molecule. This inability to form a strong, stable bond directly with MAbs using radionuclides such as technetium-99m, indium-111, yttrium-90 and rhenium-186 led to the use of bifunctional chelating agents as radionuclide-MAb linkers. Strong chelates such as diethylene triamine penta-acetic acid (DTPA) and ethylene diamine tetra-acetic acid (EDTA) (which, when coupled to the protein serve as a point of attachment for the radioactive metal) were used in order to exploit the superior physical properties of the radiometals for both imaging and therapy (156-161). The chelating agents DTPA and EDTA bind radiometals in a weak manner when compared with derivatives of the two and other

bifunctional chelates. Work which concentrated on the DTPA moiety eventually led to the use of activated derivatives including the mixed anhydride (162) and the cyclic anhydride (163). Attachment of the DTPA chelate structure is thought to occur at lysine residues throughout the protein molecule in a random fashion much like that seen with radioiodination labeling of proteins. Attachment of the chelate to the MAb molecule, then adding the radioactive metal in a form which quickly transfers the metal to the chelating group, results in a stable product with minimal loss of radionuclide *in vivo*. However, the placement of more than one chelate per MAb can interfere with antigen recognition and localization.

A more controlled, site-specific, covalent attachment of many chelate molecules on the Fc portion of the MAb structure was developed in an effort to increase the number of radioactive atoms that could be bound to the protein (specific activity), and to preserve the immunoreactivity of the MAb by keeping the chelate-radiometal combination at a distance from the antigen binding site of the MAb (164). When compared to the random placement of the chelate-radiometal complex, this method of site-specific radiolabeling through the use of a linker-chelate structure allows the stable attachment of the chelate-radiometal far removed from the antigen binding end of the MAb. In addition, the ability to attach the chelate to the MAb molecule and radiolabel at some later time enables the radiopharmaceutical kit technology, common with Tc-99m agents, to be used with radiolabeled antibodies. Currently, a number of In-111 labeled MAbs for imaging malignant and non-malignant disorders are under consideration for approval by the U.S. FDA.

The nuclear properties of Tc-99m has made this radiometal the most commonly used radionuclide in diagnostic imaging. Since its introduction over 25 years ago, a number of techniques have been described for radiolabeling proteins with Tc-99m. These methods are divided into two different strategies: indirect labeling methods and direct labeling methods. Indirect methodologies are similar to those described for labeling with In-111 in that chelating groups are first attached to the MAb structure followed by radiolabeling of the exposed chelates with reduced Tc-99m. The direct methods are unique since they attempt to stably attach Tc-99m directly to the protein structure via some exposed groups native to the protein. Various reducing agents, changes in pH, buffers, and other special conditions have been reported for the direct incorporation of Tc-99m into proteins (165). Early reports on Tc-99m labeled MAbs and their fragments indicated that the bonds formed were not stable, resulting in extensive liver accumulation (probably colloidal Tc-99m), and thyroid, stomach and intestinal uptake (probably due to the presence of free radiopertechnetate).

Several methods have been developed which use mild reducing agents at much lower concentrations to protect the protein structure and present the Tc-99m to the antibody in a reduced form weakly bound to a complex that readily tranfers the Tc-99m to sulfhydryl sites on the MAb molecule. This technique appears to increase the stability of the Tc-99m MAb bond by decreasing the non-sulfhydryl group binding of the radiometal (166).

Due to the short half-life of Tc-99m, imaging procedures need to be completed soon after administration in order to have the high count rates that produce quality images. This has led to the use of antibody fragments with Tc-99m because of the faster blood clearance of the fragments compared with the whole IgG molecule. The short physical half-life of Tc-99m and the short biological half-life of the radiolabeled antibody fragments does not permit imaging much later than 24 hours after infusion. However, the low radiation absorbed dose per mCi does allow the use of high administered dosages ranging from 20-30 mCi. A procedure for radiolabeling FAb' fragments of antifibrin antibodies that utilizes the mild reducing agent dithiothreitol and a glucose-like molecule as the transchelation agent has been used to produce a radioimmunodiagnostic agent for the imaging of forming clots (167).

The methods developed for direct labeling with Tc-99m are readily amenable to kit formulation which requires only a few minutes for incorporation of the Tc-99m into the antibody structure. Good specific activities and complete incorporation of the radionuclide without the need for post-labeling purification have made the use of Tc-99m labeled antibodies a reality. Clinically-useful images have been obtained during a number of investigational studies employing radiopharmaceutical kits for the formation of Tc-99m labeled antibodies to a variety of different antigens.

Radiolabeling of antibodies using indirect methods first employed the "workhorse" chelator DTPA (168). Although a large number of chelating groups were positioned on the MAb structure for binding of the Tc-99m, the reduced Tc-99m apparently formed weak bonds with other sites on the protein structure which led to high levels of unbound Tc-99m *in vivo*. Other chelate moieties were studied for their ability to more preferentially and more stably bind Tc-99m and similar radiometals (169,170). One indirect method that eventually led to a product stable enough for clinical investigation utilizes the diamide dimercaptide (N_2S_2) chelator that is first radiolabeled with reduced Tc-99m, then conjugated to the protein molecule (171). Early clinical trials of an Fab fragment of an antimelanoma antibody radiolabeled with Tc-99m using this technology produced images with extensive kidney, urinary bladder,

blood pool, testes, and gastrointestinal tract localization, requiring the use of a carthartic prior to imaging. Indirect methods of radiolabeling with Tc-99m are more complex, require extensive handling procedures, include a purification step, and lead to greater instability problems when compared with direct methods. Direct methods have led to *in vitro* and *in vivo* stable products with rapid and simple procedures similar to commercially-available Tc-99m kits, making radioimmunoimaging with Tc-99m invaluable in the clinical setting (44,172,173).

QUALITY ASSESSMENT OF RADIOLABELED ANTIBODIES

As in the case of other radiopharmaceuticals, radiolabeled antibodies must be tested prior to use in patients to be certain of the radiochemical purity, sterility, freedom from pyrogens, and immunoreactivity (32). Certain of these tests are completed to assure that the radiolabeling procedure has not destroyed the ability of the antibody to recognize and bind to the target antigen after infusion.

Radiochemical purity is a measure of the quantity of the radioactivity in the preparation in the desired chemical form, whole IgG or antibody fragments. Impurities arise from reaction conditions, inadequate binding of the radionuclide to the protein structure, failure of final purification methods, and decomposition due to improper storage conditions. Radiochemical impurities may exist as the unbound radionuclide, radioactive protein aggregates, and/or radionuclide-chelate in those preparations that use a chelate to bind the radionuclide to the antibody. Radiochemical impurities lead to degraded images if infused in significant quantities. A number of techniques common to nuclear pharmacy practice can be used to quickly determine the percentage of radionuclide bound to protein. Instant thin layer, thin layer, and paper chromatography, using various solvents, have been used to separate the free radionuclide from the protein-bound component. Trichloroacetic acid precipitation techniques have also been used to separate radiolabeled protein from unbound radionuclide. High performance liquid chromatography provides high resolution of radiolabeled protein components into aggregates, IgG fragments, as well as the unbound radionuclide. However, the extensive investment of equipment and time relegates this radiochemical purity determination to developmental stages of radiolabeled antibodies.

The USP sterility test, with appropriate adjustments for small volume radioactive parenteral products, is used to monitor the sterile techniques employed in the production stages. In addition, bacterial endotoxin levels of the finished product are determined prior to infusion. Immunoreactivity (the ability of the antibody to recognize and bind a specific antigen) can be changed during the radiolabeling methods used for incorporation of radionuclides into the IgG or fragment structure. Although the native antibody may be shown to bind an antigen during preliminary *in vitro* testing, structural changes in the radiolabeled antibody can occur at or near the antigen-binding region which can lead to decreased or complete loss of the ability to bind to antigen. Immunoreactivity of the radiolabeled antibody is tested to prove that the radiolabeling, purification, testing, and storage, did not decrease the antibody-antigen avidity. Immunoreactivity can be tested using cultures of live tumor cells which express the specific antigen. A small amount of the radiolabeled antibody is incubated with a known quantity of cells in suspension, and a centrifugation technique is used to pellet the cells. The pellet and supernate are then counted to determine the percentage of added radioactivity bound to the cells. Modifications to this procedure include using only the tumor cell extract containing the antigen or purified specific antigen irreversibly bound to a solid support in place of the live tumor cells which are difficult to maintain in culture (174). Determination of the percentage of added radiolabeled antibody that is bound after an incubation period provides an estimate of the immunoreactivity.

During the early development of the radiolabeling procedures leading to a radioimmunopharmaceutical for diagnosis or therapy, extensive quality assessment procedures are important to insure that a sterile, apyrogenic, radiochemically pure, immunoreactive product is produced for maximum patient efficacy. These tests are also important when determining the stability of the finished product and the shelf-life of the radiolabeled drug, or the nonradioactive kit components used to produce the radiopharmaceutical. As the product moves into the clinical trials investigational stage, and the pre-production for commercialization stage, records of these tests (which prove that a pure, sterile, apyrogenic, and immunoreactive product is formulated consistently in-house) should justify that the quality assessment testing be simplified to a quick determination of the percentage of bound and unbound radionuclide in the product just prior to patient administration.

SUMMARY

Monoclonal antibody technology involves the generation of a specific cell line capable of producing unlimited quantities of an antibody with affinity and specificity for a particular tumor-associated or other type of antigen. The availability of hybridoma cell lines, which ensures adequate levels of MAbs, has enabled researchers to conduct animal and clinical studies on a large number of MAbs for both diagnostic and therapeutic uses. These studies have proven the ability of

radiolabeled MAbs to seek out and bind to a specific target antigen which provides the specificity needed to effect "targeting" of radionuclides for nuclear medicine imaging and therapy.

Clinical use of these radiolabeled MAbs requires an understanding of many biological, chemical, and technical factors. The nuclear pharmacist, who is well-trained in the use of radiolabeled drugs for diagnosis and therapy, also has the basic knowledge of microbiology needed to understand antibody production in ascites fluid and bioreactors, the background in protein chemistry to grasp the process of antibody fragmentation, and the understanding of pharmacology to help interpret the changes in biodistribution, pharmacokinetics, and localizing properties associated with the use of smaller protein units.

Even after the commercialization of the first FDA-approved radiolabeled MAbs, continued development of radioimmunopharmaceuticals for diagnosis and therapy will occur with the movement of the investigative products into the clinical setting. Improvement of radiolabeling methods for the placement of the radionuclide on the antibody molecule in a stable, immunoreactive form without compromising the quality of the final product will provide high specific activity carriers with ideal properties for localization. The nuclear pharmacist plays an instrumental role in this development through the use of radiolabeling techniques and quality assessment methods to insure that the patient receives a sterile, apyrogenic, radiochemically pure, immunoreactive product which affords maximum benefits.

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QUESTIONS

1. Which of the following classes of human immunoglobulin is the principle class of antibody found in the blood and the type that composes monoclonal antibodies used for clinical studies?

   A. IgE
   B. IgM
   C. IgG
   D. IgB

2. Which of the following is the intricate, characteristic shape found on an antigen that provides the specific binding affinity of an antibody?

   A. amino acid sequence
   B. carbohydrate chain
   C. epitope
   D. immunogen

3. Which of the following is the tumor-associated antigen found on ovarian and colorectal cancers that is recognized by the B72.3 MAb?

   A. CEA
   B. 17-1A
   C. CA-125
   D. TAG-72

4. Which of the following regions of the antibody structure contains the areas associated with antigen binding?

   A. V_H and V_L
   B. CH_1 and CH_2
   C. C_H and C_L
   D. C_H and V_H

5. Which of the following antibody fragments results from the chemical dissection of the intact IgG mole-cule with the sulfhydryl protease enzyme, papain?

   A. one $F(ab')_2$ and one Fc
   B. two Fab and one Fc
   C. two Fab and two Fc
   D. one $F(ab')_2$ and one Fab

6. Which of the following statements concerning antibody fragments is false?

   A. Fragments have faster blood clearance half-times compared with the intact IgG molecule.
   B. Fragments have faster elimination rates.
   C. Fragments have reduced nonspecific binding properties.
   D. Fragments are eliminated primarily through the gastrointestinal tract.

7. Which of the following statements concerning "second-generation" monoclonal antibodies is false?

   A. They have higher affinity and improved ability to bind the target antigen.
   B. They recognize a different target antigen compared with their "first generation" counterpart.
   C. They are made using purified target antigen as the immunogen.
   D. Only one "second generation" monoclonal antibody can be produced for a single target antigen.

8. Which of the following is the primary goal in building chimeric antibodies?

   A. increase specificity of antigen recognition
   B. reduce human anti-mouse antibody (HAMA) response
   C. delay non-specific localization by liver
   D. increase stability of Ab-Ag binding.

9. Which of the following is the smallest of the "designer" molecules proposed for radioimmunodetection?

   A. chimeric antibodies
   B. molecular recognition units (MRUs)
   C. humanized antibodies
   D. single chain antigen binding proteins (SCABPs)

10. Which of the following radionuclides has not been used for clinical studies involving radioimmunodetection?

A. I-131
B. In-111
C. Y-90
D. Tc-99m

11. To insure the least amount of interference at the antigen-binding site of an antibody, which of the following methods for radiolabeling should be used?

A. radioiodination of MAb
B. mixed anhydride of DTPA attachment of chelate to MAb
C. linker-chelate attachment to MAb
D. N_2S_2 chelator attachment to MAb

12. Early radioimmunodetection studies were directed towards the imaging of cancer of the GI tract using radiolabeled antibodies to the _____ antigen.

A. human milk fat globulin (HMFG)
B. CA-125
C. carcinoembryonic (CEA)
D. tumor associated glycoprotein-72 (TAG 72)

13. Recent clinical investigation of the ability of radioimmunodetection to localize disease has led to detection rates near

A. 30%
B. 50%
C. 80%
D. 100%

14. Which of the following radionuclides has not been used for investigation of radioimmunotherapy?

A. Tc-99m
B. I-131
C. Y-90
D. I-125

15. In-111 labeled MAb's localize non-specifically in which of the following?

A. heart
B. kidneys
C. lungs
D. liver

16. Which of the following is a pancarcinoma antibody that binds to mucin-producing tumors of the colon, breast, ovary, pancreas and stomach?

A. MAb 17-1A
B. MAb B72.3
C. MAb OC-125
D. MAb CA19-9

17. Which of the following is the primary reason In-111 anti-myosin MAb specifically localizes in myocardial infarctions?

A. The MAb localizes in areas where high levels of calcium occur.
B. The MAb localizes in areas of inflammation due to attraction to leukocytes.
C. The large MAb protein molecule leaks from the myocardial circulation into the infarcted area.
D. After myocardial cell destruction, the antigenic substance, cardiac myosin, becomes extracellularly accessible to the MAb.

18. Which of the following antigenic substances is available for MAb binding and has been used to facilitate radioimmunodetection of venous thrombi?

A. thrombin
B. fibrin
C. transferrin
D. hemoglobin

19. Which of the following radiolabeled antibodies has been reported to be equivalent to radiolabeled antigen-specific MAb in the radioimmunodetection of focal infection or inflammation?

A. In-111 polyclonal IgG
B. Tc-99m NR-LU 10
C. In-111 ZCE-025
D. In-111 CYT-103

20. In-111 antibody for localization of sites of infection or abscess would have which of the following advantages when compared with the current practice of radiolabeling autologous leukocytes with In-111 oxine?

A. no patient blood withdrawals
B. no handling of hazardous biological specimens
C. no time-consuming manipulations involved with radiolabeling
D. all of the above

21. Which of the following is an advantage that radio-labeled MAbs would have over ricin-MAb immuno-conjugate when considered for cancer therapy?

A. The radionuclide-MAb complex can destroy tumor cells by binding to the cell or even getting nearby the cell, whereas ricin must be internalized by the cell for its killing effect.
B. Radionuclide-MAb complexes are more stable than toxin-MAb immunoconjugates and therefore deliver more tumoricidal agent at the cells.
C. The radionuclide-MAb complex only localizes at or near the tumor site and does not distribute systemically into normal tissues like the toxin-MAb immunoconjugates.
D. Radionuclide-MAb complexes would have a broader range of cancer types for therapeutic affect than the toxin-MAb immunoconjugates

22. Which of the following methods of radioiodination has become popular for iodination of MAb's due to its high yields and minimal protein damage?

A. lactoperoxidase enzyme method
B. Chloraminc-T
C. Iodine monocholoride
D. IODOGEN™

23. Which of the following chelates has been used to bind radioactive metals such as In-111, Y-90 and Tc-99m to MAb's in most early clinical investigations of radioimmuno-detection and radioimmunotherapy?

A. EDTA
B. N_2S_2
C. DTPA
D. IDA

24. Which of the following is true concerning the direct method of radiolabeling MAbs and their fragments with Tc-99m?

A. Chelates such as DTPA are first stably bound to the protein structure.
B. Reduction of Tc-99m is not necessary.
C. Tc-99m is stably attached to the protein structure via some exposed groups on the protein.
D. The most stable bond formation has been found with the nitrogen groups of the amines.

25. Which of the following quality assessment tests can be used to determine the percentage of the radionuclide bound to the MAb protein structure?

A. TCA precipitation assay
B. HPLC
C. ITLC-SG chromatography in 85% methanol
D. all of the above