ADJUNCTIVE MEDICAL KNOWLEDGE

Radioimmunodiagnosis and Radioimmunotherapy, 1982

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INTRODUCTION AND CLINICAL EXPERIENCE

The principle and the development of the method. Following the recent developments and advances in immunology, the in vivo application of radiolabeled antibodies is expanding and rapidly changing. Admittedly, routine clinical use has not yet been established, but investigators believe that this field of medical biology holds appreciable promise to provide better diagnostic methods and possibly successful therapeutic applications.

In animals or tissue cultures, antibodies have been produced against specific antigens (extracted from tumors, microorganisms, or normal organs), then labeled with suitable radionuclides and administered to animals or humans bearing the antigenic targets. Such antibodies have been shown to accumulate selectively within their specific targets and have contributed to the location and diagnosis of malignant and nonmalignant lesions (radioimmunodiagnosis). Although still in a preliminary stage of development, antibodies have been used to deliver therapeutic doses of radioactivity, selectively, to malignant tumors (radioimmunotherapy).

Passive immunotherapy was introduced clinically in 1891 by Von Behring (*1*), who developed the first horse diptheria antitoxin. Active immunization to the diptheria toxin replaced passive immunotherapy (*2*), as did the prophylaxis and treatment of tetanus (*3*). Heterologous immunospecific antibodies to pneumococcal antigens were used clinically until the introduction of the sulfa drugs and antibiotics (*4–7*).

The concept of using autologous or heterologous antibodies for cancer diagnosis and therapy has fascinated researchers for nearly 100 years. Attempts to treat cancer by passive specific immunotherapy were reported by Hericourt and Richet in 1895 (*8*) and tumor antigeneity was recognized in 1929 by Witebsky and Hierzfeld (*9–10*). Pressman, and later Bale et al., demonstrated tumor accumulation of heterologous specific antibodies (*11–12*). By means of radioiodinated antibodies, Pressman showed their usefullness for diagnostic purposes, based on tumor detection and location by external imaging (*11*). In 1960 Bale and his coworkers reported that antibodies could carry therapeutic doses of radionuclides to tumors (*12*). Following the pioneering work of these men, clinical application of radioantibodies to tumors, normal organs, and pathologic organisms has been reported by a number of investigators. Diagnostic and therapeutic applications have been investigated. All applications stemmed from the basic property of antibodies, i.e., specificity of localization that is retained even with radiotracer labeling.

A more recent development has been the papain digestion of IgG, which produces certain fragments of the

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immunoglobulin that maintain specificity and eliminate the nonspecific binding (*13*). The smaller molecule of the fragment has a more rapid blood clearance than the whole IgG, which may be important in decreasing possible sensitization of the host. Radiolabeled fragments have recently been used successfully for tumor location (*14*) and myocardial infarct imaging (*15–16*). Therapeutic applications of fragments have been reported for the treatment of patients with digitalis intoxication (*13*). Radiolabeled fragments may be useful to deliver therapeutic doses of radionuclides to malignant tissues, and these investigations are currently under way (*14*).

For the successful clinical use of antibodies (radioimmunodiagnosis and therapy), one requirement is the enrichment and purification of the specific antibody (IgG) from the original crude antiserum by chemical, chromatographic, and/or tissue-absorption techniques. On the other hand, monoclonal antibodies obtained from hybrid cell lines are chemically homogeneous, react with constant avidity to single antigenic determinants, and can be isolated in quantities previously not obtainable by conventional methods (*17–18*). Experimentally, monoclonal antibodies have been used successfully for tumor immunotherapy (*17*) and scintigraphic tumor location (*19*).

As a label for antibodies, iodine-131 has received the most attention for imaging and therapy. Iodination by either the chloramine-T or the lactoperoxidase method has been effective. For most applications other than lymphoscintigraphy, technetium-99m has a rather short half-life. Indium-111 DTPA also appears to be a promising label as suggested by its use for myocardial infarction imaging with antimyosin (*20*).

Tumor markers as related to in vivo applications. The possibility of detecting tumor byproducts (tumor markers) in the body fluids of cancer patients has stimulated significant research effort. In 1965 Gold and Freedman (*21*) reported that carcinoembryonic antigen (CEA) is a marker for cancer of the gastrointestinal tract, and Abelev (*22*) demonstrated that the oncofetal antigen, alphafetoprotein (AFP), is related to the rodent and human hepatoma. It was shown subsequently that CEA is not limited to gastrointestinal cancers and that in fact it is not specific for cancer (*23–24*), but levels of plasma CEA suggest the presence of tumors (*25–27*), and serial plasma measurements of this marker have been useful clinically (*28–31*). Other tumor-associated molecules have been isolated from the plasma of patients with tumors, and the plasma levels may be related to the presence and size of the tumors they characterize. The biologic role of these markers is not clear, other than those tumor products, such as the human hormone, chorionic gonodotropin (hCG), which has been shown to be associated with specific tumors.

Certain physical and biological parameters must be considered in view of their relationship to tumor accretion of antibodies. The combination of the extremely small plasma concentration of administered antibody and the relatively small fraction of blood flow through a tumor relative to total blood volume underscores the low probability of physical contact between antibody and antigen. The location of the antigen is an important controlling factor for the sequestration of the antibody by the tumor. If an antigen is located on a cell surface that is an integral part of a vascular wall, then localization may be markedly facilitated. Otherwise the antibody must gain access to the interstitial fluid of the tumor and randomly interact with the antigen either in the fluid (shedded) or on the cell surface. In some tumors the antigen may not be this accessible but may reside inside the cell. In this case the antibody must penetrate the cell by an appropriate process and then physically interact with the antigen. In this latter situation the probability of effectively localizing sufficient labeled antibody for purposes of external imaging is markedly decreased. If the antibody must be transported through interstitial fluid to make antigen contact, further loss of antibody may be anticipated due to metabolism (32). For most tumors, however, the extravascular space is much greater than that found in normal tissues (*33*).

Originally it was thought that circulating antigen to an intravenously administered antibody would adversely affect the opportunity for an antigen-antibody reaction at the tumor site. With the quantities of antibodies used for clinical studies, however, this has not been the experience (*34–36*).

Clinical experience in diagnostic and therapeutic use of radiolabeled antibodies. Antibodies have been used as carriers of radionuclides and cytotoxic drugs in the diagnosis and treatment of cancer (*37–38*). Organ distribution studies of radiolabeled antibodies were originally performed in laboratory animals by tissue-counting techniques (*39–40*). Subsequently, in vivo imaging was reported in 1959 by Day et al. after the i.v. administration of isolated antifibrin immunoglobulin fractions labeled with I-131 (*41*). Fibrin, a nontumor molecule, served as the target in tumor sites where its presence is due to a concurrent inflammatory process. Fibrin and ferritin (another tumor-associated marker) have been used with partial success to detect tumors in patients (*42–46*). By autoradiographic studies Conteras et al. demonstrated that the distribution of labeled antibodies to fibrin was quite irregular in tumors (*46*). In those areas of the tumor without inflammatory reaction or necrosis, the sequestration of radioactivity was sparse. Attempts to treat cases of advanced human cancer with I-131 antifibrin antibody gave short-term remissions only. An antiglioma antibody of questionable purity has shown only minimal experimental success for the detection of these tumors (*47*).

Heterologous antibodies (from goats or rabbits) to purified or crude carcinoembryonic antigen (CEA) have

been labeled with radio iodines (I-125 and I-131) following chemical or immunochemical purification. After intravenous administration into tumor-bearing animals or patients with tumors (*14,48–54*), these antibodies were found to accumulate specifically in some primary or metastatic tumor sites. Tumors of diverse histologic characteristics—including those of the gastrointestinal, genitourinary, and respiratory systems—have been successfully imaged with antibodies to CEA. The clinical usefulness of this diagnostic method, however, is limited at present because of the disadvantageous target-to-nontarget ratio of the labeled antibody (*54*). In performing the studies it was frequently necessary to apply computer enhancement by subtracting nontarget activity by means of dual-tracer techniques. By this method the sensitivity of the study can be improved by a factor of two to three (*55*).

Other tumor-associated antigens, such as hCG (*34,56*) and AFP (*36,57–58*), have been used to produce specific antibodies. Labeled with I-131, these have been used with some success to locate those tumors that secrete such antigens. Tumor-associated antigens that have not been characterized have also been used to produce heterologous antibodies for imaging. The results have been encouraging in animals with syngeneic or allogeneic tumors (*59–63*) and in humans with renal-cell carcinoma (*60,64–66*), but these are only preliminary experimental results.

Early attempts at passive immunotherapy for cancer treatment were not successful (*8*) and the use of high dosages of I-131 antibody to fibrin did not show a therapeutic effect (*11*). Following animal experimental work with nonradioactive antibodies (*67–69*), Order et al. applied therapeutic doses of heterologous specific radioantibody in humans (*14,70–72*). They have treated patients with primary or metastatic liver tumors with 100 to 150 mCi of I-131 CEA heterologous antibody (*70–72*). It is still too early to evaluate fully the results of their work, which is unique in humans. Isolated attempts at immunotherapy with antibodies to other tumor-associated antigens such as hCG (*56*) or AFP (*58*) have also been reported. Antibody as a carrier of chemotherapeutic agents (cytotoxic drugs) has been studied. It was shown that the drug preferentially affected the target's tumor cells that the antibody (to which the drug was attached) could recognize (*73*). Theoretically a more effective localized radioimmunotherapy can be achieved by neutron activation of boron-labeled antibodies after they are localized within tumors (*74*). Although the usefulness of therapeutic applications of radioantibodies has created great interest, skepticism among scientists still exists, and only a limited number of researchers offer such therapy at present (*14,72*).

In addition to investigations for the diagnostic application of radioantibodies in cancer, their application to nonmalignant diseases has been of interest. Antibodies produced in rabbits after immunization with cysticercus protein have been used for the diagnosis of cysticercosis of the central nervous system and have correctly (although not proven specifically) identified sites of infestation in six of twenty-four suspected patients (*75*). Antibodies against cocci or other bacteria could probably be used for radioimmunodiagnosis (*76*). Acute myocardial infarction can be located using whole or fragmented antibodies to cardiac myosin (*77*). The damaged cellular membranes of the myocardial fibrils permit entrance of radiolabeled heterologous antibodies into the cells and the macromolecules bind specifically to accessible myosin. This permits imaging of acutely infarcted tissue.

Autologous antibodies to normal tissues have been demonstrated in the sera of patients with glomerulonephritis (*78*), acute and chronic active hepatitis (*79*), systemic lupus erythematosus (*80*), and other autoimmune diseases (*81*). Their study has provided useful clinical information for the diagnosis and treatment of autoimmune disease. It is quite understandable that autoantibodies against tumors (*82*) or pathogenic microorganisms obtained from the patient's own serum can be used, following appropriate purification, for radioimmunodiagnosis.

ANTIGENS OF ANTIBODY PRODUCTION: SELECTION AND ISOLATION

Normal proteins as antigens. Normal cellular proteins (including embryonic) or smaller molecules have been used extensively as antigens for in vitro diagnostic tests. For the purpose of radioantibody imaging, only a limited number of normal molecules have been used as the antigen for antibody protection. For example, ferritin, a major storage form of iron, is composed of a shell of protein subunits that surrounds a micelle of ferric hydroxyphosphate (*83–86*). Although the several ferritins occurring in normal tissues differ electrophoretically, normal, embryonic, and neoplastic ferritins have close immunologic properties (*83*). For tumor location in patients, antibodies have been produced to the ferritins associated with Hodgkin's Disease (*88*). Recently, rabbit antibodies to ferritin, labeled with I-131, have been given to patients with hepatic malignancies for therapeutic purposes (*71*). Ferritins have been isolated from Hodgkin's and other lymphoma tissues by the method of Drysdale and Munro (*87*) as modified by Eshkar, Order and Katz (*88*).

Myosin from cardiac muscle is different from other myosins in some of its physicochemical properties. The extraction of cardiac myosin from fresh cardiac muscle has been described in detail by Katz et al. (*89–93*). Localization of cardiac myosin-specific antibody in acute myocardial infarction has been shown (*77*).

Human chorionic gonadotropin (hCG), a placental hormone, is produced and excreted in high concentra-

tions by nonseminomatous testicular trophoblastic neoplasms and several others. Hyperimmune goat antiserum has been prepared by Goldenberg et al. with purified urinary hCG (*34*). In transplants of human choriocarcinoma to hamsters, 2.8 times as much radioantibody to hCG accumulated in the tumors than in the livers of the same animals. In humans, trophoblastic and germinal tumors that secrete hCG have been detected scintigraphically with this labeled antibody (*34*). The hormone can be purified from the urine of pregnant women, and highly purified hCG can be obtained by the technique described by Morgan and Canfield (*94–95*).

Aberrant molecules as antigens. Malignant cells produce aberrant cellular antigens frequently found on the surface of the cell interspersed among normal transplantation antigens (*96*). These antigens are characterized by high specificity for the individual tumor, or for all tumors if caused by the same agent. For example, Burkitt's lymphoma appears to be a human tumor with tumor-specific antigens on the cell membrane. More commonly, tumor-associated antigens are used in experimental and clinical practice, but these antigens may be present in several types of cancers as well as in some normal tissues. Although ferritin, hCG, and other physiologic molecules could be considered in a broad sense as tumor-associated antigens, carcinoembryonic antigen (CEA) is a more classic example. CEA was originally isolated from carcinoma of the colon (*21*) but it occurs also in normal fetal gut and in a variety of gastrointestinal, genitourinary, and pulmonary neoplasms, as well as in some benign gastrointestinal diseases. These antigens may be malignant expressions of latent information that the cell has suppressed from embryonic stages, and they could also be induced by other than malignant pathologic states.

CEA can be extracted from liver metastases from colonic adenocarcinoma obtained at surgery or at autopsy by the method of Newman et al. (*97*). Recent work by Kimball and Brattain (*98*), however, showed that perchloric acid treatment resulted in a significant loss of CEA, as compared with material treated with saline or urea.

Alpha fetoprotein (AFP), a tumor-associated antigen, is found predominantly in hepatocellular carcinoma and certain germ-cell tumors. Nonseminomatous germ-cell testicular tumors frequently produce high levels of AFP as well as elevated levels of hCG in some patients with this kind of tumor (*99*). Radioimmunodetection of tumors producing AFP has been reported in humans (*35,57–58*), and attempts have been made to use AFP antibody for therapeutic purposes (*58*). AFP can be obtained from the blood of human fetuses ranging from 5 to 7 months gestational age (*100*).

Tumor-associated antigens can be isolated by the simplified method described by Order et al. (*67*). Several investigators have used similar methods to produce what they called tumor-specific antigens from variable tumors, but most probably they were tumor-associated antigens. Order et al. (*62,69*) extensively investigated antigens from a syngeneic ovarian carcinoma of the mouse, and achieved successful therapeutic and diagnostic applications with the antibody they produced. We have used this animal tumor model and this antibody to obtain satisfactory scintigrams. Okada et al. (*101*) identified a tumor antigen in the insoluble fraction of human nephroblastoma (Wilms tumor). Belitsky et al. (*64–66*) produced antibodies to an antigen extracted from human renal-cell carcinoma. Schultz et al. worked with a tumor-associated antigen that they found in human pancreatic cancers (*102*).

PREPARATION OF RADIOANTIBODIES AND FRAGMENTS

Antiserum preparation. A suitable quantity of the antigen is combined with complete Freund's adjuvant and injected intradermally in rabbits, goats, or other suitable animals; the work of Order provides sufficient evidence that rabbits respond with satisfactory production of antibody (*62*). The initial immunization requires 100–300 ng of the antigen (100–200 ng CEA, 240 ng ferritin, or 250 ng of the first peak from a Sephadex column for the average tumor extract). Two to four weeks later (*103*) (2 wk for CEA, 4 wk for ferritin and other tumor antigens), a booster dose with the same antigen is injected intradermally (occasionally intraperitoneally), repeating or raising the original dose. One to two weeks later, a test bleed of the rabbit provides the original serum for in vitro testing for antibodies, and if a satisfactory titer is found, regular bleeding is performed. A blood sample is collected, in a pyrogen-free test tube, from each animal demonstrating high antibody titer; it is allowed to clot at room temperature and retract overnight at 4°C, and the serum is separated by centrifugation and tested for sensitivity and specificity for the specific tumor antigen.

From the hyperimmune sera, IgG can be separated either by *recycling affinity chromatography* (*104*) or by *salt precipitation* and dialysis (*88*). Although the first method provides specific antibody, Order has worked satisfactorily with the second (*45*). Our experience has been encouraging in pilot experiments (in mice) with salt precipitation and dialysis.

Fragmentation of IgG. The in vitro cleavage of antibodies into fragments (Fab) has greatly increased our knowledge of antibodies (*13–16*). It has been shown that antigen-binding sites of the antibody lie on the Fab fragments, and the remaining fragment (the *Fc*), while not a binding antigen, has important biologically active sites involved with such processes as complement and macrophage fixation (*105*). Several proteolytic enzymes are used to cleave and digest the Fc fragment while

leaving the two Fab fragments intact and joined (*105–109*). The Fab fragment is a bivalent residue that retains its ability to bind the antigen and yield positive precipitation. The use of fragments instead of IgG would minimize several problems encountered in radioimmunodiagnosis and therapy. The long persistence of whole antibodies in the circulation prolongs the test period and increases radiation of normal tissues. Nonspecific immune complexes produced with whole antibodies may be deposited in nontarget areas, creating nondiagnostic concentrations in images and sometimes releasing vasoactive complement peptides with unwanted reactions. Undesirable immunization of the patient may occur after the use of antibodies if the quantity of administered IgG protein is sufficient, particularly if several repeated innoculations occur. The fragments have a relatively short half-time in the circulation due to rapid elimination by the kidneys, are not antigeneic, do not produce immune complexes, and do not release vasoactive complement peptides. Instead of whole IgG, these fragments may well evolve as the method of choice in radioimmunodiagnosis and radioimmunotherapy. Fragments have been successfully used for tumor location and myocardial imaging (*14,77*).

Purification of the antibody. Antibodies or fragments prepared as described are usually a mixture of the specific and nonspecific antibodies to the antigen used for immunization. The efficacy of target detection depends on the proportion of specific to nonspecific antibody present in the radiolabeled preparation. Since preparations containing a relatively low percentage of specific antibody might not produce an externally detectable level in the target tissue, it is important to purify the antibody, and the method of choice is affinity chromatography (*104*). Investigators at the University of Kentucky (*110,111*) reported that goat CEA antiserum with a radioimmunoassay titer at incubation of 2×10^6 has produced approximately 1.0 mg of affinity-purified antibody per ml of starting antiserum. If cross reaction with normal tissue occurs even after affinity chromatography, or if for other reasons this method cannot be applied, tissue-absorption techniques may be useful. The cross-reacting antibodies are eliminated by passing the preparations through normal tissue cells (erythrocytes, or cells of liver, spleen etc.) (*105*).

Monoclonal antibodies. Conventional immunization of animals with antigens, even specific and highly purified ones, produces a hyperimmune antiserum that contains a spectrum of antibodies with diverse specificities. Additionally there is variability in the affinity and specificity of the different batches of antibody produced in animals. As previously shown by the mass production of antibodies to the several types of pneumococcus bacillus, it is quite possible to produce adequate quantities of antibodies to tumors by the animal immunization technique. Nevertheless, it would be desirable to develop an in vitro method of production that provides greater certainty of mass production, eliminates the tedious steps of purification, and improves control of such parameters as specificity. A possible solution to these problems is the development of monoclonal antibodies. These, derived from lymphocyte-myeloma hybridomas, are homogeneous, require little effort for purification, and can be reproducibly prepared in large quantities. Monoclonal antibodies have been used for external scintigraphy of tumors (*19*), for myocardial imaging (*18*), and for therapy (*17*). The production of such antibodies requires expertise and facilities in tissue culture and hybridoma, identification of the desired antibody, and subsequent isolation of the antibody to obtain useful colonies. Once obtained, however, such colonies can be perpetuated.

Although the concept of using monoclonal antibodies instead of those produced in animals is very inviting, preliminary results have been equivocal (*19*). The tumor concentrations of monoclonal antibodies—i.e., target-to-nontarget ratios—are greater than those of antibodies from animals during the first 24 to 48 hr after administration. After this time, however, the T/NT ratio for monoclonal antibodies may be less. In addition, the quantity of antibody per unit volume of tissue is less than with animal-produced antibodies. The reason for these findings is thought to be the number of receptor sites for the antibody. For example, if a monoclonal CEA antibody has the opportunity of reacting with only one receptor site, A, whereas an animal-produced CEA antibody is a spectrum and can react with receptor sites A, B, and C, then probabilities of antigen-antibody reactions are greater. Background washout with the latter is slower, but after 48 hr the success of in vivo imaging becomes greater with animal-produced antibodies. The proposed solution to these problems is to develop polymonoclonal antibody preparations—i.e., to select several of the antibodies with the highest titers with a tumor-associated "spectrum" antigen such as CEA—and to use such a mixture of antibodies to increase the probability of antibody-antigen reaction. The result should be improved T/NT ratios from increased concentration of antibody in the tumor and more rapid vascular washout. If these speculations prove true, an added benefit will be earlier imaging time following administration of the tracer.

In vitro testing on antisera. Antisera are tested for specificity and titer before use clinically or for investigational purposes. Demonstration by the Ouchterlong technique of a precipitin band against the antigen, and lack of similar activity against normal spleen, liver, and kidney antigens, establishes the specificity of the antiserum (*67–69*). Gel diffusion and isoelectric focusing may be required to evaluate further the specificity of the antiserum and for more precise protein identification (*69*). Immunofluorescence studies can be performed if needed, using cell suspensions from normal organs and

tumors (*67*). If required, cytotoxicity tests can also be performed by means of standard techniques (*43,68,69*). If the antiserum shows lack of specificity and precipitates normal liver, spleen, etc., then further purification may be performed by absorption of the antiserum (*49,67,69*) with a suspension of normal human erythrocytes, viable spleen cells, or liver cells (*105*).

Titer of the antiserum can be determined using a radioimmunoassay method currently available. Antiserum titers for CEA are considered satisfactory if they are ~1 to 2 million (*49*).

Radiolabeling of the antisera. At present, so-called carrier-free iodine-131 is the most commonly used radiolabel for tumor imaging with antibodies, but only about 20% of the iodine is radioactive. The most commonly used methods for labeling are the modified chloramine-T (*112*) or the lactoperoxidase (*113*). Although I-131 has certain dosimetric and instrumental disadvantages, it is nevertheless the preferred tracer for imaging at this time because iodination is a satisfactory method of protein labeling that produces a high specific activity yet retains appreciable (as much as 70%) immunologic reactivity (*114*).

Affinity-purified goat CEA antibody has been labeled with Tc-99m, with stannous tartrate as the reducing agent. The radiochemical yield of labeled antibody ranged from 18–37% and could be increased to 60% by using small amounts of pertechnetate (*115*), with a specific activity of 1–6 μCi per μg protein. Because of its rather short half-life (6 hr) the Tc-99m label might not be suitable for tumor imaging after intravenous administration of the labeled antibodies. On the other hand, since it has no beta emission and the 140-keV gamma is efficient for current imaging equipment, it appears to have excellent potential as a lymphoscintigraphic agent (*116*).

Imaging for myocardial infarction has been performed with antibodies to cardiac myosin labeled with indium-111 diethylenetriaminepentacetic acid (DTPA). Because of the disadvantages of I-131 regarding patient dosimetry and imaging, In-111-labeled antibodies or fragments with the bi-functional chelating agent DTPA (*20*) has been proposed to take advantage of the shorter but clinically adequate half-life (67 hr), the gamma emission at 173 and 247 keV, the compatibility with conventional collimators and gamma cameras, and the lack of beta emissions. In contrast to the relatively uniform success of labeling proteins with the several iodination methods, many investigators have not realized consistently favorable results with In-111 chelation of antibodies.

Since the agents used for radioimmunodetection are from biologic sources, tests for pyrogenicity, sterility, and acute toxicity must be obtained. All three of these evaluations are needed for each new batch of antiserum, and if the time required to exhaust a batch is prolonged, these quality measures must be repeated at intervals. To date we have found it more practical to refer the batch testing to an outside laboratory. During the intervals between batch testing, each patient preparation (after labeling) is examined in house for sterility.

CLINICAL USE OF RADIOANTIBODIES FOR SCINTIGRAPHY

Twenty μCi/kg body weight of the I-131-labeled antibody is the usual dose administered for imaging purposes; it represents 150–250 μg of protein. The radiotracer is administered intravenously over a period of at least 15 min. Tumor imaging is usually performed at 24 and 48 hr following injection, whereas myocardial imaging can start at 6 hr and be completed at 24 hr. Subtraction techniques with technetium albumin, sulfur colloid, etc., to mimic nontarget radioactivity (*55*) are necessary to eliminate nonspecific accumulations.

To block thyroid accumulation of released I-131, Lugol's solution is administered orally beginning two days before the examination. Before injection of the antibody, the patients are skin-tested for hypersensitivity to IgG. In over 400 patients, only two instances of a skin reaction to the antibody have been observed, but the potential hazard of developing an immune reaction with subsequent administration of the same antibody must be considered. In more than 50 patients who have two or three imaging studies with same antibody, no reactions have been observed. It has been speculated that the quantity of protein administered is too small to stimulate an antibody reaction.

IMMUNOLOGICAL CONSIDERATIONS ON RADIOIMMUNOLOGIC DIAGNOSIS AND TREATMENT

One of the most important factors for radioimmunodiagnosis or treatment is the specificity of the radiolabeled antibody (or fragments) to the target to be studied or treated, and thus the absolute and relative accumulation of the radioindicator in the target tissue in vivo (*117–118*). Even in the case of a highly specific monoclonal antibody, however, a significant fraction of the labeled antibody is found in nontarget organs and tissues, and usually only a small percentage of the injected dose accumulates within the target. Although part of the target accumulation may be nonspecific (*118*), clinically it is sufficient to yield a high target-to-nontarget ratio, independent of the specificity of the target accumulation. As demonstrated previously, nonspecific antibodies have been used for tumor imaging; they occasionally resulted in success but frequently in failures (*53*).

The extravascular/extracellular space of tumors is much greater than that of normal tissues (*118*), and except for primary hepatomas, most or all malignant tumors probably share this characteristic. Proteins of large molecular weight, such as gamma globulins, diffuse very slowly into the extravascular/extracellular tissue

space, and when they are trapped in this space and background activity decreases, positive target-to-background accumulations are generated. This is true for iodine-131 normal gamma globulin and for certain other soluble substances with high molecular weight, including dyes (*118*) as well as antibodies to fibrin. To evaluate the specific accumulation of the radioantibody for purposes of consistent results and good sensitivity in clinical diagnosis and therapy, the double-antibody technique is important. Pressman et al. have worked with the double-nuclide technique in animals and have proven that there is specific accumulation of the radioantibody within tumors (*118*). Researchers using monoclonal antibodies and the double-nuclide technique (tumor-specific antibody labeled with iodine-131 and nonspecific labeled with iodine-123), were able to show that specific accumulation of the radioantibody does occur in vivo (*19*).

For the detection of small colonies of tumor cells, it is theoretically beneficial to produce an antibody to specific antigens that are expressed on the membranes of these cells. The possibility that the process of eluting the membrane antigen from the cell may alter its specificity prompted investigators to consider the production of antibodies to intact tumor cells by the hybridoma (monoclonal) technique (*117–119*). Intracellular tumor antigens extracted from destroyed tumor cells could have the theoretical limitations of cell destruction for tumor detection by antibody.

Although relatively uncommon, nonspecific accumulation of the radioantibody can result in false-positive studies (*50*). On the basis of previous evidence (*120–121*), it has been postulated by investigators who found CEA antibody accumulation in lymph nodes (by lymphoscintigraphic techniques), without histologic evidence of metastases, that antigen transferred to regional lymph nodes from tumors could accumulate antibody, specifically from the immunological point of view, but nonspecifically from the clinical point of view, resulting in "false?" positive studies (*50,53*). Since the radioimmunodetection method depends on an antibody-antigen reaction, the question arises in antibody lymphoscintigraphy whether the detection of antigen sequestered by lymph nodes draining a cancer is misleading, or whether it has implications not yet appreciated. Clinically it is of course a very important finding in cases of suspected tumor recurrence. In a patient with previous extirpation of neoplasm, the identification of a tumor-associated antigen signals the presence of tumor with drainage into that particular group of lymph nodes. Although thorough clinical investigations are still required, the demonstration of antigen in different groups may well influence further diagnostic and therapeutic approaches to a particular case.

The classic example of radioimmunologic diagnosis of nonmalignant tissues is the detection of myocardial infarction by radiolabeled antibodies (fragments) to myosin (*15,16*). Following acute myocardial infarction, the irreparable damage to the cells results in increased permeability of the cell membrane, making it possible for large molecules to gain entrance to the cells. This penetration of the cellular membrane is not possible in the normal myocardium, nor in merely ischemic myocardium. In a patient with acute myocardial infarction intravenously administered antibodies to myosin enter the damaged cells, react specifically with the myosin molecules, and are trapped. The differential accumulation of the labeled antibody provides a significant diagnostic target-to-nontarget ratio. In this instance intracellular components are used as antigens for immunodiagnosis of the disease. There are other diseases characterized by cellular damage with increased permeability of the cellular membrane.

CLINICAL CONSIDERATION OF RADIOIMMUNOLOGIC DIAGNOSIS AND TREATMENT

Specific tumor imaging could be the method of choice for the evaluation of patients with known or suspected cancers. All but a small minority of the existing methods of tumor diagnosis are indirect, based on tumor differences in vascularity, x-ray absorption, ultrasound reflection, mass effects, hypermetabolic activity, or increased protein catabolism (*122*). Frequently the results are not specific for tumors, and confusion with inflammatory or vascular disorders is not uncommon. Radioimmunodiagnosis offers a possible solution to these problems, and in addition permits imaging of the entire body in search of unsuspected sites of tumor involvement (*123*). Specific diagnosis of nonneoplastic diseases by radioimmunologic methods can also be achieved in clinical practice (*16*). For therapy, the significance of this method, if successful, is even greater, since existing treatments are often ineffective or only partially or temporarily effective (*71*).

Concerning clinical applications at this time, the most successful use of radioimmunologic diagnosis is reportedly that of acute myocardial infarction using radiolabeled antibody to myosin (*16*). Clinical applications of tumor imaging have been met with varied success but the method, in addition to the technical difficulties, is hampered by the fact that the sensitivity is variable (40–90%) although the specificity is high. Recent and future developments in the methods of antibody production, purification, fragmentation, and labeling, together with improvement in nuclear medicine instrumentation, will undoubtedly influence these results (*118*).

Finally we note that—even under the most ideal conditions from the biological point of view—there are theoretical limitations to immunodiagnostic imaging. Based on mathematical formulations, Rockuff and his

colleagues determined that transmission computerized tomography using antibody labeled with stable iodine is a theoretically discouraging approach (*124*). According to the same authors, the tomographic approach to immunological imaging of tumors with antibodies carrying radiotracers is quite feasible: one-cc tumors, located as deep as five centimeters or more from the body surface, appeared detectable with target-to-nontarget ratios of the order of 5. Smaller and deeper tumors require higher uptake ratios to be detectable, but even these ratios are possible, granted a highly specific radioantibody imaged with emission tomography, facilitated probably by background-subtraction techniques.

Order et al. have initiated clinical applications of radioantibodies for therapy (*71,72*). The preliminary results are encouraging and open an exciting area in the treatment of tumors. As with all new approaches, certainly difficulties lie ahead, but the probabilities are that the difficulties will be overcome by an improvement of methods and enrichment of our knowledge.

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