

# Monoclonal Antibodies in Nuclear Medicine

Andrew M. Keenan, John C. Harbert, and Steven M. Larson

*Department of Nuclear Medicine, Clinical Center, National Institutes of Health, Bethesda, Maryland; and Division of Nuclear Medicine, Georgetown University Hospital, Washington, DC*

Monoclonal antibodies have been a part of biomedical research for nearly a decade, yet a comparable period of time may be required before radiolabeled monoclonal antibodies come into standard practice in the diagnosis and therapy of human disease. Difficulties in identifying appropriate antigens, developing the optimal antibody species, attaching the most effective radiolabel and choosing the best method of administration all confound present investigations. The problems confronting therapeutic applications are, for the most part, an extension of those associated with diagnostic techniques. Still, there is much well-deserved optimism, and progress in all areas of investigation will ultimately come together to resolve most if not all of these problems. It is inevitable that monoclonal antibodies will have significant impact on the practice of nuclear medicine, and presented here is a review of the basic knowledge as well as an update on the current status of radiolabeled monoclonal antibodies in medicine.

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Monoclonal antibodies have heralded a revolution in radiopharmaceutical strategy. Recent advances in biotechnology, particularly recombinant genetics, cell cloning, new radiolabeling techniques, and the characterization of cell receptors and antigens have raised new hope for achieving the long-cherished goal of employing site-specific radiopharmaceuticals for medical diagnosis and therapy. The concepts underlying these developments as they relate to immunology and the special language that defines them are the subject of this discussion.

## BASIC CONCEPTS

Antibody molecules, or *immunoglobulins*, are produced by *plasma cells* in higher animals in response to the introduction of foreign substances (*antigens*) that are generally large molecules of 1,000 daltons or more. Immunoglobulins possess specific binding regions that recognize the shape of particular sites, or *determinants*, on the surface of the antigen. The combination of an antibody with its specific antigen initiates a complex

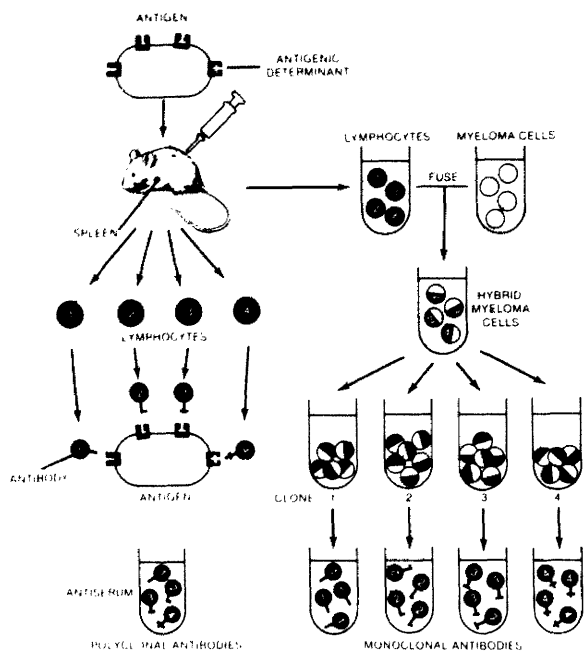
immunological response that usually results in the destruction or elimination of the antigen.

An antigen may have several determinants, or *epitopes*, each of which can stimulate one or more *B lymphocytes*. Each B lymphocyte has the capacity to differentiate into plasma cells that secrete a single, specific immunoglobulin in response to one antigenic determinant, and each different antibody is produced by the family of plasma cells that stems from one of the B lymphocytes. Antigenic challenge thus evokes a heterogeneous antibody response resulting from a mix of antibody-producing plasma cells.

If a suitable animal recipient, typically a mouse or rabbit, is immunized with an antigenic agent, serum taken from the sensitized host will contain antibodies to different epitopes of the antigen. Since these antibodies are derived from a population of stimulated B lymphocytes and their daughter plasma cells, the term *polyclonal antibodies* is applied. However, if individual lymphocytes or plasma cells could be extracted and cloned in tissue culture, each clone would have the potential to manufacture a single species of antibody molecule, or a *monoclonal antibody* (Fig. 1). Unfortunately, normal antibody-producing cells do not survive in culture media. It was the genius of two recent Nobel laureates, Georges Kohler and Cesar Milstein, to recognize that *myeloma* cells, which are cancer cells that

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For reprints contact: Andrew M. Keenan, MD, Dept. of Nuclear Medicine, Building 10, Rm. 1C401, National Institutes of Health, Bethesda, MD 20205.



**FIGURE 1**

Injection of antigen into mouse or other higher animal elicits heterogeneous antibody response due to stimulation of several B lymphocytes by various determinants on antigen, resulting in polyclonal antibodies in serum (left). If sensitized lymphocytes are removed from spleen of immunized animal and induced to fuse with myeloma cells, individual hybrid cells can be cloned, each producing monoclonal antibodies to single antigenic determinant (right) (Adapted from *Sci Am* 243(4):66-74, 1980)

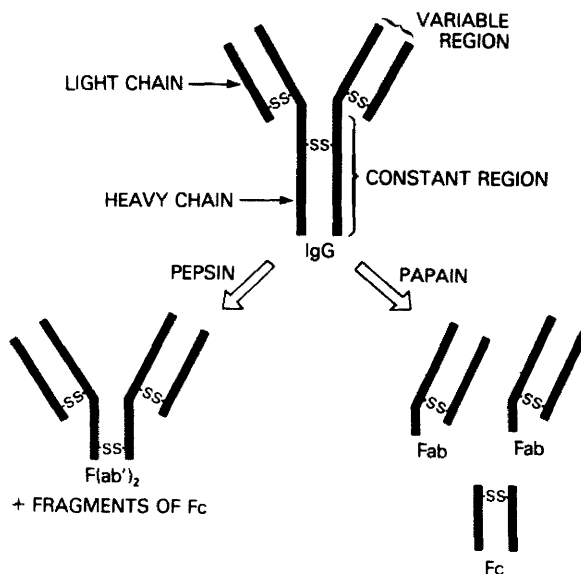
produce large amounts of identical but nonspecific immunoglobulins and which survive in cultures indefinitely, might be altered by the new techniques of recombinant genetics to construct immortal clones that secrete useful immunoglobulin products (1).

Kohler and Milstein developed a method of producing such monoclonal cell strains by fusing splenic lymphocytes from immunized mice with a mouse myeloma cell line, giving rise to clones of hybrid cells called *hybridomas* (2). Although stimulated lymphocytes do not survive in cell culture and myeloma cells cannot be induced to secrete antigen-specific antibodies, the fusion of these cells, mediated by polyethylene glycol, results in clones that "express both the lymphocyte's property of specific-antibody production and the immortal character of the myeloma cells" (2). Hybridoma cells are selectively grown in hypoxanthene-aminopterin-thymidine (HAT) medium, which supports neither the unfused lymphocytes nor the myeloma cells. Hybridomas produced in this manner are then ready for assessment of antibody production and further selective cultivation.

Immunoglobulins (Ig) are designated IgG, IgM, IgE, IgA, or IgD according to their general structure and can be further subdivided on the basis of internal attributes.

Hybridomas usually generate IgG of the IgG<sub>1</sub> or IgG<sub>2a</sub> subclass (*isotype*), although IgM monoclonal antibodies have also been produced. IgG molecules are comprised of two long and two short amino acid chains, referred to as *heavy* (H) and *light* (L) chains, having a total molecular weight of ~150,000 daltons (Fig. 2). The integrity of the molecule is maintained by disulfide bridges linking the chains together. The IgG isotypes differ structurally in the number of disulfide bonds linking the two heavy chains together, and they differ functionally in their ability to fix complement and to interact with effector cells such as macrophages and mast cells. The *constant* region of the antibody molecule includes the *Fc fragment*, so called because of its tendency to crystallize in vitro. This region is the same for all IgG molecules of the same isotype and can activate various components of the immune system. Two antigen-binding *Fab fragments* contain the *variable* regions, which are unique for each monoclonal antibody; antigen-specificity resides at these sites.

Two sets of genes control antibody synthesis: a pair of *V genes* encodes the variable region and a pair of *C genes* encodes the constant region. Each plasma cell synthesizes an antibody based on only one of many sets of V and C genes in the cell. Each gene of a given pair is designated an *allele* and has the same location on the corresponding, *homologous* chromosomes of diploid cells. The alleles of a gene pair can be different from each other, and if both were expressed, different antibodies



**FIGURE 2**

Immunoglobulin G molecules consist of two heavy and two light protein chains held together by disulfide bonds. Two variable regions can bind to specific antigenic sites, and constant region interacts with host immune system. Enzymatic digestion with pepsin removes part of constant region to produce an F(ab')<sub>2</sub> fragment, whereas papain splits molecule into Fc fragment and two Fab fragments

would result from the same cell; however, only one allele is active in antibody production, thus each lymphocyte produces a single species of antibody molecule. Hybridoma cells contain chromosomes from both the myeloma-cell parent and the spleen-cell parent, and both sets of these *heterologous* chromosomes may be expressed by these hybrid clones, resulting in hybrid antibody molecules with H and L chains from different parent cells. The aim is to find the clone that expresses only the H and L chains of the immunized mouse lymphocyte.

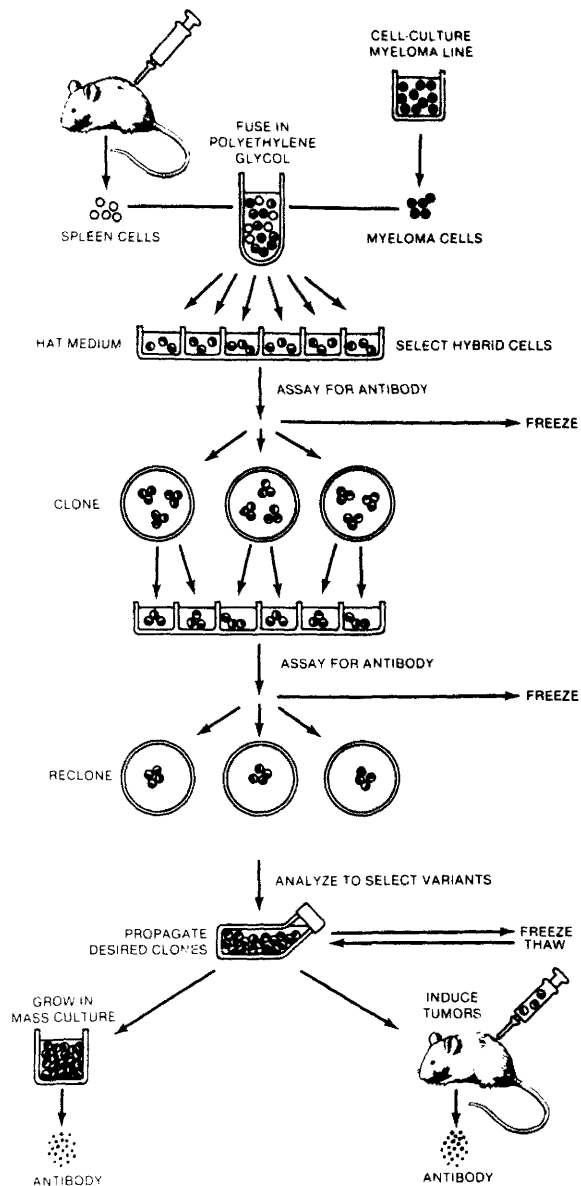
The process of developing a viable hybridoma that manufactures antibodies with the right properties and specificity is painstaking. Hundreds of fusions may be required before a single hybrid that secretes the desired antibody is formed. Individual hybridoma cells must be manually separated into culture dishes and analyzed to identify clones generating antibodies to the selected antigen; further testing is then necessary to evaluate the binding characteristics of antibodies that have diagnostic and therapeutic potential (Fig. 3).

*Affinity* refers to the strength of attraction between the antigen and antibody, and *avidity* is a measure of the integrity of the antigen-antibody complex once formed. These qualities can be measured using standard radioimmunoassay techniques. Affinity constants are determined by plotting bound-to-free antigen ratios against the bound-antigen concentration and extrapolating back to zero antigen concentration (*Scatchard analysis*). The affinity constant of a useful monoclonal antibody should be at least  $10^8$  l/mole; highly specific preparations can reach as high as  $10^{10}$  l/mole (3).

When all the proper steps have been completed, the resultant hybridoma clone elaborating unlimited quantities of the desired antibody can be maintained in tissue culture, from which the supernatant can be separated as a dilute source of antibodies. Alternatively, the clones can be grown in mouse ascites fluid to yield a more concentrated source of antibodies. Monoclonal antibodies harvested in this manner are reproducible, relatively pure and uniformly immunoreactive, and hybridomas can be frozen for storage or transport.

Most experience has been gained with rodent, or *murine*, antibodies, but monoclonal antibodies from human-human hybridomas have also been developed (4). Murine antibodies are foreign to humans; human anti-murine antibodies (HAMA) are commonly induced following injection, some of which can interact directly with the binding site (5) and are thus designated *anti-idiotypes* (6). HAMA can interfere with subsequent murine antibody administrations and may evoke allergic reactions. Human monoclonal antibodies may help reduce this potential, but currently only murine sources are readily available for clinical use.

Since the Fc region of the antibody molecule is most likely to trigger allergic responses, fragmentation has been used to remove this portion from the antibody



**FIGURE 3**

Fusion of antigen-stimulated spleen cells with myeloma cells in polyethylene glycol results in hybrid cells that can be cloned in HAT medium. Those clones that generate immunoglobulin are further propagated, and those producing antibodies to desired antigen are selected to find variant that produces antibody with desired specificity and binding properties. Hybridomas can be maintained in mass culture or mouse ascites, and clones at any stage of development can be frozen for later use (Adapted from *Sci Am* 243(4): 66-74, 1980)

molecule (7,8). The proteolytic enzyme *pepsin* acts adjacent to the disulfide bonds bridging the H chains, cleaving off most of the Fc region and leaving the two Fab fragments bound together in a divalent structure referred to as the  $F(ab')_2$  fragment. The enzyme *papain*

removes a larger portion of the H chains, resulting in two monovalent Fab fragments and an intact Fc fragment (Fig. 2).

Whole IgG is metabolized in the liver and reticulo-endothelial system whereas Fab fragments, weighing ~50,000 daltons, are principally cleared by the kidneys (8). Since Fab fragments are monovalent, binding to cell-bound antigens is weaker than the divalent binding of the corresponding intact immunoglobulin. F(ab')<sub>2</sub> fragments, on the other hand, have the avidity of divalent binding without the immunogenicity of the Fc region, and may prove superior to either whole immunoglobulins or Fab fragments (9).

Monoclonal antibodies can be labeled with radionuclides using established radioiodination techniques or by newer conjugation methods. Chloramine-T, Iodogen, lactoperoxidase and Bolton-Hunter reagent can all be used to covalently bond iodine-131 (<sup>131</sup>I), iodine-125 (<sup>125</sup>I), or iodine-123 (<sup>123</sup>I) to antibodies, and one or more iodine atoms can be attached per molecule depending on the number of tyrosine moieties available to iodinate. Iodine-125 is most often used to determine relative tissue distribution in animals. Scintillation camera imaging can also be used with <sup>125</sup>I in small animals, but <sup>131</sup>I is more appropriate for imaging patients. Unfortunately, despite its low cost and ready availability, the beta radiation and 8-day half-life of <sup>131</sup>I severely limit diagnostic doses in humans. The 13-hr half-life of <sup>123</sup>I restricts its use to short-term studies, but excellent images can be obtained from its favorable gamma emission (10).

Recently, diethylenetriaminepentaacetic acid (DTPA) has been used to chelate metallic cations such as indium and technetium to antibody molecules. The best results have been achieved with <sup>111</sup>In (11), and kits containing antibody-DTPA conjugates designed for in-house labeling with <sup>111</sup>In are under investigation (12). Technetium-99m (<sup>99m</sup>Tc) has shown less promise for antibody labeling with DTPA, and direct labeling with <sup>99m</sup>Tc by stannous reduction results in antibody preparations which at best retain only 30 to 40% of the original immunoreactivity (13). Gallium-67 (<sup>67</sup>Ga) offers little advantage over <sup>111</sup>In for single photon imaging, but gallium-68 may be useful for studies with positron emission tomography. Various stable elements, such as manganese and gadolinium, may be of value in nuclear magnetic resonance imaging.

Several alpha- and beta-emitting radionuclides have potential for the radioimmunotherapy of cancer. Iodine-131 has most commonly been used, and its gamma radiation can be used to measure in vivo activity scintigraphically (8,14). The Auger electrons of <sup>125</sup>I may be effective for therapy when used in conjunction with antibodies that are internalized rather than remaining on the cell surface (15). Bismuth-212 (<sup>212</sup>Bi) has been proposed as a therapeutic radiolabel due to the extremely high linear energy transfer of its alpha emissions (16)

and is available as a generator product of lead-212 (<sup>212</sup>Pb) (17). The 1-hr half-life of <sup>212</sup>Bi limits its applications, but interestingly, <sup>212</sup>Pb, with its 11-hr half-life, might better serve as the primary antibody label. Copper-67 is under investigation as a therapeutic beta-emitter with its 62-hr half-life and gamma radiation that can be imaged to monitor biodistribution (18). Palladium-109, scandium-47 (<sup>47</sup>Sc), and yttrium-90 have also shown potential for radioimmunotherapy, but of these, only <sup>47</sup>Sc has useful gamma radiation (160 keV) for imaging in conjunction with therapy (19-21).

Radiolabeling can cause significant alteration in the biological activity of antibodies. The determination of the *immunoreactive fraction*, or the percent of the radiolabeled antibody preparation that retains its original antigen-binding specificity, is an important quality control measure, and accurate binding assays have been developed for this purpose (22). Furthermore, the behavior of antibodies in vivo is subject to additional influences, such as blood flow, capillary permeability and host metabolism. The degree of *immunospecificity* can be determined using the dual tracer technique of Pressman and associates (23) by giving animals injections of specific and nonspecific antibodies of the same subclass labeled with different radioisotopes of iodine and then measuring relative tissue concentrations. Nonspecific binding results in nearly unit ratios, whereas target-specific uptake is associated with high tissue ratios of specific-to-nonspecific antibody label.

Successful imaging requires target-to-background ratios of at least 2:1, and ratios of 5:1 or more are necessary to detect deeper and smaller lesions (24). Some improvement in imaging can be achieved by digital subtraction of background activity, which is usually performed on scintigraphic data from <sup>131</sup>I-labeled antibodies and technetium-99m simulated background activity; however, this method can lead to higher false-positive rates (25). Background subtraction should not be necessary with many current monoclonal antibody preparations, which yield tumor-to-tissue ratios up to 7:1 in patients (26) and up to 20:1 in mice (27,28). Calculations of the maximum achievable uptake in vivo for high affinity antibodies indicate that ratios between 100:1 and 1000:1 are theoretically obtainable (3). Furthermore, since antibody fragments are cleared more rapidly than whole immunoglobulins, less background activity persists, resulting in better images and more favorable dosimetry for therapy (8,9).

Targeting of human tumors requires the identification of tumor-associated, or preferably, tumor-specific antigens that are expressed on tumor cell surfaces in over 100-fold concentration compared to normal tissues, and that are present in quantities of at least 100,000 molecules per cell (3). Most antibodies under study are directed at oncofetal proteins such as carcinoembryonic antigen (CEA), found in adenocarcinomas arising from

the endodermally derived digestive system epithelium (29), and p97, found primarily on the cell surface of human melanomas (30). Dozens of monoclonal antibodies to a variety of antigens, including antigens associated with cancer of the colon, breast, ovary, lung, liver, melanoma, and lympho-proliferative disorders, are now entering into clinical research trials, and increasingly more will follow.

### CLINICAL STATUS OF RADIOLABELED MONOCLONAL ANTIBODIES

The first clinical study of radiolabeled monoclonal antibodies was reported in 1981 by Mach and associates (31), who used  $^{131}\text{I}$  labeled anti-CEA antibodies in patients with CEA-bearing colon and pancreatic carcinomas. Results of this and other early studies showed only slightly better sensitivity than similar studies with polyclonal anti-CEA antibodies (31-33). More recently, Larson and colleagues have demonstrated greater sensitivity with anti-melanoma antibodies and Fab fragments (26), and Moldofsky and associates have used  $^{131}\text{I}$ -labeled  $\text{F}(\text{ab}')_2$  fragments to image metastatic colon carcinoma in retroperitoneal lymph nodes that was undetectable by other diagnostic modalities such as x-ray computed tomography (34). Most current tracer studies have been designed to use i.v. doses of 0.5 to 50 mg of antibody with radioactivities ranging from 1 to 5 mCi of  $^{131}\text{I}$  or  $^{111}\text{In}$ , thus high specific activities are not always required. However, since best results have been obtained several days after injection, count rates are often low, especially for single photon emission computed tomography (31). Higher target-to-background ratios will help overcome this limitation.

Early therapeutic trials with  $^{131}\text{I}$ -labeled monoclonal anti-p97 Fab fragments have shown that patients can be given cumulative doses of up to 500 mCi before reaching dose-limiting toxicity, and that single doses of 200 mCi can be safely administered, resulting in tumor radiation doses of over 10 rad per mCi compared to 0.3 rad per mCi to the bone marrow (8). If maximal target-to-background ratios of 1,000:1 could be achieved, then it would be theoretically possible with  $^{131}\text{I}$  to deliver 100,000 rad of highly localized radiation to tumor tissue for each 100 rad to the bone marrow (3). Hematologic toxicity, particularly thrombocytopenia, appears to be dose-dependent and dose-limiting, and results from circulating radioactivity rather than bone marrow localization of labeled antibodies. Fab fragments may offer an advantage in that they clear from the blood more rapidly than whole antibodies. Curative radioimmunotherapy has yet to be accomplished, and the optimal parameters, such as the milligram dose of antibody administered, the most suitable antibody species or fragment, the best radiolabel and dose scheduling remain to be established.

### PROBLEMS AND FUTURE DIRECTIONS

Many factors can impair antigen-specific localization and contribute to excessive background activity. Radiolabeling can destroy antibody integrity or interfere with antigen binding in the Fab region, and molecular damage at any stage of preparation can result in non-specific localization. Cross-reactivity with nontarget antigens can result in nonselective antibody binding. Interaction of the Fc region and deposition of antigen-antibody complexes may further contribute to non-specific uptake. Insufficient perfusion and poor antigen accessibility in the target tissue can decrease delivery and hinder uptake. Metabolism of antigen-antibody complexes can influence the fate of correctly localized antibodies. Variable expression of antigens on target tissues can result in false-negative scintigrams or incomplete therapy.

Many of these problems will be overcome by improved labeling and purification techniques and better tissue screening procedures. Hybridoma technology provides consistent preparations that can be used for the pharmacokinetic studies needed to design optimal antibody regimens. The problems associated with successful radioimmunotherapy are for the most part an extension of those facing radioimmunodiagnosis, and a variety of clinical studies are now under way. Alternative routes of administration, such as intra-arterial and intra-cavitary infusion, may improve delivery to organs, tumors and body spaces, and s.c. injection and intralymphatic administration can direct antibodies to regional lymph nodes (35,36). The use of different monoclonal antibodies in combination has shown improvement over single antibody preparations (37), most likely by effectively expanding the number of potential binding sites. Greater knowledge of the structure and function of antigens will aid in target selection, and methods to influence antigen expression may revolutionize immunology once again.

Monoclonal antibodies clearly indicate the direction in which the field of nuclear medicine is moving: site-specific radiopharmaceuticals directed at molecular targets will supersede nonspecific agents such as colloids that are phagocytized or macroaggregates that lodge in capillaries (38). Although most work has centered on tumor-associated antigens, monoclonal antibodies can also be directed at sequestered antigens such as cardiac myosin (39), foreign antigens such as microorganisms (40), and various normal antigens for localization studies (41). Batteries of monoclonal antibodies should become widely available for ready selection and tissue cross-matching. Precise radiolocalization at the molecular level affords an endless array of applications, and further advances in biotechnology will continue to bestow nuclear medicine with a new generation of radiopharmaceuticals to study, diagnose, and ultimately treat human disease.

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