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Therapeutic Applications of Monoclonal Antibodies

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ABSTRACT: Researchers have sought therapeutic applications for monoclonal antibodies since their development in 1975. However, murine-derived monoclonal antibodies may cause an immunogenic response in human patients, reducing their therapeutic efficacy. Chimeric and humanized antibodies have been developed that are less likely to provoke an immune reaction in human patients than are murine-derived antibodies. Antibody fragments, bispecific antibodies, and antibodies produced through the use of phage display systems and

genetically modified plants and animals may aid researchers in developing new uses for monoclonal antibodies in the treatment of disease. Monoclonal antibodies may have a number of promising potential therapeutic applications in the treatment of asthma, autoimmune diseases, cancer, poisoning, septicemia, substance abuse, viral infections, and other diseases.

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In 1975, Kohler and Milstein revolutionized the field of immunology by developing monoclonal antibodies (MAbs). Since that time, many MAbs have been developed for use in diagnostic procedures and in immunotherapy. Ever since it was observed that the therapeutic use of heterologous MAbs elicited immunogenic responses in humans, significant research efforts have been devoted toward creating chimeric and humanized antibodies for use in human patients. Major achievements have been in the production of MAbs in transgenic plants and animals. The use of phage display libraries has created customized antibodies with defined affinity and specificity. This review describes how rodent, chimeric, and humanized antibodies have each been used, with varying degrees of success to treat cancer, septicemia, autoimmune disorders, and infectious diseases. We also describe here recent applications of antibody engineering, such as the use of bispecific antibodies and antibody fragments in immunotherapy.

History of MAb Development

Von Behring and Kitasato discovered in 1890 that the serum of vaccinated persons contained certain

substances, which they termed “antibodies.” In 1895, they treated diphtheria with an antiserum raised against the toxin. On the basis of research on tetanus toxin and trypanosome parasites, Ehrlich proposed in 1900 the “side-chain theory” of antibody formation, which hypothesized that physiologically active substances, including toxins, attach to cell surface receptors that are produced in response to toxin-cell interactions and then ejected from the cells into the bloodstream, leading to circulating antibodies.^{1,2}

Not until the 1950s, however, did scientists’ understanding of antibodies become sufficient to lay the foundation for the development of MAbs. Jerne³ postulated in 1955 a theory of natural selection for antibody formation. Animals vaccinated with an antigen were expected to produce several distinct antibodies against several epitopes of the antigen. Frank Macfarlane Burnet subsequently refined and expanded Jerne’s theory.⁴ Burnet’s “clonal selection theory,” as it is generally known, postulates that cells specific for synthesizing 1 type of antibody are spontaneously generated due to random somatic mutations during the maturation of the immune system and that these cells proliferate when exposed to an antigen. At about the same time, Porter isolated fragment antigen binding (Fab) and fragment crystalline (Fc) from proteolytically cleaved rabbit γ -globulin.⁵

Until the 1960s, antibody-producing cells were difficult to maintain in culture, because they died after a few days. In addition, only polyclonal antibodies could be obtained. In 1964, Littlefield⁶ developed a way to isolate hybrid cells from 2 parent cell lines using the hypoxanthine-aminopterin-thymi-

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dine (HAT) selection media. In 1975, Kohler and Milstein⁷ provided the most outstanding proof of the clonal selection theory from results of heterokaryons—cell hybrids formed by the fusion of normal and malignant cells. Twenty-five years after Kohler and Milstein produced the first monoclonal antibodies, dramatic progress has been made in using antibodies for diagnostic purposes, but the uses of MABs to treat disease have—until recently—remained somewhat limited.^{8,9} However, as this review indicates, the potential of MABs to aid in the treatment of a wide range of diseases is now beginning to be realized.

Antibody Structure

Antibodies are Y-shaped proteins composed of peptides called heavy and light chains, the ends of which vary from antibody to antibody. Each combination of heavy and light chains binds to a particular antigenic site. These glycoprotein chains are folded into domains of about 110 amino acids that become twisted into the immunoglobulin (Ig) fold and are stabilized by disulfide bonds. Structurally, each Ig molecule consists of 2 50-kD heavy chains and 2 25-kD light chains, linked by disulfide bonds.

Human immunoglobulins are divided into 5 classes or isotypes based on the amino acid composition of their heavy chains: α , δ , ϵ , γ , and μ , denoted IgA, IgD, IgE, IgG, and IgM, respectively. There are 2 kinds of light chains, κ (k) and λ (l), which are common to all 5 classes. Four subclasses of IgG (IgG1, IgG2, IgG3, and IgG4) and 2 subclasses of IgA (IgA1 and IgA2) exist, each with a distinct function. Secretory IgA exists in dimeric form held together by a J chain and is associated with a secretory component that helps it pass through the cell membrane.^{10,11}

Each chain has a constant domain to bind host effector molecule and a variable domain to bind to the target antigen. Light chains have 1 variable (V_L) and 1 constant domain (C_L), whereas heavy chains have 1 variable (V_H) and either 3 (α , δ , and γ chains) or 4 constant domains (ϵ and μ heavy chains) depending upon the isotype class. Each variable domain contains 3 regions known as “hypervariable loops,” also known as complementarity-determining regions (CDRs), that identify the antigen. The other amino acids in the variable (Fv) domain are known as framework residues and act as a scaffold to support the loops. The V_L and the V_H , the C_{H1} and the C_L , and the 2 C_{H3} domains are paired; the 2 C_{H2} domains have carbohydrate side chains attached to them and are not paired. The folded constant domains may be homologous among different species, allowing hybrid domains (eg, mouse-human) to be produced. The variable domain confers specificity and affinity. The CDR amino acid sequences are extremely variable and play a large role in interac-

tion with the targeted antigen. The chain type, region, and distance from the amino terminus characterize the domains. Thus, C_{H2} domain refers to the second constant domain of the heavy chain.

Upon digestion with papain, the antibody molecule is cleaved on the amino-terminal side of the disulfide bridges into 2 identical Fabs and an Fc fragment, whereas pepsin cleaves the antibody on the carboxy-terminal side of the disulfide bridges into 1 F(ab')₂ fragment containing both the arms of the antibody, and many small pieces of the Fc fragment. Currently, the following are available: whole antibodies, enzymatically produced 50-kD Fab fragments, engineered 25-kD single-chain Fv (scFv) antibodies consisting of the V_H and V_L connected by a flexible peptide linker, diabodies (noncovalent dimers of scFv), minibodies (scFv- C_{H3} dimers), and heavy chain IgGs found in species of the Camelidae family, which are devoid of light chains and are referred to as V_{HH} .

Conventional MAB Production

The first MAB described by Kohler and Milstein was created by the fusion of murine myeloma cells with murine-antibody-secreting lymphocytes.^{7,8} Myeloma cells are immortalized B lymphocytes capable of secreting homogeneous antibodies. The immortal myeloma cell lacks the enzyme hypoxanthine guanosine phosphoribosyl transferase (HGPRT) and is sensitive to the HAT media. However, a hybrid cell known as a hybridoma, generated by the fusion of myeloma cell and an antibody-producing B cell, can survive in the HAT media. The spleen B lymphocytes contribute the *HGPRT* gene to the hybrid cell and, hence, unfused myeloma cells and spleen cells die in the HAT media.

The conventional method of generating MABs is the hybridoma technology in which spleen cells from immunized mice are fused with murine myeloma cells. Whereas the myeloma cell imparts immortality to the hybridoma allowing cells to be cultivated indefinitely, the immune spleen B-cell confers antigen specificity. Because each hybridoma is derived from a single cell, the cells within a hybridoma cell line are identical and make the same antibody molecule with same antigen-binding site and isotype, hence it is called a MAB. Among several excellent reviews that detail MAB production with hybridomas is a recent review by Dean and Shepherd.¹² Initial attempts to bypass the mouse to make human MABs involved fusion of human immune spleen lymphocytes with nonsecreting human myeloma partners to obtain hybrid cells that continually secrete a specific antibody. However, poor fusion of human myelomas, unsatisfactory performance of the hybrid cells, and the difficulty in accessing immune lymphocytes have prevented success. Although heteromyelomas—which are fusions of hu-

man and mouse myelomas—work better, these hybrids are usually unstable. Attempts to use mouse myeloma cells to create hybrids and derive human MAbs led to the loss of human chromosomes and the inability to make human Igs.¹³

Unfortunately, *in vitro* immunization is limited by its inability to produce a secondary response and by the absence of the affinity maturation process that occurs *in vivo*.¹³ Affinity maturation process is a complex phenomenon, a consequence of intense B-cell proliferation, somatic hypermutation of Ig variable domain genes, and selection for B cells with high-affinity antigen binding, all occurring in the specialized microenvironment of the germinal center within the lymphoid tissue. Thus, the search for an ideal fusion partner for generating human MAbs has been difficult, which is why the Epstein-Barr virus (EBV) technique for immortalization of B lymphocytes is preferred. But immortalized B cells do not always replicate exactly the *in vivo* antibody response, because the tissues from which these cells were selected and the manipulations to which they are subjected to in the laboratory may alter the antibody specificity.^{14,15} Protocols for the preparation of EBV virus, B cells, and cell fusion have been described elsewhere.^{14,16,17}

Methods used for large-scale production of MAbs may include the generation of ascites tumors in mice or *in vitro* mammalian cell culture fermentation by using bioreactors and continuous perfusion culture systems.^{18,19} The key issues in scale-up productions are the growth media, fermenter size, fermentation time, and purification procedures.¹⁹ Purification or downstream processing is accomplished by chromatography, fragmentation, conjugation with chelating agents, ultrafiltration, and controlled precipitation.¹⁸

New Approaches and Developments

A more recent technique for producing antibody-like molecules uses what is known as the Phage Display Library. It involves the construction of V_H and V_L gene libraries and their expression on the surface of a filamentous bacteriophage. Developed in the 1990s, the phage display method requires repeated “panning” or screening of different antibodies based on their affinity for a specific antigen. Antibody genes are linked to bacteriophage coat protein genes and the bacteriophages with the fusion genes are used to infect bacteria to create the phage display library. The resulting bacteriophages express the fusion proteins and display them on their surface, and the phage display library comprises recombinant phages, each displaying a different antigen-binding site on its surface. The phage expressing an antigen-binding domain specific for a particular antigen can be detected and isolated by binding to the surface coated with that antigen. Libraries of V_H and V_L genes may be generated from nonimmunized

donors, immunized donors who have an immune response against a particular antigen or from a synthetic library consisting of antibody fragments.^{20,21}

One promising way to increase antibody yield or develop new antibodies may be by using genetically altered animals and plants. Abgenix, a company in Fremont, CA, has developed the transgenic “Xeno-Mouse,” in which the mouse antibody-producing genes have been inactivated and functionally replaced by approximately 90% of the human Ig gene loci in germline configuration, coding for the heavy and κ light chains.^{22,23} Upon immunization with any specific human or nonhuman antigens, the “Xeno-Mouse” generates MAbs, which are fully human Igs, with high affinities and antigen-binding specificities. “XenoMouse” strains producing specifically IgG1, IgG2, or IgG4 isotypes have also been created to generate panels of diverse and highly specific MAbs.

Kirin Brewery Company, Japan, has developed another transgenic mouse known as the “Trans-Chromo” mouse. The endogenous IgH and IgG loci of the “Trans-Chromo” mouse were inactivated, but it harbors 2 individual human chromosome fragments, derived from human chromosomes 2 and 14, that contain whole human Ig light- and heavy-chain loci, respectively.²⁴ These mice are capable of producing every subtype of fully human Ig, including IgA and IgM. In these transgenic mouse models, human antibodies with high affinity to an immunized antigen are naturally selected by the murine immune system via an affinity maturation process, and thereby show increased diversity of the MAbs.

Transgenic mice may be a suitable alternative to chimeric or humanized antibody production or the use of phage display systems to create less immunogenic or novel antibodies.²⁵ For instance, transgenic mice that express MAbs to coronavirus in their milk have been developed.^{26,27} Because ruminant animals, such as cows, goats, and sheep, produce relatively large amounts of milk, genetically-modified members of these species could also be used to produce large quantities of therapeutic proteins, including MAbs.²⁸

Plants may be a potential source of recombinant proteins, including MAbs.^{29–31} Plant virus vectors, such as the tobacco mosaic virus, may be used to make MAbs. Transgenic tobacco plants may also be used for large-scale production of recombinant IgA, which is used in passive mucosal immunotherapy.^{30,31} This MAb could be added to toothpaste to effectively protect against bacteria that cause tooth decay.^{31,32}

Recombinant antigens obtained from plants may also have therapeutic applications. For instance, attempts to immunize mice with *Escherichia coli* heat labile enterotoxin B produced in transgenic tobacco and potato plants have proved promising.³³

Hepatitis B viral surface protein produced in transgenic tobacco plants has been shown to be immunogenic in mice.³² The genes coding for murine malignant B-cell specific markers are inserted into tobacco mosaic virus to cultivate an immunogenic protein in tobacco plants that may eventually be used in developing a vaccine against non-Hodgkin lymphoma.^{34,35} Other immunotherapeutic proteins under development include Norwalk virus capsid proteins produced in tobacco and potatoes, cholera toxin and CT-B produced in potatoes, hepatitis B antigen produced in tobacco, anti-human IgG used to detect nonagglutinating antibodies produced in alfalfa, and humanized anti-herpes simplex virus (HSV)-2 grown in soybeans.^{36–39}

FDA Regulation of Therapeutic Monoclonal Antibodies

The Food and Drug Administration (FDA) considers antibodies to be “biopharmaceuticals”; as such, MAb applications are regulated by the agency’s Center for Biologics Evaluation and Research (CBER) and the Center for Drug Evaluation and Research.^{40,41} The FDA has created a “Points to Consider” document advising manufacturers of factors to consider in the production and testing of MAbs intended for human use and identified information that should appear in Investigational New Drug or biologics license applications.⁴⁰ The “Points to Consider” document serves to “indicate the agency’s current thinking on MAb products for human use.”⁴⁰ The agency’s recommendations are aimed at protecting human health because viruses and cellular DNA from antibody-producing cells with malignant phenotypes may be integrated into host cells after transformation. Accordingly, among the Points to Consider are several steps—such as taking care to ensure purity of immunoconjugates and demonstrating the ability of any purification scheme to remove adventitious agents—designed to prevent contamination of the final product by human pathogens.⁴⁰ Manufacturers must also adhere to animal care standards and detail steps to prevent contamination of cell culture.

The Points to Consider document includes a list of normal human tissues used in cross-reactivity testing, tests for murine viruses, and organs to be considered in dosimetry estimates. The FDA recognizes that because of species differences, animal models expressing the antigen of interest or cross-reactive epitopes are not always available.

The agency has also published a guidance entitled *S6 Preclinical Safety Evaluation of Biotechnology Derived Pharmaceuticals* based on International Conference of Harmonization technical requirements.⁴¹ Before beginning phase 1 clinical studies—conducted to assess the safety of the drug and its mechanism of action—the FDA recommends that

researchers conduct in vivo and in vitro testing of the MAb to assess cross-reactivity with human tissues or non-target-tissue binding. Preclinical safety testing aims to evaluate the immunogenicity, cross-reactivity, stability and effector functions of MAbs. The metabolism, carcinogenicity, and genotoxicity of the product should also be evaluated.⁴¹

Guidance has also been published for MAbs used as reagents in drug manufacturing.⁴² The guidance emphasizes biological safety, performance characteristics of the reagent, and potential presence of residual amounts of the reagent in the final product.

The FDA general principles for testing and manufacturing are broadly applicable to many classes of antibodies, including those produced by phage display systems or transgenic plants and animals. Depending on the expression system being used, other FDA guidance documents, such as CBER’s *Points to Consider in the Production and Testing of Therapeutic Products for Human Use Derived from Transgenic Animals*, *Points to Consider in the Production and Testing of New Drugs and Biologicals Produced by Recombinant DNA Technology*, and *Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals* should also be referenced.^{43–45}

Humanizing Monoclonal Antibodies

Rodent MAbs with excellent affinities and specificities have been generated using conventional hybridoma technology, but their use in clinical medicine is limited due to the immune responses they elicit in humans. For instance, the human anti-mouse antibody (HAMA) response can compromise the clinical effectiveness of murine MAbs. Although HAMA responses are directed against the murine constant regions, which represent the major antigenic features of the mouse Ig, significant responses are also directed toward the murine variable regions. As a result, patients may mount an immune response against the injected murine antibodies, leading to allergic or immune complex hypersensitivities, rapid clearance of the antibody, and reduced clinical efficacy.^{13,46–51}

Initially, Morrison and colleagues^{48,52} introduced chimeric MAbs in 1984, which showed several advantages over unmodified rodent antibodies. Generally, chimeras combine the human constant regions with the intact rodent variable regions, replicating the rodent antibody variable regions by PCR and then cloning them into eukaryotic expression vectors containing human constant regions.⁴⁹ Ideally, this allows better interaction with human effector cells and the complement system. Because the Fc region has little influence on the structure of the Fv region, the chimeric constructs’ affinity and specificity are virtually unchanged, and both rodent and chimeric antibodies cause apoptosis at a similar rate

and intensity against target cells *in vitro*.^{50,51,55} Although chimeric antibodies have helped solve some of the problems associated with the use of rodent MAbs, they still show significant immunogenicity in humans; because of their approximately 30% mouse sequence, they cause an human-antichimeric antibody response.

Humanized antibodies containing only the CDRs of the rodent variable region grafted onto the human variable region framework have been introduced to overcome these deficiencies.⁵³ The early work on recombinant, chimeric, and rodent/human antibodies happened during the mid 1980s and, by the late 1980s, Greg Winters and his colleagues demonstrated that a functional human-like antibody could be created by grafting the antigen-binding CDRs from variable domains of rodent antibodies onto human variable domains. Numerous humanized antibodies have now been designed and constructed, and many are currently being evaluated in clinical trials.^{13,14,39,47,54}

Efficient procedures for constructing humanized antibodies have been developed.^{13,14,47,55} The first step is to clone and sequence the complementary DNAs (cDNAs), coding for the variable domains of the mouse antibody to be humanized. The mouse hybridoma cell line is grown in an appropriate culture medium, and cells are harvested for RNA isolation. Polymerase chain reaction (PCR) primers that hybridize to the 5' ends of the mouse leader sequences and to the 5' ends of the mouse constant regions are designed for cloning κ light chain variable regions and heavy chain variable regions. cDNA is synthesized from total RNA, followed by PCR amplification with light and heavy chain specific primers. Positive bacterial colonies containing mouse variable regions are then screened.

Construction of a chimeric antibody involves modifying the cloned mouse leader-variable regions at the 5'- and 3'- ends, using PCR primers to create restriction enzyme sites for convenient insertion into expression vectors, to incorporate sequences for efficient eukaryotic translation, and to incorporate splice-donor sites for RNA splicing of the variable and constant regions. The adapted mouse light and heavy chain leader-variable regions are inserted into vectors containing, for example, human cytomegalovirus enhancer and promoter for transcription, a human light or heavy chain constant region, a neomycin gene for selection of transformed cells, and the simian virus 40 origin of replication in COS cells. These vectors are designed to express chimeric or reshaped human light and heavy chains in mammalian cells.

The design and construction of an engineered human antibody require an analysis of the primary amino acid sequences of the mouse variable regions to identify the residues most critical in forming the antigen-binding site. A structural model of the

mouse variable region is built on the basis of homology to known antibody variable regions. The framework regions (FRs) of the new variable regions are modeled on FRs from structurally similar immunoglobulin variable regions. The design process involves selecting human light and heavy chain variable regions that will serve as templates for the construction of a reshaped human antibody. The mouse CDRs are then joined to the FRs from selected human variable regions. The primary amino acid sequences are then carefully analyzed to ascertain whether they would recreate an antigen-binding site that mimics the original mouse antibody.

Within the FRs, the amino acid differences between the mouse and the human sequences are examined, and the relative importance of each amino acid in the formation of antigen-binding site is evaluated. Minimum changes in the FRs are desirable and should closely match the sequences from natural human antibodies. Any potential glycosylation site in the FRs of either mouse or human sequence needs to be identified and its influence on antigen binding considered.

The DNA sequences coding for the reshaped human variable regions, either made synthetically or based on an existing sequence that is very similar to the newly designed reshaped human variable region, are modified by PCR with specially designed oligonucleotide primers. The human variable regions together with their leader sequences are then cloned into a mammalian expression vector that already contains human constant regions. Each human variable region is linked to the desired constant region via an intron.

Preliminary expression and analysis of the reshaped human antibodies are done by cotransfection of mammalian cell-expression vectors, 1 coding for human light chain and 1 coding for human heavy chain. The vectors will replicate in the COS cells and transiently express and secrete reshaped human antibodies. The concentration of the antibody produced can be analyzed by using an enzyme-linked immunosorbent assay. Specific changes in the amino acids of the framework region may also be required to preserve the orientation and structure of the rodent CDR required for binding. Computer modeling using databases containing human variable genes will identify sequences homologous to the rodent V regions.^{13,47} A computer model of the rodent Fv can identify the non-CDR residues that interact with the CDR sequences, and choices can be made regarding which residues need to be included in the variable region.

Antibodies humanized in this way may have binding affinities up to one-third greater than the corresponding murine antibodies.⁴⁹ Allergenicity is also significantly reduced. About 20 to 40% of patients exhibit a HAMA reaction to murine antibodies,

whereas only about 7% have a similar reaction to humanized antibodies.^{47,53,56–58}

Humanization of MAbs still has several practical difficulties. First, a detailed knowledge of the antibody structure and function is required. Second, methods for efficient construction of humanized MAbs are limited. Third, unpredictable immunogenicity may result when a new amino acid sequence is introduced to balance affinity retention. Fourth, the antibody repertoire is limited to the animal in which the progenitor MAb originated. Fifth, the rodent MAb producing hybridoma *must* be isolated and thoroughly characterized.

However, obstacles to humanization are gradually being surmounted. Karpas et al¹⁵ reported creating a HAT-sensitive and ouabain-resistant human myeloma cell line that can fuse with human lymphoblast cells. A HAT-sensitive subline of the myeloma cells that secreted only λ light chains was fused with EBV-transformed white blood cells that produced IgG MAbs to HIV-1 gp41. Eventually, a clone was isolated that was polyethylene glycol-resistant and would not revert when placed in HAT medium. Standard polyethylene glycol fusion protocol was followed to fuse the myeloma cells with both EBV-transformed white cells and fresh white cells obtained from the peripheral blood of adults and tonsil cells from 2 children. The authors reported promising rates of hybridoma formation, stable Ig production, and high yield of secreted antibodies compared with antibody-producing cell lines from mouse myeloma cells. The authors have now developed 40 hybridomas that have been secreting cells for more than 5 months.

A human myeloma cell line may eventually prove useful in creating MAbs against certain autoimmune diseases and cancers. This technique may also make it easier for other researchers to generate human MAbs for use in therapy.

Antibody Function in Immunotherapy

Antibodies may act directly when binding to a target molecule by inducing apoptosis, inhibiting cell growth, mimicking or blocking a ligand, or interfering with a key function.^{53,59} In addition, antibodies may modulate or potentiate drugs or other therapies. The antibody may itself act as an effector—as in antibody-dependent cellular cytotoxicity (ADCC) or antibody-dependent complement-mediated cascade—or it may involve effector elements such as cytotoxins, enzymes, radioactive isotopes, signals for other parts of the immune system, and/or cytotoxic drugs.^{49,50,60–62} ADCC occurs if Fc regions on the antibodies are recognized by receptors present on cytotoxic cells, such as natural killer cells, macrophages, granulocytes, and monocytes. Complement-mediated cytotoxicity ensues if the antibody binding prompts a complement cascade to

occur. Active immunotherapy can be accomplished if the antigen can provoke a long-lasting T-cell response, which may be achieved by administering whole cancer cell extracts or by using small antigenic peptides isolated from tumors in experimental patients or animals.⁵⁸ For instance, MAbs mimicking breast cancer-specific antigens elicit anti-idiotypic antibodies; this is another way of creating active immunity, which can lead to a humoral auto antibody-like immune response.⁵⁸

The use of bispecific molecules that recognize antigens on both the target cell and effector cell can increase ADCC. The most effective mechanisms are blockade of a crucial ligand or growth factor or ADCC in which tumor cells are killed by Fc receptor-bearing cytotoxic effectors. Cell killing by ADCC is proportional to the amount of antibody bound to a cell, whereas the blockade of an essential growth factor may not show effects until most of its receptor is saturated. A higher antigen expression by the target cell will increase antibody binding and subsequent ADCC. But high receptor expression will also make it difficult to prevent the binding of a cytokine or ligand at minimum threshold.^{53,59,63}

With a bispecific antibody, the specificity of MAb is combined with the cytotoxicity of immune effector cells, for instance, to neutralize a tumor.^{9,45,63,64} Bispecific antibodies link the tumor cell directly to the killer cell via cytotoxic trigger molecules, such as T-cell receptors or Fc receptors, leading to lysis and/or phagocytosis by the effector cells. Although bispecific antibodies can enrich effectors at the tumor site and activate tumor bound effector cells by enabling cross-linking between effector and target cells, they can cause system-wide immune activation because of T-cell receptor cross-linking. Bispecific antibodies can also mediate cellular cytotoxicity via various effector cells, including phagocytes, natural killer cells, and T-lymphocytes.

Another way to use the binding properties of antibodies is by conjugating antibodies to cytotoxic drugs, radioisotopes, or toxins.^{49,58,63,65,66} Techniques for conjugation have been described in several recent reviews and articles.^{49,58,67,70} MAbs can be conjugated to chemotherapeutic drugs such as doxorubicin, mitomycin, and methotrexate. Chemotherapeutic agents constitute cytotoxic or cytostatic drugs that can be conjugated to antibodies; thus far, however, these drugs have shown poor specificity for target cells and frequently lead to toxicity.^{55,62,65} Often, antibodies may lose reactivity upon conjugation with such agents. Immunotoxins used in cancer therapy are conjugated antibodies that combine plant (ricin, gelonin, saponin, abrin, pokeweed antiviral protein) or bacterial (diphtheria toxin and pseudomonas toxin A) toxins with antibody specificity.^{52,59,62,63,65,66,68,69} Toxins may inhibit protein synthesis even at picomolar concentrations. Natural toxins,

such as diphtheria toxin, can act at low concentrations, enter cells, and disrupt key cellular processes.⁶⁹

Enzymes may also be conjugated to antibodies in antibody-directed enzyme-prodrug therapy.^{55,61,62,66,71} An enzyme that can convert a prodrug to an active form may be conjugated to an antibody and targeted to a desired location using the antibody-binding region. They may more effectively localize tumors than chemical conjugates, and a prodrug is converted to an active drug by the enzyme at the target cells.

Another therapeutic technique relies on using antibody fragments, which may be produced by the traditional method (proteolytic digestion with papain) or with newer methods that may reduce damage to the binding sites.^{66,71} Some newer methods attempt to construct mimetics that combine multiple CDRs from several antibodies into single molecules with molecular weights substantially lower than single chain fragments. Because of their smaller size, antibody fragments may be able to penetrate tissues and tumors more readily and be less immunogenic than whole MAbs. However, although antibody fragments may have better penetration than whole antibodies, their shorter half-lives may compromise clinical usefulness.⁶⁸

Heteropolymerized antibodies have recently been developed by chemically linking a mouse IgG MAb, specific to a complement receptor site (CR1) on the human or nonhuman primate erythrocyte, to a second mouse IgG MAb that is specific to the targeted antigen.⁷² The heteropolymer technique is based on immune adherence, in which antibody-antigen immune complexes bind to a complement receptor on the red blood cell, facilitating the phagocytosis of these complexes.⁷² Once introduced into the patient, the heteropolymers will bind to both the red blood cells and the targeted antigen. The antigen-antibody heteropolymer complexes are then transported to the liver and spleen, where the complexes are destroyed by macrophages, whereas the red blood cells return to the circulation unharmed.⁷²⁻⁷⁵ A wide variety of conditions, of both autoimmune and foreign origin, may potentially be treated with this method, including HIV infection, systemic lupus erythematosus, Marburg virus infection, and myasthenia gravis.⁷⁶⁻⁷⁸ Although mouse antibodies are currently being used with this technique, chimeric mouse-human antibodies could also be constructed.⁷² It may also be possible to use the heteropolymers as "sentinels" by injecting them before antigen exposure. In 1 recent study, multiple infusions of heteropolymers provided nonimmunized monkeys with protection against an antigen (Φ 174) for as long as 2 weeks.⁷⁹

Use of Unconjugated MAbs in Cancer

When Milstein and Kohler announced their isolation of a MAb in 1975, many thought MAbs would

provide an effective cancer treatment. However, early clinical trials were disappointing.⁸⁰⁻⁸¹ Producing MAbs to tumor antigens is a complex process. First, proteins peculiar to human cancer cells are identified; then, mice are injected with human tumor cells (antigen) to stimulate an immune response. After about 30 days, the mouse lymphocytes are removed and fused with myeloma cells to isolate and reproduce hybrid cells specific to a predetermined antigen. Rodent antibodies alone have been used in several trials, but the clinical effects are often minimal because of poor activation of human effector or complement cells by the Fc region of the murine antibody and the accompanying HAMA responses.^{55,57} Substituting the rodent Fc region with a human Fc fragment may help overcome these effects.⁸¹

With the discovery of proto-oncogenes, second-generation trials in the 1990s turned to more specific tumor antigens that could be potential targets.⁵⁸ In 1994, MAb 17-1A, an antibody to epithelial cell surface antigen expressed on human colorectal carcinomas, was approved for the identification of adenocarcinomas; MAb17-1A may reduce the mortality and occurrence rate of colorectal cancer.^{82,83} In 1997, rituximab, a mouse-human chimeric anti-CD20 antibody, was approved for the treatment of non-Hodgkin B cell lymphoma.^{53,83} Rituximab binds to CD20 antigen on B cells and B cell tumors and then elicits a natural immune response that can kill malignant cells. Recent animal trials have shown the potential of MAb therapy in cancer.⁵² Increases in epidermal growth factor (EGF) receptor expression have been found on many human cancers, and a fully human anti-EGF receptor IgG2 MAb has been shown to inhibit human cancer growth *in vitro* and *in vivo*.²²

Other efforts are focusing on antibody to HER2 antigen in breast cancer. Patients with cancer often mount an immunologic response to tumor-associated antigens with increases in cytotoxic lymphocytes and antibodies.¹⁴ Several tumor-reactive antibodies have been cloned against melanomas, colon carcinomas, ovarian, breast, and lung tumors. Often, these antibodies cross-react with other malignant tissues or cell lines.¹⁴ Recently, trastuzumab (Herceptin), a humanized MAb that binds directly to the C-erbB2 protein (HER2), has been approved by the FDA for the treatment of breast cancer.^{52,84-89} Herceptin can be used to treat metastatic breast cancer in patients whose tumors overexpress the HER2 protein (about 30% of breast cancer patients) and may be used in conjunction with other therapies such as paclitaxel (Taxol).⁸⁹ Antibody was developed by humanizing murine MAb 4d5 by inserting antigen-binding regions of MAb 4d5 into the framework of a consensus human IgG1, resulting in rhuMab-Her2 or trastuzumab.⁸⁵

Cancer cells have antigens that are specific to the

tumor (tumor-specific antigens) or are present in greater concentration than normal (tumor-associated antigens). Antibodies may eliminate target cells by complement action or through ADCC. The variable region of the antibody recognizes and attaches to a specific antigen and the constant region, then joins with an effector cell capable of killing the targeted cancer cell. When the antigen and antibody bind, precipitation and agglutination may isolate the complex.

Anti-idiotypic MABs that mimic-tumor associated antigens also may be used in cancer therapy.⁸² The idiotypic network hypothesis, formulated by Lindemann and Jerne, suggests that because each mature B cell secretes an antibody with unique antigen-binding specificity in the variable domain (referred to as the idiotypic), anti-idiotypic MABs can be generated and used as surrogate antigens or vaccines for immunization against the tumor.^{82,90}

A murine anti-idiotypic MAB, ACA125, which mimics the tumor-associated antigen CA125, was recently found to induce a specific anti-anti-idiotypic immune response in 28 of 42 patients with platinum-pretreated recurrent ovarian cancer enrolled in a phase I/II clinical trial.^{90,91} A positive immune response was associated with statistically significant ($P < 0.0001$) prolonged survival times (19.9 ± 3.1 months in patients shown to have an anti-anti-idiotypic response compared with 5.3 ± 4.3 months in patients who were anti-anti-idiotypic negative).^{90,91} In addition, peripheral blood lymphocyte mediated lysis of CA-125 expressing tumor cells increased in 9 of 18 patients after vaccination with the MAB.⁹¹ Despite the use of a murine antibody, both this study and a previous phase I study involving patients with ovarian cancer found minimal side effects.^{90,91}

Promising early results have also been demonstrated in patients with advanced colorectal carcinoma who received a murine anti-idiotypic MAB that mimics an epitope of carcinoembryonic antigen (CeaVac)^{82,90} and in patients with malignant melanoma who received an anti-idiotypic MAB (TriGem) that mimics disialoganglioside GD2⁹²

Despite promising developments, however, there are still several obstacles to effective cancer therapy with MABs. Problems with the tumor, the MAB, or conjugate characteristics all continue to challenge researchers.^{52,63,84} Thus far, chemotherapeutic MAB therapies have faced obstacles due to the poor ability of agents to affect tumor cells preferentially over healthy cells, the intrinsic insensitivity of many tumors to these drugs, and the rapid development of resistance in tumor cells.^{65,66,93,94} MABs often decrease the size of tumors, but rarely lead to complete remission of solid tumors.⁵⁹

At the cellular level, MABs at low doses ideally should bind with excellent affinities. Because no antigens have been identified that are expressed

exclusively on tumor cells, cross-reactivity of MABs must be examined with histochemical tests on tissue sections or in animal models. Careful testing must also be done to ensure that the effector molecules such as drugs, toxins, or isotopes in the conjugates do not inadvertently target healthy cells. Although humanized antibodies have greater affinity than murine antibodies, this potential has yet to be translated into improved clinical outcomes against cancer.⁵² However, recent success using the Xenomouse technology to develop a fully human IgG2 MAB specific to epidermal growth factor receptor indicates the potential for future advances in this area.²²

Recombinant Immunotoxins in Cancer Therapy

Recombinant immunotoxins, fusion proteins containing the Fv of a MAB and a bacterial toxin, are under development for cancer therapy. Because immunotoxins exploit only the variable region-binding function of the MAB, in theory just a fragment of the binding region, such as the bivalent F(ab')₂ fragment, monovalent Fab, scFV, or disulfide-stabilized Fv fragments may be used as opposed to the larger and probably more immunogenic whole antibody. Disulfide-stabilized fragments may be more stable or have higher affinity for the antigen than scFV, in which the linker may interfere with binding or fail to stabilize the fragment.⁶⁶ Recombinant immunotoxins derived from *Pseudomonas* enterotoxin have been shown to be active against lymphomas, solid tumors, and leukemias.⁶⁶ Because binding specificity and affinity are the key elements, it is not known which form of the immunoconjugate is usually more effective in treating human tumors; some animal tests suggest that the whole antibody, with its longer half-life and bivalent binding, is more effective than the antibody fragments with decreased binding affinity.

Bispecific antibodies and single chain FVs are being developed that may be more effective because they clear faster from nontumor tissues and more deeply penetrate tumors than whole antibodies.^{49,64,70} Bispecific antibodies may be used to target tumor vascular endothelial cells, thereby limiting the tumor's blood supply and possibly inhibiting its spread.⁷⁰

Use of MAB in Cancer: Radioimmunotherapy

Advances in radiolabeling have allowed immunoconjugates to be delivered to cells and showed promise in clinical trials.^{14,63,80,95,96} Radioimmunotherapy uses a radiolabeled MAB to deliver radioactive isotopes to targeted cells. Radioisotopes such as iodine-131 and yttrium-90, which are β emitters, can cause damage not only to the bound cell but also to cells adjacent to tumor cells that antibodies may not be able to reach within the tumors. Lack of knowledge about the appropriate dose, biodistribution, and shedding of antigen hinders use of radioisotopes.

Radiolabeled MABs may also affect normal cells, depending on the extent to which reticuloendothelial cells expressing Fc receptors bind to the constant regions of intact antibody molecules. Using antibody fragments or constructs may modify this nonspecific uptake.^{63,68,82,93}

Radiotherapy exerts most of its effect by emitting a low dose, which exponentially decreases continuous radiation, but the antibody per se may have a cytotoxic effect. The duration of radiotherapy is determined by the half-lives of the antibody and the isotope used. The success of radioimmunotherapy is affected by the specificity, affinity, dose, and immunoreactivity of the antibody, heterogeneity of antigen expression, diffusion rate, tumor volume, blood supply and tumor location, dose rate effects, and variability in dose deposition. The choice of radionuclide, selection of the chelate used to link the MAB to the radionuclide, and the MAB selected are critical in development of radiolabeled MABs.⁸⁰ Suitable radio nuclides include yttrium-90, iodine-131, and copper-67. Iodine-131 was the first radioisotope to be used in treatment of Hodgkin disease and other lymphomas.⁶⁸ Yttrium-90 is potentially useful for lymphoma therapy because it decays with β , but not γ , emissions that may kill other tumor cells in a cross-fire effect.⁶⁸ The energy released by yttrium is 5 times higher than that of iodine-131, which degrades rapidly after uptake into a tumor cell, causing toxicity.⁸⁰

In addition to their uses in radiotherapy, radiolabeled MABs can also be used to diagnose cancer.^{18,56,57,82,94} Radioactive isotopes linked with MABs may help localize tumors in a form of diagnostic imaging called immunoscintigraphy. For instance, OncoScint, a MAB coupled to indium-111, may be used to detect an antigen (TAG-72) found on colorectal adenocarcinomas.⁵⁶

Use of MABs in Therapy of Autoimmune Diseases: Graft-versus-Host Disease, Crohn Disease, and Rheumatoid Arthritis

MABs may potentially be used to suppress the immune system after transplant or to induce tolerance to transplanted organs or tissues. Thus far, however, only antibodies to CD3 and CD25 are licensed for clinical use.⁹⁷ OKT3, a murine IgG2a antibody to human CD3, and antibodies to CD25 (IL-2 receptor) have been used to reduce allograft rejection.⁹⁷

Graft-versus-Host Disease (GVHD) is a complication of allogeneic stem cell transplant that occurs despite histocompatibility testing and use of cyclosporine and its analogs.⁹⁸ GVHD is a frequent cause of illness or death in allogeneic transplant patients and occurs when alloselective donor T cells recognize and interact with major and minor histocompatibility antiigens in the host, leading to cytokine release.

MAB therapy against GVHD is most effective when it is administered after a bone marrow transplant but before GVHD development by targeting T-cells before their activation.⁹⁹ For instance, 1 target is the interleukin-2 receptor α -chain (IL-2R α Tac protein or CD25), whose expression is a crucial step in the activation of alloreactive T cells.^{97,100} Recently, it was reported that daclizumab, a humanized anti-IL-2a antibody, was an effective complement to dual immunosuppression therapy in renal transplant patients.¹⁰¹ Because only activated cells express IL-2, antibodies to this cytokine might inhibit T cells during allograft rejection and prevent generation of cytotoxic T cells. The development of HAMA response in patients and the decreased effectiveness of murine MAB relative to human MAB have thus far compromised the effectiveness of this approach. However, humanized anti-Tac MABs, with better human effector functions, may survive longer in vivo and may prove less immunogenic than its murine counterpart. Short-term CD4 antibody therapy may also contribute to long-term acceptance of skin and islet allografts, even after gaining immunocompetence.¹⁰²

More generally, the ability of MABs to induce tolerance to transplanted tissues and self-antigens may hold great therapeutic potential.^{97,102} MABs to CD4 and CD8 have been studied, although thus far these have not resulted in clinical success.⁹⁷ Depletion or blockade of T cells by antibodies may facilitate tolerance by preventing T cells from attacking the graft, and recent T cell depletion studies in primates have proven promising.⁹⁷

MAB treatments for other complications after transplants are also under study, including potential treatment of posttransplant lymphoproliferative disorder with the anti-CD20 MAB rituximab and the use of anti-LFA-1 MAB (odulimomab) to protect against ischemia-reperfusion injury after kidney transplants.^{103–105} Daclizumab, a humanized antibody that targets the anti IL-2 receptor, may reduce the risk of acute rejection of a renal transplant and also lower cytomegalovirus infection rates among transplant recipients.¹⁰⁶

The inflammatory bowel disorder known as Crohn disease has also been treated with MAB therapy.¹⁰⁷ A chimeric IgG1k antibody, infliximab (Remicade), acts by binding to soluble and transmembrane tumor necrosis factor α (TNF α), preventing it from binding to its receptors on activated macrophages. The anti-TNF α antibody may provide relief to patients with moderately to severely active Crohn's disease. In addition, the FDA recently approved infliximab for the treatment of rheumatoid arthritis in combination with methotrexate.^{108–111}

Use of MABs in Therapy of Asthma

High levels of IgE may cause bronchial hyperresponsiveness, a risk factor for asthma.^{112–114} Im-

mune responses mediated by IgE are important in the pathogenesis of allergic asthma. In 1 recent study with subjects reporting moderate to severe allergic asthma, twice-weekly injections of recombinant humanized anti-IgE antibody (rhuMAB-E25), which forms complexes with free IgE and blocks its interaction with mast cells and basophils led to a fall in serum IgE levels and slightly decreased asthma symptom scores relative to the placebo group.¹¹² Patients receiving anti-IgE were able to reduce reliance on corticosteroids. Although the study must be interpreted cautiously, it is nonetheless a promising step in finding more effective asthma therapies.^{112,114,116}

Use of MAbs in Therapy of Septicemia

Approximately 400,000 cases of sepsis in the US and about 25,000 cases in the UK are reported each year.^{116,117} The mortality rate could be as high as 40 to 70%, depending on the population studied.^{117,118}

MAbs have been targeted to TNF α and TNF α receptors, which are key elements of the inflammatory response. Unfortunately, this may inhibit many cytokines, which can impair the patients' ability to fight infection and increase the risk of secondary sepsis; also inhibiting a few cytokines may not be sufficient. Although MAbs targeted toward components of the host immune system, such as individual receptors or mediator cells, help in the treatment of septic shock, targeting the bacterial endotoxin or lipid A of gram-negative bacteria with MAbs may be more efficacious. Lipopolysaccharide endotoxin, a component of the outer cell membrane of Gram-negative bacteria, consisting of a highly variable O-linked polysaccharide chain, an R core region, and lipid A, which in turn is composed of a glucosamine disaccharide backbone substituted with amide- and ester-linked long-chain fatty acids, is believed to be important in many cases of septic shock and septicemia, and can trigger an inflammatory cascade that can cause serious injury and even death.^{119,120} Lipid A is linked by the core region to the O-linked side chain, and variations in the O-linked side chains result in an enormous diversity among Gram-negative bacteria, making adoptive immunotherapy against the O-linked antigenic determinants difficult.¹¹⁴

Directing antibodies to the core region or lipid A, which tend to be more conserved, would allow for therapy against a diverse array of Gram-negative bacteria. Favorable results with polyclonal J5 antibody have spurred attempts to develop a MAb.^{116,117} A murine IgM MAb, E5 (XOMA, Berkeley, CA) binds to an epitope on lipid A of *E coli* J5. A human IgM, HA-1A (Centoxin) is derived from a heterohybridoma fused from spleen cells of a patient vaccinated with *E coli* J5 before splenectomy.^{116,117,119,121} Both the antibodies have thus far shown mixed success in

patients with sepsis and neither is currently cleared by the FDA for therapeutic use. Because patients with bacteremia often have endotoxemia, HA-1A, an IgM antibody to endotoxin may be effective against endotoxin in the bloodstream as well. Unfortunately, the high cost of antibody (\$3500/dose in Europe) combined with the uncertainty that the sepsis is caused by Gram-negative bacteria has hindered the widespread use of HA-1A.^{112,115,117} Although it is less efficacious, the only other option may be to target TNF α , but 1 recent study reported a high (54.1%) rate of adverse reactions in patients receiving an anti-TNF α antibody.¹¹⁸ The antibody was no more effective than the placebo given to control subjects. However, research on a MAb for the treatment of septicemia continues.

Use of MAbs in Therapy Against Complications of Viral Infections

Cytomegalovirus (CMV) causes serious illness affecting the immunocompromised, such as patients with AIDS and those undergoing organ transplants. Infection rates may be up to 75% in those negative for CMV who receive kidneys from seropositive patients.¹²² CMV infection can result in retinitis and gastroenteritis in HIV-infected patients and may also cause chronic intrauterine infection.^{122,123} Up to 40,000 cases of congenital CMV infection are reported each year; mental retardation and hearing loss may occur in about 25% of these cases.¹²² Currently, there is no vaccine against CMV. Ganciclovir, foscarnet, and (S)-1-[3-hydroxy-(2 phosphonylmethoxy)propyl]cytosine are some of the potential treatments for CMV infection.¹²² Another mode of treatment is via administration of anti-CMV hyperimmunoglobulin, derived from pooled sera of CMV-seropositive persons. Passive immunization has been shown to reduce the severity of CMV and prevent mother-to-infant transmissions.¹²² Moreover, antibodies may be able to clear the virus from infected tissues, a function previously thought to be exclusive to cytotoxic T lymphocytes. Many physicians use a combination of antiviral agents and immunoglobulins in patients at risk for CMV infection. MAbs may also decrease the amount of antiviral agents required for treatment. MAbs against murine CMV polypeptides have been shown to be protective in animal models.¹²⁴

Although MAbs may act synergistically with foscarnet or ganciclovir in vitro, it is unclear whether this advantage could be extrapolated to in vivo conditions. Experiments in which murine CMV is used as a model system suggest that high concentrations of antibody, along with ganciclovir or (S)-1-[3-hydroxy-(2 phosphonylmethoxy)propyl]cytosine, synergistically inhibit the growth of murine CMV in cell culture, whereas lower antibody concentrations produce only an additive effect. However, antibody ad-

ministered along with ganciclovir in severe combined immunodeficient mice also produced only an additive effect.¹²³ The extent to which a MAb can protect the host from CMV infection cannot reliably be predicted from its immunoreactivity, neutralizing titers in vitro, or from its antigen-binding data, because the mechanism of action of a MAb in vitro and in vivo may be entirely different.¹²⁴

If an antibody is used along with drugs, not only is the treatment efficacy improved but also the side effects of a high drug concentration may be avoided. For instance, 25 mg of ganciclovir/kg of body weight, along with an antibody, was almost as effective as 50 mg/kg ganciclovir administered alone in CMV-infected severe combined immunodeficient mice.¹²⁴ The MSL109 antibody, a human IgG1 directed against human CMV glycoprotein, is marginally synergistic in inhibiting CMV in vitro when conjugated to ganciclovir or foscarnet. In a recent trial involving 209 AIDS patients with active CMV retinitis, the rate of retinitis progression was similar in the group receiving the antibody (60 mg, intravenous) for 2 weeks and the placebo control group.¹²⁵ Surprisingly, mortality increased in the MSL-109 group, and the poor performance of MSL-109 in this trial may have been due to the inability of the antibody to neutralize the established CMV infection in AIDS patients or its failure to cross the blood-ocular barrier in sufficient quantities.

Liver transplant patients are also at risk of CMV infection and may require treatment with the murine MAb OKT3, an anti-T cell antibody that is directed to CD3 antigen, along with ganciclovir to mitigate transplant rejection.¹²⁶ Human MAbs neutralizing CMV were tested for their safety and pharmacokinetics over a period of 3 to 73 days in bone marrow transplant recipients and were found to be safe and efficacious, as evidenced by a steady increase in the neutralizing activity.¹²⁷ Also, a human anti-CMV MAb designated C23 that was purified from hybridomas generated by the fusion of human lymphocytes with mouse myeloma cells has virus neutralization titers about 1000-fold higher than those produced after conventional human γ globulin used in humans.¹²⁸ A humanized MAb has been developed that binds to the 86-kd glycoprotein, gpUL75 (gH), of CMV, recognizes a variety of virus strains, and neutralizes clinical isolates of CMV; it could be used as a potential agent for the prevention or treatment of CMV infections in humans.¹²⁹

Respiratory syncytial virus (RSV) causes serious lower respiratory tract disease requiring considerable supportive care, administration of humidified oxygen, and respiratory assistance. The FDA approved the use of a humanized MAb against RSV, which afflicts mostly infants and children younger than 24 months of age.^{130,131} Known as palivizumab, the antibody (produced by Synagis and MedImmune, Inc.) treatment showed a 55% reduction in

RSV infection in hospitalized patients.^{130,131} The American Academy of Pediatrics' Committee on Infectious Diseases has published recommendations on the use of this antibody in children at risk for RSV infection.^{130,131}

MAbs have also been used in the therapy of HSV infections. The incidence of neonatal herpes is 30 to 50% in babies born vaginally to mothers with primary infection but only 1 to 3% in babies born to mothers with recurrent infections.¹³² This difference is attributed to the passive transfer of protective antibodies to the fetus. Antibodies to HSV-1 may also confer partial protection against type 2 strains. Based on this observation, the potential of MAb therapy against herpes has been studied for more than a decade. Experiments in mice, for instance, indicate that microgram quantities of antibodies may promote healing of corneal opacity and blepharitis caused by herpes simplex.¹³³ However, attempts to confer immunity in humans and other higher animals by passive immunization alone have been disappointing, because antibodies to HSV are restricted to only a few specific epitopes.¹³³ Among the 11 glycoproteins expressed by the herpes virus on its surface, 5 glycoproteins (gB, gD, gH, gK, and gL) are thought to be crucial to infection. The initial step in HSV infection seems to be the binding of gC to heparan sulfate proteoglycans followed by the binding of gB, and the binding of gB and gC is stabilized by gD. gH is involved in initiation of viral fusion and other glycoproteins may help in expanding the fusion process.

Evidence that neutralizing antibodies to gH, gD, and gB prevent membrane fusion but not viral attachment is consistent with this model.¹³³ Most human antibodies are directed against gD and gB epitopes. MAbs can target at least 7 different antigenic sites on HSV glycoprotein D in the murine model.^{133,134} Glycoprotein D is necessary for virus replication in tissue culture. Yamamoto et al¹³⁵ reported the antibody-dependent cellular cytotoxicity effect of HS1, a neutralizing MAb to glycoprotein B of HSV, in athymic nude mice inoculated with HSV intracutaneously. When HS1 is administered, the skin lesions healed in 50% of the mice given the antibody. Also, latent infection in the ganglia was prevented in mice that survived, as evidenced by the failure to detect HSV upon co-cultivation with Vero cells. Administration of HS1 after development of zosteriform lesions (5 to 9 days after infection) reduced the virus in the ganglia and prolonged survival time; however, the disease was not totally avoided and eventually the mice succumbed to the disease. The antibody therapy could prevent or decrease the severity of herpetic keratitis, iritis, and blepharitis after corneal infection by HSV.^{136,137} Thus, MAbs could potentially be used to prevent congenital herpes and sexual transmission of the herpes virus.^{47,133,138,139}

Currently, no treatments exist for the hemorrhagic fever caused by the filovirus Ebola. Recently, a team of researchers at the US Army's Medical Research Institute identified protective antibodies directed against epitopes on Ebola virus membrane-anchored glycoprotein. Some of the antibodies protected mice as long as 2 days after exposure; doses were equivalent to acceptable (3 to 5 mg/kg) human levels. Antibody specificity is important, because some MAbs studied bound to Ebola Zaire but not to the Ivory Coast or Sudan serotypes. Much research remains to be done before the potential of antibodies in the treatment of Ebola will become realized.¹⁴⁰

Use of MAbs in Therapy of Natural and Synthetic Toxins

Toxins are poisonous proteinaceous substances. Antibodies were first clinically used in the 1970s for protection against the toxin digitalis.^{48,102} Today, antidigitalis immunotoxicotherapy is a standard therapy. Digoxin is produced from sheep immunized with digoxin-serum albumin conjugate. IgG antibody specific for digoxin is purified and cleaved into Fabs, because the smaller molecular mass of the fragments allows faster renal clearance and more rapid action than the whole antibody. The Fab fragments bind to circulating digitalis molecules and generate a complex that is unable to bind to receptors. Adverse effects are rare, and the immunotherapy is generally effective against digoxin and digitoxin within 1 hour.^{120,141} However, because polyclonal antibodies may be difficult to produce and may cause hypersensitive reactions, researchers have attempted to develop humanized MAbs against digoxin and other toxins.¹⁴¹ For example, 1 group has used a transgenic mouse to produce hybridoma-secreted human MAbs against digoxin.¹⁴¹ More recently, the effect of goat anti-colchicine IgG antibodies has been studied in mice exposed to a lethal intraperitoneal dose of anti-inflammatory colchicines, and preliminary studies in a 25-year-old woman with colchicine poisoning showed promise.^{102,141}

Attempts have also been made to use drug-specific goat antibody fragments to treat anti-tricyclic antidepressant (TCA) overdose.^{120,141} Antidepressant overdose is the most common cause of intentional drug overdose in the US. However, lethal doses of tricyclics are 10- to 100-fold higher than those of colchicine, digoxin, and snake venom, which require higher antibody doses (up to several g/kg) to reverse the toxicity.^{120,142} High-affinity tricyclic antidepressant-specific MAbs have been shown to reverse the cardiovascular toxicity of antidepressant desipramine in rats and prolong their survival, and tricyclic antidepressant-specific Fab fragments, along with sodium bicarbonate, a standard treatment for tricyclic overdose, also minimizes the required dose of

antibody.¹⁴² The use of smaller antibody fragments, such as single chain Fv fragments, half the size of a Fab, may also be a promising approach because the single chain fragment retains affinity and has shorter half-life in the body.

Monoclonal antibodies may also help to alleviate poisoning due to environmental contamination. Hexachlorobiphenyl, paraquat, atrazine, 3,5,6-trichloro-2-pyridinol, the chief degradation byproduct of the insecticides chlorpyrifos, triclopyr, and chlorpyrifosmethyl, and other chemicals may soon be detected and remediated with the help of antibodies.^{139,143-145} Antibodies may also be used in the treatment of poisoning due to paraquat, hexachlorobiphenyl, domoic acid [amnesic shellfish poisoning], and other contaminants that are otherwise difficult to remove from the body or surrounding environment.¹⁴⁵⁻¹⁴⁸

Use of MAbs in Therapy of Substance Abuse

The primary target of many abused drugs is the central nervous system (CNS), and immunotherapy against such chemicals must be able to penetrate the CNS.¹⁴⁹ PCP or phencyclidine (angel dust) is a type of arylcyclohexamine that affects multiple sites in the brain.^{150,151} It is linked to violent psychotic episodes that are similar to schizophrenia.¹⁴⁹ Treatment is difficult because there is no known antagonist identified, PCP has a high volume of distribution, and about 95% of it is cleared after being metabolized.¹⁵¹ Recently, MAbs have been described that could bind to cocaine or PCP and act like sponges in the bloodstream to prevent them from reaching the brain.¹⁵² It has been shown that a single dose of antibody reduced PCP effects for up to 2 weeks in animals, which might be equivalent to a several months in humans. High-affinity antibody 6b5 fragments against PCP function better in that the fragments with bound PCP are more quickly cleared from the body than the whole antibody-bound PCP.¹⁵³ The crystal structure of the antibody fragment complexed with PCP has been studied in detail.¹⁵⁴ Antibody fragments are also preferable to whole antibodies in the treatment of PCP addiction because of their lower antigenicity and improved pharmacokinetics.^{149,151} Anti-idiotypic antibodies could potentially be produced against the drug-MAb binding site and mimic drug structural features, although these antibodies do not cross the blood-brain barrier.¹⁵¹ By increasing protein binding in the vascular compartment and lowering the drug's volume of distribution, the antibody acts like a pharmacokinetic antagonist.¹⁵⁰ Evidence that the antibody can also reverse effects of other potent arylcyclohexylamine drugs suggests that antibody medications can be used to treat different classes of drugs.^{150,151} An anti-PCP IgG has been produced

from a hybridoma cell line using both ascites and bioreactor methods, and the pharmacological and immune specificity of the antibody has been confirmed by administering the anti-PCP fragment to rats in three different studies without producing observable effects in behavior.¹⁵⁵ Active immunotherapy against methamphetamine addiction is also being contemplated.¹⁵⁶

Antibody therapy for cocaine addiction is also a possibility, especially with the development of catalytic antibodies having enzyme-like characteristics that can be used to inactivate drugs.^{151,157–159} Antibodies with esterase activity have been successfully mimicked, and because cocaine is believed to be metabolized by *in vivo* esterases, such antibodies would be useful in the treatment of cocaine addiction.¹⁵¹ Catalytic antibodies probably would not be as useful as high-affinity anti-cocaine antibodies, but could be used in medical emergencies or as part of a withdrawal therapy.¹⁵¹ Potential antibody applications against cocaine addiction have been tested in rats with a vaccine approach by (1) attaching cocaine molecules to a carrier protein for immunizing rats to produce antibodies against cocaine and (2) humanizing anticocaine antibodies derived from rats and producing them in bacteria.^{152,160} A catalytic MAb (15A10) has recently been reported to attenuate cocaine's cardiovascular effects in mice.^{155,159}

Conclusion

About 200 years have elapsed since Edward Jenner vaccinated a young child against smallpox. Since that time, the field of immunology has evolved at a rapid pace and has yielded many critical developments. Although vaccination has thus far proven to be the most cost-effective method of preventing diseases worldwide, the development of MAbs that use the specificity of immunological responses is one of the most successful applications of immunology to date. Chimeric and humanized antibodies have reduced the risk of allergenicity from exposure to nonself antibodies and heightened the clinical effectiveness of MAb treatments. Developments in radiology and pharmacology have allowed radiolabeled and immunoconjugated antibodies to be produced. Antibody fragments, heteropolymers, and bispecific antibodies are now available in addition to whole MAbs. These promising developments may soon allow MAbs to be used to treat afflictions as varied as substance abuse, cancer, asthma, viral infection, septicemia, and poisoning. As Hedy Zola observed in 1995, "The therapeutic application of MAbs, whilst still limited in scope, promises to break its substantial shackles and realize the potential forecast by its proponents."⁴⁶

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