Monoclonal Antibodies in Diagnosis and Therapy

Thomas A. Waldmann

Monoclonal antibodies have been applied clinically to the diagnosis and therapy of an array of human disorders, including cancer and infectious diseases, and have been used for the modulation of immune responses. Effective therapy using unmodified monoclonal antibodies has, however, been elusive. Recently, monoclonal antibody–mediated therapy has been revolutionized by advances such as the definition of cell-surface structures on abnormal cells as targets for effective monoclonal antibody action, genetic engineering to create less immunogenic and more effective monoclonal antibodies, and the arming of such antibodies with toxins or radionuclides to enhance their effector function.

THE DEVELOPMENT OF MONOCLONAL ANTIBODY TECHNOLogy by Köhler and Milstein (1) provided an enormous opportunity for examination of a range of previously elusive issues. For example, monoclonal antibodies are being used in radioimmunoassays, enzyme-linked immunosorbent assays, immunocytopathology, and flow cytometry for in vitro diagnosis, and in vivo for diagnosis and immunotherapy of human disease. However, in the area of immunotherapy, monoclonal antibodies are just beginning to fulfill the promise inherent in their great specificity for recognizing and selectively binding to antigens on cells.

Monoclonal antibodies have largely been applied clinically to the diagnosis and therapy of cancer and the modulation of the immune response to produce immunosuppression for treatment of autoimmune and graft versus host diseases (GVHD) and for prevention of allograft rejection. Human monoclonal antibodies have also been applied clinically against cytomegalovirus, Varicella zoster virus, and the various specific serotypes of Pseudomonas aeruginosa, Escherichia coli, and Klebsiella pneumoniae. For example, in a multicenter clinical trial involving 200 patients with Gram-negative bacteremia, mortality was reduced in patients who received an immunoglobulin M (IgM) monoclonal antibody (HA-1A) that binds specifically to the lipid A domain of endotoxin (2). In other studies, monoclonal antibodies that are specific for leukocyte adhesion molecules, such as lymphocyte functional antigen–1 (LFA-1) or intercellular adhesion molecule–1 (ICAM-1), the adhesion partner of LFA-1, have been used to inhibit accumulation of neutrophils and thereby reduce tissue damage in animal models of bacterial meningitis, hemorrhagic shock, and myocardial reprofusion injury (3).

The use of monoclonal antibodies has also been proposed for therapy of myocardial infarction (4), for reversal of drug toxicity (digitalis intoxication), and for fertility control. Despite this wideranging interest, the "magic bullet" of antibody therapy that has been the dream of immunotherapists since the time of Paul Ehrlich has proved to be elusive (5). Only one monoclonal antibody, OKT3, has been licensed for clinical use. Furthermore, the initial use of unmodified murine monoclonal antibodies in human patients with cancer was disappointing, with only 23 partial and 3 complete remissions reported among the initial 185 patients included in 25 clinical trials (6). A number of factors explain the low therapeutic efficacy observed. Unmodified murine monoclonal antibodies are immunogenic and elicit a human immune response to the murine antibodies. Moreover, most mouse monoclonal antibodies are not cytocidal against neoplastic cells in humans because the antibodies do not participate in human complement or cell-mediated cytotoxicity. In most cases, the antibodies were not directed against a vital cell-surface structure such as a receptor for a growth factor required for tumor cell proliferation. In an attempt to circumvent these problems, researchers have developed human and humanized antibodies, prepared by genetic engineering, that are less immunogenic than murine antibodies. Cytotoxic action has been augmented by arming the antibodies with toxins or radionuclides. Finally, cell surface antigenic targets have been defined for effective monoclonal antibody action.

In this article I summarize information on in vivo use of monoclonal antibodies for diagnosis and therapy, with special reference to the most seminal discoveries and recent advances in (i) definition of cell-surface structures on abnormal cells as targets for effective monoclonal antibody action, (ii) development of genetic engineering approaches for creating more effective agents, and (iii) development of techniques for arming monoclonal antibodies with radionuclides or toxins and thereby increasing effector function. Comprehensive recent reviews provide more detail (7–9).

Cell Surface Antigenic Targets

The ideal immunosuppressive monoclonal antibody would be one that abrogates responses to a defined antigen and preserves responses to all others. However, the monoclonal antibody that is most widely used clinically (OKT3) is directed against the CD3 (cluster of differentiation) antigen of the T cell receptor complex that is expressed on virtually all circulating T cells. The Food and Drug Administration licensed the use of OKT3 for the treatment of acute renal allograft rejection on the basis of randomized clinical trials showing the superiority of this treatment (93% reversal of acute rejection episodes) to more conventional, broad-spectrum immunosuppressive agents (75% reversal) (10). There are, however, toxicities associated with the use of OKT3. Antibodies to CD3 initiate in vivo T cell activation that is accompanied by release of tumor necrosis factor, interferon γ, and, in some cases, interleukin-2 (IL-2), which leads to an acute clinical syndrome that involves high fever, vomiting, diarrhea, and occasionally respiratory distress.

The author is in the Metabolism Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

Furthermore, therapy with antibody to CD3 leads to broad immunosuppression that is associated with an increased incidence of infections and B cell neoplasms.

Monoclonal antibodies have been used to target the polymorphic α and β subunits of the human T cell antigen receptor (TCR). In pilot studies, one monoclonal antibody (BMA 031) specific for the constant region of the TCR was used successfully in treatment of acute organ allograft rejection and GVHD (11). Monoclonal antibodies that specifically identify the hypervariable antigen-binding region of the TCR represent more antigen-specific agents. Janson and co-workers (12) prepared a murine monoclonal antibody reactive with an idiotypic determinant of the hypervariable region of the TCR expressed by a patient's malignant leukemic cells. There was an 80% reduction in the number of leukemic cells after infusion of this monoclonal antibody. However, associated toxic side effects included fever, chills, nausea, vomiting, diarrhea, and shortness of breath. Monoclonal antibodies directed toward idiotypic determinants of the TCR may also be useful in the treatment of autoimmune disease because it has been observed that there is an extreme restriction in the TCR variable region usage in the T cells responsible for experimental allergic encephalomyelitis (EAE), a murine model of multiple sclerosis. The majority of T cells specific for myelin basic protein that can transfer the disease express a specific Vβ8 phenotype (13). Furthermore, in vivo treatment with a monoclonal antibody to Vβ8 provided protection from disease transfer by encephalitogenic clonal T cells. Evidence for such restricted usage of the TCR variable region is being sought in spontaneous human neurological diseases, including multiple sclerosis and tropical spastic paraparesis.

Most anti-idiotypic antibodies utilized in treatment of human lymphoid malignancies are directed toward B cell immunoglobulin idiotypes. Idiotypic monoclonal antibodies to immunoglobulin were used (14) for treatment of patients with B cell lymphomas. The results were encouraging; one patient manifested an uninterrupted remission for more than 4 years after therapy. A partial response was observed in approximately half of the remaining patients. However, there were certain difficulties. During maturation, B cells use somatic hypermutation to increase their diversity. Thus, there is a tremendous idiotypic heterogeneity within the B cell population, which permits some neoplastic B cells to escape the attack of an antibody directed toward an individual idiotype. The TCR does not undergo somatic hypermutation and is less susceptible to this problem.

Monoclonal antibodies that recognize surface molecules that facilitate cell-cell interactions are also effective immunosuppressive agents. For example, antibodies specific for LFA-1 or its adhesion partner, ICAM-1, inhibit a series of T cell functions and thereby inhibit allograft rejection and GVHD (15). The CD4 and CD8 proteins, which are important in T cell activation, recognition of major histocompatibility complex gene products, and cooperative cellular interactions, are also useful targets. In animal models, antibodies to CD4 or CD8 suppressed allograft rejection, GVHD, and autoimmune reactions (16). Certain antibodies to CD4 are of special interest because they induce immunological tolerance in mice to simultaneously administered human, rat, and rabbit immunoglobulins. This form of tolerance was induced in T helper cells but not in B cells (17), and thus CD4 cells may be made tolerant when simultaneously confronted with certain antigens and monoclonal antibodies to CD4. Effective CD4 monoclonal antibody tolerance therapy is not confined to special protein antigens but is exploitable in transplantation. For example, short-term administration of a monoclonal antibody to CD4 induced cellular depletion and led to indefinite survival of adult pancreatic islet cell transplants in mice without subsequent immunotherapy (18). Furthermore, administration of antibodies to CD4 ameliorated Type II collagen-induced arthritis in rats, murine EAE, and autoimmune myasthenia gravis (16).

The monoclonal antibodies to primate CD4 examined to date are not effective in tolerance induction in subhuman primate models. Despite this limitation, the administration of monoclonal antibodies to CD4 has led to improvement in preliminary clinical trials involving patients with severe rheumatoid or psoriatic arthritis (19).

The inducible α chain of the interleukin-2 receptor (IL-2Rα) expressed on the surface of activated or abnormal T cells is also a target for effective monoclonal antibody immunotherapy (20). Interleukin-2 receptor (IL-2R) is a useful target because resting cells do not express the IL-2Rα, whereas the T cells participating in allograft rejection and abnormal T cells in certain autoimmune disorders express this receptor. To exploit this difference in expression, we used anti-Tac, a monoclonal antibody that blocks the binding of IL-2 to IL-2Rα, in the treatment of patients with human T cell leukemia/lymphoma virus–I (HTLV-I)–associated, IL-2Rα–expressing adult T cell leukemia (ATL) (20). The 20 patients treated in this study did not suffer any untoward reactions. Of the 20 treated patients, seven had remissions, three of these partial, one mixed, and three complete, lasting from 1 to more than 17 months after anti-Tac therapy. Unmodified anti-Tac has also been used for prevention of early allograft rejection episodes in patients receiving renal allografts. The IL-2/IL-2R system offers a variety of other possibilities for relatively specific immune intervention strategies that will be considered below.

Genetically Engineered Antibodies

Although murine antibodies are of value in therapy of human diseases, their effectiveness is limited because rodent monoclonal antibodies have a short survival time in humans and induce an immune response that neutralizes their therapeutic effect. Furthermore, the responses induced by murine antibodies are limited because they only weakly recruit human effector elements and are relatively ineffective as cytocidal agents. To circumvent these difficulties, genetically engineered antibody variants were produced that combine the rodent variable or hypervariable regions with the human constant or constant and variable framework regions (21–25). The ability to genetically engineer antibodies represents a quantum leap in immune intervention that is comparable to the immunological revolution initiated by the introduction of monoclonal antibodies.

After the demonstration that lymphoid cells can express cloned transfected immunoglobulin genes, mouse-human chimeric monoclonal antibodies to tumors were generated with specificities directed toward antigens expressed by colorectal, mammary, pancreatic, and B cell and T cell malignancies (21, 22). Jones et al. (23) proposed that because V domains represent a framework of β sheets topped with antigen-binding loops and because β-framework structures of most crystallized antibodies are nearly invariant, the specificity of the antibody combining site might be independent of the framework region. Thus, to further reduce the immunogenicity of rodent elements, Winter and colleagues generated humanized antibodies that retained only the antigen-binding complementarity-determining regions (CDRs) from the parent rodent monoclonal antibody in association with human framework regions (23, 24). Unfortunately, in some cases humanized antibodies produced by this approach have reduced binding affinity for antigen when compared to the original rodent antibody. Queen and co-workers (25) addressed this problem in two ways. First, the human framework was chosen to be as homologous as possible to the original mouse antibody in order to reduce deformation of the transplanted mouse CDRs. Second, computer modeling was used to identify several amino acids in the mouse antibody framework that, although outside the CDRs, were

likely to interact with the CDRs or antigen. These specific amino acids were retained in the humanized antibody.

One of the goals in the generation of humanized antibodies is the reduction of their immunogenicity. Although humanized antibodies are less immunogenic than their murine counterparts (26–28), the idiotypic element may be immunogenic. Furthermore, the presence of allotypes on the human immunoglobulin G (IgG) framework may provide foreign carrier determinants, thereby enhancing immune responses to the idiotypic element (29). In the case of anti-Tac, the humanized version was dramatically less immunogenic than the parent murine monoclonal antibody when administered to cynomolgus monkeys (26). Similarly, in the first clinical trial of a chimeric antibody, a murine human chimeric IgG1 antibody specific for a gastrointestinal tumor antigen elicited an antibody response against the chimeric antibody in only one of ten patients studied (27). Furthermore, no anti–monoclonal antibody response was detected in two patients with non-Hodgkin's lymphoma studied who received CAMPATH-1, an antibody recognizing an antigen expressed by human lymphocytes (28). The pharmacokinetics of an antibody molecule may also be altered genetically. The survival time of a monoclonal antibody can be altered to increase its period of effective action or, alternatively, to accelerate its clearance. For example, the pharmacokinetics of radiolabeled humanized anti-Tac differed substantially from that of murine anti-Tac when administered to normal cynomolgus monkeys, with a prolongation of the survival half-time of humanized anti-Tac to 103 hours, as compared to 38 hours for murine anti-Tac (26). Prolonged survival of humanized monoclonal antibodies in humans was observed by LoBuglio and co-workers (27). The catabolic rate of an immunoglobulin is determined by the CH2 domain of the Fc region of the immunoglobulin. Thus, the longer survival of humanized antibodies observed probably reflects replacement of the murine IgG CH2 domain with the human IgG CH2 domain (30, 31).

Antibodies or their fragments can also be genetically engineered to have more rapid clearance. This might be desirable when a monoclonal antibody is conjugated to a radionuclide for use in radioimmunoscanning. For example, antigen-binding fragment (Fab), $F(ab')_2$, or single chain Fv fragments of monoclonal antibodies have survival half-lives of less than 5 hours. Rapid turnover can also be accomplished by the deletion of the CH2 domain as demonstrated for an antibody reactive with the disialoganglioside GD2 expressed on human tumors of neuroectodermal origin (32).

Effector functions can be improved by introduction of human constant regions that impart biological activity to a murine antibody that lacks effector function but has the desired binding specificity. The human IgG subclasses differ in their antitumor activity and in their capacity to induce complement or antibody-dependent cell-mediated cytotoxicity. The IgG1 subclass appears to be superior to the other subclasses in most functions (33). The human IgG1 versions of the CAMPATH-1 antibody and the L6 monoclonal antibody to a carcinoma-associated antigen were more effective in antibody-dependent cell-mediated cytotoxicity (ADCC) than the parent rodent antibodies (8, 24). Furthermore, the humanized version of anti-Tac induces ADCC with human mononuclear cells, a function absent from the original mouse monoclonal antibody (34).

Human Monoclonal Antibodies

One solution to the problems of immunogenicity and poor recruitment of effector functions characteristic of rodent monoclonals is to produce human monoclonal antibodies. Human antibodies of appropriate specificity and of high affinity have been difficult to isolate. The use of mouse myeloma cells as fusion partners for human cells often leads to preferential loss of human chromosomes and instability of the hybrids. For ethical reasons one cannot immunize humans with certain tumor antigens. An alternative, the immortalization of human cells by Epstein-Barr virus, often generates lines that produce only low amounts of IgM-type antibodies. Human antibodies may be produced more easily with the use of SCID-hu mice (35), that is, immunodeficient mice reconstituted by human peripheral blood or human fetal thymus, bone marrow, and lymph nodes. When peripheral blood is used, antibodies may be produced if the donor has already been primed with antigen. Such antibodies have not yet been used in therapeutic trials.

An alternative approach to the production of human monoclonal antibodies has been reported that bypasses hybridoma technology (7, 36, 37). The immunoglobulin V-region genes from B cells were cloned with the use of the polymerase chain reaction technique. The antibody derivatives were then expressed in E. coli and screened for ability to bind antigen. Initially, heavy chain V regions were expressed alone or with an irrelevant light chain V region. However, a large combinatorial library of the immunoglobulin repertoire of the mouse in phage lambda has now been generated (36). Heavy and light chain libraries were prepared in phage lambda and used to generate a large array of random heavy plus light chain pairs expressed in bacteria in the form of Fab molecules. The screening for binding of antigens to hapten was rapid and permitted the analysis of many monospecific Fab-producing clones. In similar studies, Mullinax and co-workers (37) identified human antibody fragment clones specific for tetanus toxoid in a bacteriophage lambda immunoexpression combinatorial library prepared using messenger RNA derived from human peripheral blood lymphocytes. The bacteriophage clones are directly amenable to genetic manipulation for preparing complete immunoglobulins of the desired isotype. Further studies will be required to determine whether this approach will allow the efficient identification of human monoclonal antibodies of sufficiently high affinity for clinical studies.

Bifunctional Antibodies

Antibodies with two distinct binding activities have been generated to deliver radionuclides, toxins, cytotoxic drugs, or host cytotoxic cells to specific cellular targets (38). Their predominant use has been to direct cytotoxic cells to target and lyse cells that they normally would not lyse. Such bifunctional antibodies have been prepared by chemical cross-linking, disulfide exchange, or the production of hybrid-hybridomas (quadromas). Bispecific antibodies have also been produced by introduction of two sets of immunoglobulin heavy and light chains into myeloma cells or by construction of single-peptide bispecific antibodies with the use of peptide linkers between the variable domains of two distinct monoclonal antibodies. To be effective, the bispecific antibody must retarget the cytotoxic cell from its natural ligand to the ligand identified by the monoclonal antibody. Furthermore, the antibody must activate the cytotoxic cells into functional effectors without the normal major histocompatibility complex– and antigen-specific restrictions. The most effective bispecific monoclonal antibodies have the CD3 antigen on cytotoxic T cells or the CD16 Fc γ R III receptor on natural killer cells as their nonantigen specificity. Bispecific antibodies with specificity against both tumor targets and CD3 or CD16 effector cells are effective in mediating the killing of tumor cells in vitro and in vivo. For example, murine anti-Tac does not participate in ADCC with human mononuclear cells. In contrast, murine anti-Tac–anti-CD3 and anti-Tac–anti-CD16 bifunctional agents used in conjunction with peripheral blood mononuclear cells killed targets that express IL-2 receptors. A universal

bispecific antibody for retargeting effector cells to tumor cells can be generated with the use of a bispecific hybrid antibody with dual specificities for CD3 and for a rat immunoglobulin light chain allotype (39). This bispecific antibody mediates retargeting of effector cells to a range of tumor cells, each coated with rat monoclonal antibodies bound to surface antigens.

Human T cells that had been retargeted by bifunctional antibodies were used to treat established human ovarian carcinoma in a nude mouse model (40). Peripheral blood lymphocytes from patients were incubated overnight with IL-2 and treated with heteroconjugates containing antibody to CD3 cross-linked to an appropriate antibody to tumor and were injected interperitoneally into tumor-bearing mice. Tumor growth was inhibited. Bispecific antibodies have also been used in nontumor systems. For example, thrombolysis was enhanced by targeting of tissue plasminogen activator (tPA) by bispecific antibodies to tPA and fibrin (4). Thus, although problems associated with their manufacture remain to be resolved, bispecific antibodies show great promise as therapeutic agents.

Monoclonal Antibody–Cytotoxic Agent Conjugates

The limited efficacy of many unmodified monoclonal antibodies led to an alternative approach, the use of these agents as carriers of cytotoxic substances. An array of toxins of bacterial and plant origin have been coupled to monoclonal antibodies for production of immunotoxins (8, 41). The strategy is to select from nature a toxic protein and then to modify the toxin so that it will no longer indiscriminately bind and kill normal cells but will instead kill only the cells expressing the antigen identified by the monoclonal antibody. The majority of toxins targeted to cell surfaces by immunoconjugates act in the cytoplasm, where they inhibit protein synthesis. After binding to cell surface antigens, immunotoxins are taken up by endocytosis and delivered to endosomes. Fragments of some toxins (for example, diphtheria toxin) are then translocated across the membrane of this organelle. Other immunotoxins (for example, ricin) are routed further to the trans-Golgi network, where a minority undergo translocation to the cytoplasm. Unfortunately, most are routed to lysosomes, where they are degraded. In the cytoplasm, the toxins used clinically act either to adenosine diphosphate (ADP)–ribosylate elongation factor 2 (for example, Pseudomonas exotoxin) or to inactivate the 60S ribosomal subunit so that it has a decreased capacity to bind elongation factor 2 (for example, ricin). Less than ten toxin molecules in the cytoplasm are sufficient to kill the cell; however, more must bind to the cell surface to compensate for the inefficiencies in internalization and translocation.

Although immunotoxins are simple in concept, the first-generation immunotoxins were relatively ineffective. Several requirements must be fulfilled for an immunotoxin to be effective (41). In particular, (i) the immunoconjugate should be specific and should not react with normal tissues. Binding to tissues that do not express antigen can be reduced by removal of the nonspecific natural cell–binding subunits or domains of the toxin. Furthermore, because plant glycoprotein toxins contain mannose oligosaccharides that bind to cells of the reticuloendothelial system and, in some cases, also contain fucose residues that are recognized by the receptors on hepatocytes, deglycosylation of plant toxins may be required to avoid rapid clearance and potential cytotoxic effects on these cells. (ii) The linkage of the toxin to the antibody should not impair the capacity of the antibody to bind antigen. (iii) The immunotoxin must be internalized into endosomic vesicles. Thus, toxins directed by monoclonal antibodies to surface receptors that are normally internalized may be more active than those directed

toward noninternalizing cell surface molecules. (iv) The active component of the toxin must translocate into the cytoplasm. These various goals can be in conflict; thus, the removal of the B chain of ricin reduces nonspecific binding but also reduces the capacity of the residual A-chain monoclonal antibody to translocate across the endosomic vesicle membrane. (v) For in vivo therapy, the linkage must be sufficiently stable to remain intact while the immunotoxin passes through the tissues of the patient to its cellular site of action. The first generation of heterobifunctional cross-linkers used to bind the toxin to the monoclonal antibody generated disulfide bonds that were unstable in vivo. This problem was solved in part by the synthesis of more stable cross-linkers, which used phenyl or methyl groups, or both, adjacent to the disulfide bond to restrict access to the bond.

The development by Pastan and co-workers (41) of IL-2R–directed Pseudomonas exotoxin (PE) conjugates for the treatment of IL-2R–expressing ATL demonstrates recent progress in the development of effective immunotoxins. PE, chemically coupled to anti-Tac, showed specificity in vitro (42). However, only a few milligrams of this agent could be given to patients without producing liver damage because the toxin had not been sufficiently changed so that it would no longer bind to normal liver cells. Functional analysis of deletion mutants of the 66-kD PE (43) showed that Domain III was responsible for ADP-ribosylation of elongation factor 2; Domain II helped in translocation of the toxin to the cytosol, whereas Domain I was responsible for unwanted ubiquitous cell binding. A PE molecule from which the 26-kD Domain I had been deleted (PE40) had full ADP-ribosylating activity but extremely low cell-killing activity when used alone. PE40 conjugated to anti-Tac inhibited protein synthesis in T cell lines expressing Tac but not in lines not expressing the IL-2R. However, immunotoxins made by chemical attachment of this truncated PE to anti-Tac yielded a product that was heterogeneous. Active single-chain Fv fragments of antibodies have been produced in E. coli by linking of the light and heavy chain variable domains with a peptide linker (44). Chaudhary and co-workers (45) used this genetic engineering approach to produce a single-chain antibody toxin fusion protein [anti-Tac (Fv)-PE40] in which the variable regions of anti-Tac were joined in peptide linkage to PE40 to generate an immunotoxin that was cytotoxic to human cell lines bearing IL-2R and to freshly obtained ATL cells but not to receptor-negative cells.

The antitumor activity of immunotoxins has been evaluated in animal models since their introduction (46). Treatment usually delayed the appearance of tumors and prolonged the lifespan of the animals. In a few cases, there was complete regression of the tumor.

Several in vivo clinical trials in humans have involved the parenteral administration of immunotoxins. In certain cases, for example, with monoclonal antibody–toxin conjugates directed toward breast or ovarian cancers, severe neurological toxicity was observed because of an unanticipated crossreactivity of the monoclonal antibody with an antigen in the central nervous system (47). The most common toxicity observed was capillary leakage that resulted in hypoalbuminemia, edema, fatigue, and myalgia.

The results of in vivo clinical trials in patients with cancer with first-generation immunotoxins did not fulfill the hopes engendered by in vitro and animal model studies. There were only two complete remissions and eight partial remissions among the 127 patients treated in ten clinical trials with toxin-conjugated monoclonal antibodies directed toward ovarian, breast, colorectal, and lymphoid neoplastic cells. More encouraging results were obtained when benign diseases were treated with immunotoxins or when modified immunotoxins were used in therapy of patients with cancer. For example, 9 of 22 patients manifested a mixed or partial response, and one had a complete remission after treatment of a refractory B

cell leukemia-lymphoma with a monoclonal antibody to CD19 conjugated to a modified ricin toxin that had galactose-binding sites blocked sterically (48). Furthermore, 22 of 32 evaluable patients with GVHD had a favorable early response in at least one organ after therapy with monoclonal antibody to CD5 conjugated to ricin A (49).

An alternative approach for the delivery of cytotoxic agents to cancer cells involves the use of monoclonal antibodies as carriers for enzymes to tumor cell surfaces (50). The enzymes are chosen for their ability to convert drug precursors injected parenterally into active antineoplastic drugs. The active cytotoxic agents formed can then penetrate nearby tumor cells and cause the death of these cells. A number of prodrugs (drugs in an inactive form that can be transformed at the tumor into active anticancer drugs by antibody-enzyme conjugates) have been developed. The antibody-enzymes conjugates were shown to localize to tumors through the activity of the monoclonal antibodies that bind to tumor-associated antigens. In vivo studies showed that prodrug administration after monoclonal antibody–enzyme infusion can result in antitumor activities significantly greater than the activities of the prodrugs, drugs, or monoclonal antibodies given alone.

Radiolabeled Monoclonal Antibodies

Toxin conjugates do not pass easily from the endosome to the cytosol. Furthermore, the toxins are immunogenic and thus provide only a short therapeutic window before the development of antibodies directed toward the toxin. Radiolabeled monoclonal antibodies have been developed as alternative immunoconjugates for delivery of a cytotoxic effector to target cells and for radioimaging (8, 51). Radioimmunodetection with the use of radiolabeled monoclonal antibodies, most often with monoclonal antibodies to carcinoembryonic antigen, is widely used to complement other approaches for tumor detection. Although intact IgG antibodies are retained better by tumors and thus appear to be better for therapy, F(ab')$_2$ and Fab fragments are preferred for imaging because both targeting and blood clearance are more rapid, which reduces the background. Tumors as small as 0.5 cm, which are sometimes missed by other radiological methods, can be imaged with antibodies or antibody fragments labeled with suitable radionuclides.

One advantage in the use of radiolabeled monoclonal antibody conjugates for therapy is that with the appropriate choice of radionuclide, radiolabeled monoclonal antibodies can kill cells from a distance of several cell diameters and may therefore kill antigen-negative cells adjacent to antigen-expressing cells. Furthermore, the radiolabeled antibody need not be internalized to kill the tumor cell.

In a radiolabeled monoclonal antibody, the radionuclide must be tightly linked to the antibody either directly or by a bifunctional chelate. For a monoclonal antibody–chelate complex to be effective, it must meet criteria in addition to those that are true for all monoclonal antibodies: (i) the chelating agent coupled to the monoclonal antibody should not compromise antibody specificity; (ii) the chelation and radiolabeling procedure should not alter the distribution and catabolism of the monoclonal antibody; and (iii) the bifunctional chelate should not permit elution and thus premature release of the radiolabeled metal in vivo. Failure to fulfill this last requirement has led to unacceptable toxicity and reduced efficacy. There are a number of suitable α-, β-, and γ-emitting radionuclides. Isotopes emitting β particles, although superior to γ-emitting radionuclides, are not optimal because their low linear energy transfer released over a relatively long distance results in inefficient local killing of target cells coupled with toxicity to distant normal tissues. Nevertheless, β-emitting radionuclides such as ^{131}I,

^{90}Y, ^{186}Re, ^{188}Re, and ^{67}Cu have been useful in immunotherapy. For example, hepatoma-bearing patients have been successfully treated with ^{131}I-labeled antibodies to ferritin (52). Furthermore, ^{90}Y-labeled antibodies to ferritin combined with autologous marrow transplantation resulted in complete remissions in four of eight patients with Hodgkin's disease (52). ^{90}Y-labeled anti-Tac was effective in prolonging the survival of cardiac allografts and xenografts in a subhuman primate model (53). In a subsequent trial, ^{90}Y-labeled anti-Tac was evaluated for the treatment of patients with HTLV-I–associated, Tac-expressing ATL. At the doses used (5 and 10 mCi per patient), no toxicity was observed in five of six patients studied; modest granulocytopenia and thrombocytopenia were observed in one patient. Five of these six patients underwent a sustained partial or complete remission after ^{90}Y-labeled anti-Tac therapy.

Future development of isotopic monoclonal antibody chelates may focus on α-emitting radionuclides, which may be the most effective at killing tumor targets without damaging distant normal tissues (51, 54). Radionuclides emitting α particles release high energy emissions (6 to 9 MeV, ten times as great as β- or γ-emitters) over a short distance (40 to 80 μm) and are efficient at killing individual target cells, such as those found in leukemia, without significantly penetrating normal tissues. Under hypoxic conditions that permit little cellular repair, α irradiation is efficient at killing cells. Suitable α-emitting nuclides available for immunotherapy include ^{211}At, ^{212}Pb, and ^{212}Bi. ^{212}Bi chelated to anti-Tac was used with a limiting dilution colony-forming assay to demonstrate that ^{212}Bi conjugated to anti-Tac was well suited for immunotherapy (54). Activity levels of 0.5 μCi, or the equivalent of 12 rad/ml of α irradiation, targeted by ^{212}Bi-labeled anti-Tac eliminated greater than 98% of the proliferative capacity of IL-2R–expressing cells with only a modest effect on IL-2R–negative lines. Thus, one of the most promising directions for future development of armed monoclonal antibodies for the treatment of cancer involves the chelation of α-emitting radionuclides to human or humanized monoclonal antibodies. Such conjugates may prove to be relatively nonimmunogenic agents that are effective in eliminating malignant cells when used alone or as part of multimodality treatment with conventional chemotherapy.

Since the development of monoclonal antibody technology, the medical community has applied these agents to in vivo diagnosis and therapy of human disease. Recent advances in linkage of toxins and isotopes to monoclonal antibodies and in genetic engineering of antibodies has led to reduced immunogenicity and has improved effector function, thus providing new potential for the prevention of allograft rejection and for the treatment of neoplastic, infectious, and autoimmune diseases. We have come close to fulfilling the vision of Ehrlich who, in ending his Croonian lecture, *On Immunity with Special Reference to Cell Life* in 1900, stated, "It is to be hoped that such immunisations as these, which are of great theoretical interest, may also come to be available for therapeutic application. The idea has already been mooted by v. Dungern, of attacking epithelial new formations, particularly carcinoma, by means of specific 'antiepithelial sera.' . . . I trust, my lords and gentlemen, that from what I have said you may have obtained the impression, to allude again to my quotation from Bacon, that we no longer find ourselves lost on a boundless sea but that we have already caught a distinct glimpse of the land which we hope, nay, which we expect, will yield rich treasures for biology and therapeutics." (5).

REFERENCES AND NOTES

1. G. Köhler and C. Milstein, *Nature* **256**, 52 (1975).
2. E. J. Ziegler *et al.*, *N. Engl. J. Med.* **324**, 429 (1991).
3. N. B. Vedder *et al.*, *J. Clin. Invest.* **81**, 939 (1988); P. J. Simpson *et al.*, *ibid.*, p. 624.

4. E. Haber, T. Quertermous, G. R. Matsueda, M. S. Runge, *Science* **243**, 51 (1989).
5. P. Ehrlich, *Proc. R. Soc.* **66**, 424 (1900).
6. R. Catane, D. L. Longo, J. Isr, *J. Med. Sci.* **24**, 471 (1988).
7. G. Winter and C. Milstein, *Nature* **349**, 293 (1991).
8. J. Schlom, in *Molecular Foundations of Oncology*, S. Broder, Ed. (Williams and Wilkins, Baltimore, in press).
9. R. D. Mayforth and J. Quitans, *N. Engl. J. Med.* **323**, 173 (1990); L. Pirofski *et al.*, *J. Clin. Immunol.* **10**, 5S (1990); H. Waldmann, Ed. *Monoclonal Antibody Therapy*, (Karger, Basel, Switzerland, 1988), pp. 1–173; C. A. K. Borrebaeck and J. W. Larrick, Eds., *Therapeutic Monoclonal Antibodies* (Stockton, New York, 1990), p. 1; E. J. Wawrzynczak and A. J. S. Davies, *Clin. Exp. Immunol.* **82**, 189 (1990); R. O. Dilman, *Ann. Intern. Med.* **111**, 592 (1989); L. Chatenoud, *Curr. Opin. Immunol.* **2**, 246 (1989).
10. Ortho Multicenter Transplantation Group, *N. Engl. J. Med.* **313**, 337 (1985).
11. H. J. Schlitt *et al.*, *Transplant. Proc.* **20**, 103 (1988).
12. C. H. Janson *et al.*, *Cancer Immunol. Immunother.* **28**, 225 (1989).
13. H. Acha-Orbea *et al.*, *Cell* **54**, 263 (1988); J. L. Urban *et al.*, *ibid.*, p. 577.
14. R. A. Miller, D. G. Maloney, R. Warnke, R. Levy, *N. Engl. J. Med.* **306**, 517 (1982); T. C. Meeker *et al.*, *Blood* **65**, 1349 (1985).
15. A. Fisher *et al.*, *Lancet* **ii**, 1058 (1986).
16. H. Waldmann, *Annu. Rev. Immunol.* **7**, 407 (1989); B. M. Hall, *Am. J. Kidney Dis.* **14** (Suppl. 2), 71 (1989); D. Wofsy, *Prog. Allergy* **45**, 106 (1988).
17. R. J. Benjamin *et al.*, *Eur. J. Immunol.* **18**, 1079 (1988).
18. D. S. Kaufman *et al.*, *Transplantation* **46**, 210 (1988).
19. C. Herzog *et al.*, *Lancet* **ii**, 1461 (1987).
20. T. A. Waldmann, *J. Natl. Cancer Inst.* **81**, 914 (1989); *J. Biol. Chem.* **266**, 2681 (1991); *Science* **232**, 727 (1986); _____ *et al.*, *Blood* **72**, 1805 (1988).
21. S. Rudikoff *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 1979 (1982); M. Brüggemann, A. Rabruch, K. Rajewsky, *EMBO J.* **1**, 629 (1982).
22. V. T. Oi, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 825 (1983); M. S. Neuberger *et al.*, *EMBO J.* **2**, 1373 (1983); S. L. Morrison, V. T. Oi, *Adv. Immunol.* **44**, 65 (1989).
23. P. T. Jones *et al.*, *Nature* **321**, 522 (1986).
24. L. Riechmann, *ibid.* **332**, 323 (1988).
25. C. Queen *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 10029 (1989).
26. P. Brown *et al.*, *ibid.* **88**, 2663 (1991).
27. A. F. LoBuglio *et al.*, *ibid.* **86**, 4220 (1989).
28. G. Hale *et al.*, *Lancet* **ii**, 1394 (1988).
29. R. G. Mage, *Nature* **333**, 807 (1988).
30. T. A. Waldmann and W. Strober, *Prog. Allergy* **13**, 1 (1969).
31. D. Yasmeen *et al.*, *J. Immunol.* **116**, 518 (1976).
32. B. M. Müeller *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 5702 (1990).
33. M. Brüggemann *et al.*, *J. Exp. Med.* **166**, 1351 (1987); Z. Steplewski *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 4852 (1988).
34. R. P. Junghans *et al.*, *Cancer Res.* **50**, 1495 (1990).
35. J. M. McCune *et al.*, *Science* **241**, 1632 (1988); D. E. Mosier *et al.*, *Nature* **335**, 256 (1988).
36. W. D. Huse *et al.*, *Science* **246**, 1275 (1989).
37. B. L. Mullinax *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 8095 (1990).
38. U. D. Staerz, O. Kanagawa, M. J. Bevan, *Nature* **314**, 628 (1985); S. Songilvilai, P. J. Lachmann, *Clin. Exp. Immunol.* **79**, 315 (1990).
39. L. K. Gilliland *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 7719 (1988).
40. M. A. Garrido *et al.*, *Cancer Res.* **50**, 4227 (1990).
41. I. Pastan *et al.*, *Cell* **47**, 641 (1986); E. S. Vitetta *et al.*, *Science* **238**, 1098 (1987); E. S. Vitetta and P. E. Thorpe, in *Biological Therapy of Cancer: Principles and Practice*, V. DeVita, S. Hellman, S. Rosenberg, Eds. (Lippincott, Philadelphia, in press); D. C. Blakey *et al.*, *Prog. Allergy* **45**, 50 (1986).
42. D. J. P. FitzGerald *et al.*, *J. Clin. Invest.* **74**, 966 (1984).
43. J. Hwang *et al.*, *Cell* **48**, 129 (1987).
44. J. S. Huston *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 5879 (1988); R. E. Bird *et al.*, *Science* **242**, 423 (1988).
45. V. J. Chaudhary *et al.*, *Nature* **339**, 394 (1989).
46. F. L. Moolten and S. R. Cooperband, *Science* **169**, 68 (1970).
47. B. Gould *et al.*, *J. Natl. Cancer Inst.* **81**, 775 (1989).
48. L. Nadler *et al.*, *Proc. of the Second Int'l. Sym. on Immunotoxins* (1990), p. 58.
49. V. Byers *et al.*, *Blood* **75**, 1426 (1990).
50. K. D. Bagshawe, *Br. J. Cancer* **60**, 275 (1989); P. D. Senter, *FASEB J.* **4**, 188 (1990).
51. R. W. Kozak *et al.*, *Trends Biotechnol.* **4**, 259 (1985); D. M. Goldenberg, *Semin. Nucl. Med.* **19**, 332 (1989); D. M. Goldenberg, *J. Natl. Cancer Inst.* **83**, 78 (1991).
52. S. E. Order *et al.*, *J. Clin. Oncol.* **3**, 1573 (1985); S. E. Order, J. L. Klein, P. K. Leichner, *Natl. Cancer Inst. Monogr.* **3**, 37 (1987).
53. R. Kozak *et al.*, *Cancer Res.* **49**, 2639 (1989); M. M. Cooper *et al.*, *Transplantation* **50**, 760 (1990).
54. R. Kozak *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 182 (1986).
55. I thank B. Holmlund for her excellent editorial assistance.

Vaccinia Virus: A Tool for Research and Vaccine Development

BERNARD MOSS

Vaccinia virus is no longer needed for smallpox immunization, but now serves as a useful vector for expressing genes within the cytoplasm of eukaryotic cells. As a research tool, recombinant vaccinia viruses are used to synthesize biologically active proteins and analyze structure-function relations, determine the targets of humoral- and cell-mediated immunity, and investigate the immune responses needed for protection against specific infectious diseases. When more data on safety and efficacy are available, recombinant vaccinia and related poxviruses may be candidates for live vaccines and for cancer immunotherapy.

Vaccinia virus was successfully used for the prevention of smallpox and the eradication of variola virus, the causative agent of that disease. The protective effect of vaccination was reported by Edward Jenner in 1798 (1). Vaccinia virus was initially isolated directly or indirectly from infected cows (Fig. 1, top). Subsequently, some preparations of vaccine came from pox lesions of horses and other lots were purportedly mixed with smallpox virus to increase their "potency"; after a nearly 200-year history, in man and animals, the derivation of present-day vaccinia virus is obscure (2). DNA analyses indicate that vaccinia virus is closely related to, but distinct from, variola virus, cowpox virus, and other members of the poxvirus family that occur naturally (3).

The novelty of the vaccination procedure led some contemporaries of Jenner to view the idea of using cowpox material with alarm (Fig. 1, bottom), a response similar to the greeting recombinant DNA methodology received in our own time. Nevertheless, the safety of vaccination compared to the practice of variolation (inoculation with small quantities of live variola virus) and its prophylactic value were soon evident. In 1801, Jenner predicted "that the annihilation of the Small Pox, the most dreadful scourge of the human species, must be the final result of this practice [vaccination]" (4). Implementation of the Intensified Smallpox Eradication Programme by the World Health Organization (WHO) began in 1967, a year in which there were 131,776 reported cases of smallpox in 31 different