Pharmaceutical Research, Vol. 5, No. 1, 1988

Review

Monoclonal Antibodies as Magic Bullets

Frances M. Brodsky[1]

Monoclonal antibodies have been used as experimental therapy in humans since the beginning of the 1980s (A. N. Houghton and D. A. Scheinberg. *Semin. Oncol.* 13:165–179, 1986). They have been hailed as the prototypical magic bullet drug because of their inherent capacity for specificity. Consequently, monoclonal antibodies have many possible therapeutic applications with varied potential for successful outcome. Current review articles discussing monoclonal antibody therapy deal with the application of monoclonal antibodies within specific areas of medicine. The aim of this review is to summarize the current reviews and provide a broader perspective on the medical applications of monoclonal antibodies along with some general principles by which their therapeutic success or failure might be understood.

KEY WORDS: monoclonal antibody; protein drugs; immunotherapy.

HISTORICAL PERSPECTIVE

The concept of magic bullet drugs was first developed by Paul Ehrlich at the turn of the century (2). Ehrlich proposed that it would be possible to find drugs that would specifically bind to infectious organisms and not bind to the host tissue, thereby being toxic to the organism and not the host. In his search for such a treatment for syphilis, he tested over 600 arsenic derivatives before he found compound 606 (Salvarsan) which was effective. Although Salvarsan seemed to cure most patients of syphilis, it was occasionally lethal to some patients. He spent much of the remainder of his career cataloging the lethal effects and trying to determine their cause (3,4). Thus, in addition to providing the concept of a magic-bullet drug, Ehrlich's work illustrated that specificity of the magic bullet is crucial or side effects can be extremely serious.

Ehrlich also made another contribution to the development of the concept of magic-bullet drugs. This evolved from his studies with Emil Behring on immune activity of the blood. Behring had discovered a natural antitoxin to diphtheria toxin in the blood of animals that survived diphtheria infection (2–4). Behring and Ehrlich studied the properties of this natural antitoxin, which, of course, was a neutralizing antibody. They discovered that similar blood substances could be produced by injecting foreign material into rabbits and that these substances had binding specificity for different foreign materials as though they bound to specific receptors. Analysis of serum from rabbits injected with either goat or ox red blood cells indicated to Ehrlich that each serum contained antibodies recognizing receptors shared by the two types of red blood cells and some antibodies that bound to unique receptors on each type of cell (Fig. 1). Thus, Ehrlich and Behring identified antibodies with binding affinities for tissue-specific receptors. Ehrlich proposed that these were the body's natural magic bullets and might be harnessed as highly specific drugs.

As the properties of the antibody molecule have been defined, they have revealed that Ehrlich's speculations were correct. The antibody molecule is ideally designed to be a magic bullet. A typical antibody molecule of the immunoglobulin G class is a Y-shaped protein composed of four disulfide-linked polypeptides which are organized into two functional regions (Fig. 2) (5). The arms, called the Fab domains, contain the variable regions which create unique binding sites for the antigen to which the antibody is directed. The stem, called the Fc domain, contains invariant sequences that contribute to the immunological functions of the antibody (6). The stem has recognition sequences for complement proteins which bind to antibodies and lyse the cells to which they are attached. The stem also contains recognition sequences for cytotoxic lymphocytes which destroy cells to which the antibodies are attached. Finally, the stem contains a binding site for the antibody receptor on macrophages which ingest antibody with antigen bound to it. Thus, antibodies are the immune system's magic bullet because they bind foreign antigen and mediate its destruction. In the process of vaccination, antigen in altered form is used to elicit antibody production so that the immunized host produces appropriately targeted antibodies. However, in some situations, sufficient or effective antibody is not produced or is produced too late. In these cases, it would be ideal to be able to administer exogenous antibody to substitute for or supplement the body's immune response. It is this therapeutic application of exogenous antibody which is considered in this review with regard to its potential applications, limitations, and current status.

MONOCLONAL ANTIBODIES

Before considering how antibodies could be used as therapy, it is important to consider how they can be pro-

[1] Department of Pharmacy, School of Pharmacy, University of California, San Francisco, California 94143.

0724-8741/88/0100-0001$06.00/0

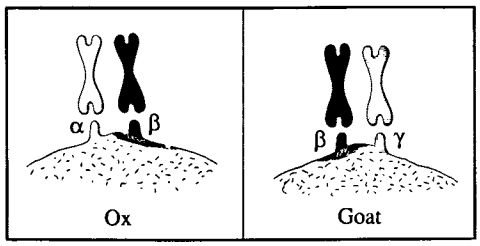

Fig. 1. Ehrlich's diagram of cell-specific receptors. This diagram is reproduced from Ehrlich's *Collected Studies on Immunity* published in 1906 (2). Here Ehrlich explains the reactivity of two antisera, one produced by immunizing a rabbit with ox red blood cells and the other produced by immunizing a rabbit with goat red blood cells. The sera contain antibodies which bind to receptors (antigens) on the target blood cell. The target blood cells share one type of antigenic receptor but each also has a unique antigenic receptor. One type of antibody recognizes the common receptor. Other antibodies recognize unique receptors. This demonstrates the potential cell specificity of a single antibody. (Reproduction of the figure is with the permission of Chapman and Hall.)

duced for this application. Prior to the development of monoclonal antibodies, the only option was to isolate antibodies from the serum of an immunized animal. However, antibodies isolated from serum are not that useful because

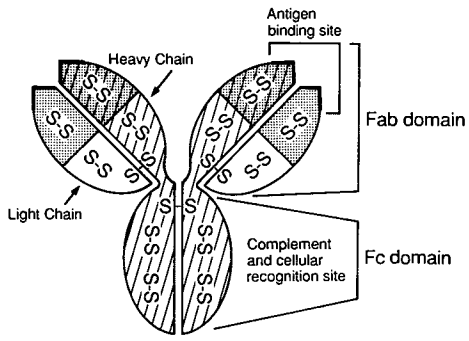

Fig. 2. Immunoglobulin G structure and function (5,6). The immunoglobulin G molecule is composed of two identical heavy chains and two identical light chains with molecular weights of 50 and 25 kilodaltons, respectively. All four polypeptide chains are cross-linked by disulfide bonds (S−S) and are composed of 12.5-kilodalton folded regions with internal disulfide bonds. Proteases can cleave the molecule into two types of functional domains. The two Fab domains each have an antigen binding site formed by amino acid residues from hypervariable regions of the heavy and light chains. These hypervariable residues (dark black) are located among additional variable sequences (stippled region). The rest of the protein sequence is relatively constant and shared between subclasses of IgG molecules. The constant-region residues in the Fc domain form recognition sites for complement, cytotoxic cells, and phagocytic cells.

they have multiple binding specificities and are available in limited amounts. Consider the case of an animal immunized with an antigen with multiple recognizable sites (Fig. 3). Antibody-producing cells will be generated in the spleen, such that each cell produces a single type of antibody recognizing one of the antigenic sites (7). These antibodies are all secreted into the serum, which becomes a mixture of antibodies recognizing all the sites characteristic of this antigen. The serum will also recognize any other antigens that share common sites. Furthermore, the amount of this mixed antibody which could be obtained is limited by the volume of the animal's serum. In addition, a second animal immunized with the same antigen would produce a different set of antibodies.

However, the development of monoclonal antibody technology made it feasible to isolate monospecific antibodies in unlimited amounts. This technology was initiated in 1975 by Köhler and Milstein (8), who were awarded the 1984 Nobel Prize in Medicine for their work. In the production of monoclonal antibodies, spleen cells are isolated from an immunized mouse and fused with a mouse tumor cell which confers immortality on the spleen cells, making it possible to grow the spleen cell hybrid in culture outside of the animal (Fig. 3). The immortalized spleen cells can be cloned into individual cultures such that each culture produces a single specific antibody. Clones producing antibody to a unique site on an antigen can be selected and produced in culture. These antibodies are easily isolated from the cultures and can be generated in large amounts. Thus, by immunizing a mouse with a chosen antigen it is possible to generate a monospecific antibody reacting with that antigen which can be produced in amounts sufficient for therapeutic purposes.

OVERVIEW OF THERAPEUTIC APPLICATIONS

Monoclonal antibodies can be used for therapy in *native* or *conjugated* form (summarized in Table I). Native antibodies have been applied to situations where their activity depends simply on binding the target antigen or where they are involved in both binding to targets and mediating their destruction by activating other biological systems. To date, the most straightforward and successful application of native antibody therapy is during drug toxicity, in which the antibody blocks the effect of the toxic substance (9). For example, antibodies are currently being used as therapy for digitalis poisoning. The blocking activity of native antibodies has also been used for treatment of conditions that result from destructive cellular immune responses. Particular antibodies seem to block the activity of these destructive cells or cause their removal. Currently, antibodies, such as Orthoclone OKT-3, are being used to block destruction of kidney transplants by cytotoxic T cells, which are ultimately responsible for kidney rejection (10). Similarly, native antibodies can be used to block the activity of cells involved in production of the self-reactive antibodies which are characteristic of autoimmune diseases (11). This particular application has so far been demonstrated only in experimental animals. Finally, taking advantage of the antibody's capacity to trigger cell destruction, antibody therapy has been applied to tumor elimination. Native antibodies recognizing target antigens on tumors could bind to the tumors and me-

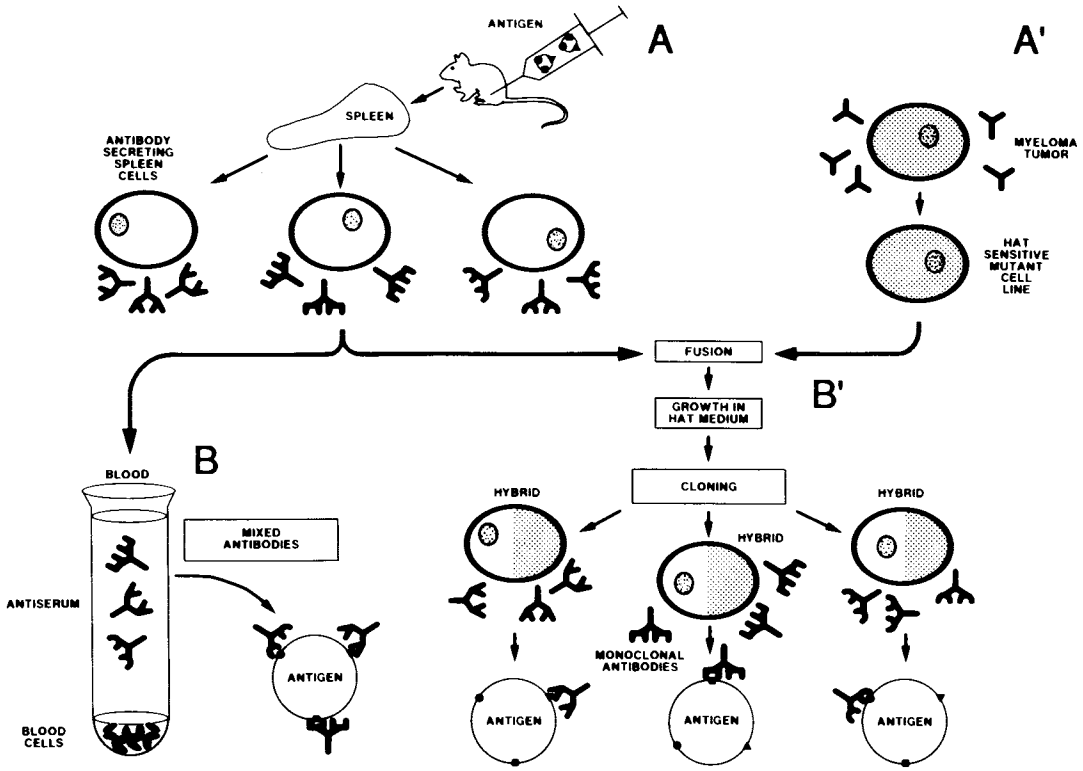

Fig. 3. Monoclonal antibody production. (A) A mouse is immunized with an antigen bearing three antigenic determinants (distinct sites which can be recognized by an antibody). Antibodies to each determinant are produced in the spleen. One spleen cell produces a single type of antibody. A spleen cell has a finite lifetime and cannot be cultured indefinitely *in vitro*. (B) In the mouse, the antibody-producing cells from the spleen secrete into the blood. The liquid portion of the blood (serum) therefore contains a mixture of antibodies reacting with all three sites on the antigen (antiserum). (A') A mutant cell derived from a mouse myeloma tumor of an antibody-producing cell has stopped secreting antibody and is selected for sensitivity to the drug aminopterin (present in HAT medium). This mutant tumor cell can grow indefinitely *in vitro* but is killed by HAT medium. (B') The mutant myeloma cell is fused by chemical means with spleen cells from an immunized mouse. The resulting hybrid cells can grow indefinitely *in vitro* due to properties of the myeloma cell parent and can grow in HAT medium because of an enzyme provided by the spleen cell parent. The unfused myeloma cells die because of their sensitivity to HAT and unfused spleen cells cannot grow indefinitely *in vitro*. The hybrid cells are cloned so that individual cultures are grown up from a single hybrid cell. These individual cells produce a single type of antibody because they arose from a single spleen cell. The monoclonal antibody isolated from these cultures is specific for only one antigenic determinant on the original antigen. An earlier version of this figure was published by the author in Ref. 40 and was modeled after the figures in Ref. 41. (This material is reproduced with permission from the McGraw-Hill Book Company.)

diate their destruction. To date native monoclonal antibodies have been used to treat leukemias and lymphomas and a few solid tumors, including melanomas (1,12,13). These treatments have resulted in tumor regression in about 25% of the cases treated. In most of these cases, regression was transient. One, now famous, case of complete remission of B-cell lymphoma induced by a monoclonal antibody was reported several years ago by Levy and his colleagues (14).

Monoclonal antibodies (MAbs) can also be applied to therapy as specific carriers for other substances such as drugs/toxins/radioactivity and enzymes. Such antibody conjugate therapy is still primarily in the experimental stage using animal models, whereas native antibody therapy has been tried in a number of human cases. Attachment of a drug or toxin to an antibody should increase its ability to kill targeted tumor cells (15,16). In animal models, a number of tumors have been treated using this strategy. In a recent summary by Pastan and colleagues they estimate that in nine different experimental systems, toxin/MAb conjugates were successful in prolonging survival in five systems and actually inhibiting tumor growth in four systems (17). Monoclonal antibodies conjugated to toxins can also be used to eliminate cells from bone marrow transplanted into cancerous patients. This removal has been efficient and resulted in successful transplants into experimental animals (18). This procedure is currently being tested in human bone marrow transplantation (16). Ultimately monoclonal antibodies could be used to concentrate a drug or enzyme in a specific location in the body. This would be particularly useful in cases where systemic distribution of the drug or enzyme would have detrimental side effects. For example, anti-fibrin monoclonal antibodies conjugated to urokinase could help localize this enzyme to blood clots, where it could activate conversion of plasminogen to plasmin, which

Table I. Application and Limitations of Monoclonal Therapy

Antibody form	Condition/disease treated	Trials in humans	Success rate	Limitations	Ref. No.
Native	Drug toxicity	Yes	High	Minor	9
	Organ transplantation	Yes	Moderate	Anti-immunoglobulin	10
	Bone marrow transplantation	Yes	Moderate	Graft failure	12
	Autoimmune disease	No	N/A[a]	Immunosuppression	11
	Cancer	Yes	Variable	Anti-immunoglobulin	1, 13, 14
Conjugated to					
Drug/toxin	Cancer	No	N/A	Toxicity/specificity	15–17
	Bone marrow transplantation	Yes	Variable	Efficacy	16
Enzyme	Thrombosis	No	N/A	Specificity	19
Radioactivity	Cancer (imaging)	Yes	Moderate	Toxicity/specificity	20

[a] Not applicable or available.

degrades fibrin (19). Finally, the one case in which antibody conjugates are already being used in humans is for radiological imaging (15,20). Coupling a radioactive marker to a tumor-specific antibody allows localization of the tumor and definition of its size, which contributes to its diagnosis. Radioactive antibody conjugates reacting with fibrin could also be used to localize blood clots (9). Using radioactive tracers on antibodies will also allow assessment of the ability of the antibody to localize to the target and evaluate its use as a carrier for a drug, enzyme, or cytotoxic agent.

LIMITATIONS TO ANTIBODY THERAPY

There are limitations to the use of antibodies for therapy, and some of these limitations are specific to the form of antibody therapy. The use of native antibodies is limited by the natural capabilities of the antibody—whether it efficiently triggers destruction of the target or whether it has effective blocking or neutralizing activity. Furthermore, when a mouse monoclonal antibody is injected into a human patient, it is recognized like any foreign antigen and the patient will make antibodies which react with the therapeutic monoclonal antibody.

The use of antibody conjugates has the same limitations as native antibodies plus additional problems. Application of antibody conjugates is more strictly limited by the definition of specific target molecules, to which the antibody can bind. This is a particular problem if the conjugated material is highly toxic. The conjugate must be directed to unique target molecules so that it does not bind to and destroy the wrong tissue. A further problem arises when antibody binding to a target molecule induces the disappearance or modulation of that molecule from the surface of the target cell. Thus, ideally it is useful to define more than one unique target receptor on a cell so that when one receptor disappears, therapy can be continued, directed at the second receptor. Even for *in vitro* depletion of bone marrow cells, the most effective application of therapeutic antibody conjugates seems to require a cocktail of monoclonal antibodies recognizing several receptors unique to the target cell (21). In addition, antibodies carry a signal in their Fc domain for their uptake by the macrophage-like cells in the reticuloendothelial system (18). These cells are present in many tissues in the body and their function is to ingest foreign antigen with antibody bound to it (7). Should these cells ingest toxic antibody conjugates, they would be killed off along with the target tissue, resulting in destruction of an important arm of the immune system.

Thus, in the use of monoclonal antibodies as magic bullets, one confronts the same problem that Paul Ehrlich faced. That is, it will be critical to learn how to predict and deal with serious side effects. Lowder and Levy have compiled a list of the toxic effects of native monoclonal antibody therapy and the number of cases in which they have been observed (13). The occurrence of these effects seems to be related to the amount of antibody–antigen complexes present in the patient's blood. They can be reversed by stopping the administration of antibody and controlled with some drugs. Most of these effects result in physical discomfort but not in obvious long-term damage. The most serious effect is anaphylaxis, which is an acute allergic reaction to the administered antibody. This has been observed only rarely and in cases where the anti-monoclonal antibody response was previously activated. A second serious effect of producing an anti-monoclonal antibody response is that the antibodies recognizing the therapeutic "foreign" monoclonal antibody can neutralize its effect, inactivating the therapy. In about 50% of cases where tumors have been treated with native antibodies, the patients developed an anti-antibody response which, in many cases correlated with further inefficacy of the therapy.

Thus, use of native monoclonal antibodies for therapy presents a dilemma. Either the treatment must be effective following the administration of a single dose or the normal response of the immune system must be circumvented to allow the administration of repeated doses. In the case of using antibody conjugates, there is no single-dose alternative because the reticuloendothelial arm of the immune system must be avoided even during the first antibody administration. For this reason, use of toxic antibody conjugates in humans has not yet been attempted. However, given the present limitations of single-dose administrations and using native MAbs, some successful antibody treatments have been achieved and promising models for native antibody therapy have been developed.

SUCCESSFUL THERAPY WITHIN LIMITATIONS

Treatment of cancer with monoclonal antibodies is still in an experimental stage in both animals and humans. Most patients treated are terminally ill so that the therapeutic effects of the antibody are difficult to assess. However, the treatment of cancer patients has revealed useful information about the immune response to the therapeutic antibody and indicated that those treatments involving a single administration of native antibody are most likely to succeed (13).

In keeping with this prediction, one of the most successful applications of antibody therapy has been in the treatment of drug toxicity (9,22). This involves either a single or multi-dose administration over a short time span so that antibody reactions to the therapeutic antibody have no opportunity to affect the treatment. In addition, the treatment is just as effective with fragments of antibody containing only the Fab portion which binds antigen. This smaller fragment is cleared more rapidly from the system than whole antibody, so is less toxic and less immunogenic. Some of the work on this type of antibody therapy has been for treatment of digitalis poisoning and has been carried out by Haber and colleagues. Their current approach uses therapeutic antibodies isolated from serum rather than monoclonal antibodies. However, they have recently produced monoclonal antibodies that have been shown to be equally as effective in an experimental animal model (23).

Digitalis glycosides are administered to improve cardiac contractility and/or control supraventricular arrhythmias. An excess of digitalis, however, can cause life-threatening ventricular arrhythmias. Excess occurs when overdoses have been accidentally or intentionally ingested or a patient's renal function changes markedly. Antibodies to digoxin, a digitalis glycoside, neutralize the effects of digoxin by binding free drug in the blood and preventing it from binding to its receptor in cardiac tissue. Ventricular arrhythmias due to digitalis toxicity can be rapidly reversed following administration of anti-digoxin Fab fragments (22). Another index of digitalis effect is AV conduction or the ventricular response rate to atrial fibrillation. Administration of anti-digoxin Fab fragment reverses this digitalis effect as seen in Fig. 4. This patient with atrial fibrillation and digitalis toxicity received 520 mg of Fab between 4:45 and 5:15 PM. Within two hours, the patient's ventricular response increased, representing a reversal of the digoxin-induced AV conduction delay. When the serum concentrations of digoxin are measured before and after Fab administration in patients with digitalis excess (Fig. 4), the measured total serum digoxin concentration is actually higher than prior to antibody administration, but virtually all of the digoxin is antibody bound and therefore neutralized. The rise in serum concentration suggests that, although inactivation by the antibody is immediate, some digoxin is extracted from surrounding tissues by the equilibrium effects of antibody binding. Slower clearance would also be expected for an antibody–drug complex compared to the drug alone.

Clearly, the effects of administering a neutralizing antibody are quite dramatic in the case of digoxin toxicity. Another application that is currently under development is antibody therapy for septicemia in which Fab fragments could neutralize effects of bacterial endotoxin. This also has the potential for therapeutic success since it could depend on single-dose administration under acute conditions.

Another area where native MAb treatment has been successful is in the reversal of kidney transplant rejection (10). A large factor in rejection of kidney transplants is the generation of cytotoxic T cells by the recipient, which attack the transplanted kidney. Goldstein and his colleagues have demonstrated that these T cells can be removed from the circulation by the administration of a therapeutic monoclonal antibody specific for the CD3 protein on the surface of T cells. The antibody which they have used is called OKT3. The reversal of graft rejection by administration of this antibody has been so effective that it has been approved by the FDA and is now on the market as a treatment. The treatment protocol involves the infusion of 5 mg/day for 14 days at the first signs of a rejection episode. Simultaneously, the amount of immunosuppressive drugs (azathioprine and prednisone) the patient receives is lowered. In a study by Goldstein *et al.* where first rejection episodes were treated with either OKT3 antibody and reduced steroids or standard levels of steroids (methylprednisolone, 500 mg/day), rejection was reversed in 94% of the patients receiving antibody and 75% of the patients receiving steroids (10). In addition, the transplanted kidneys survived for a longer period of time following treatment with antibody as compared to treatment with steroids alone.

These patients did develop antibodies to the OKT3 antibody (24). However, the peak of antibody development was at 2–3 weeks after the initiation of therapy. When the drug, Cytoxan, was given simultaneously with the OKT3 antibody, the time at which reactive anti-antibody was produced was prolonged and fewer patients developed antibody (Fig. 5). Cytoxan also reduced the potency of the antibody response to OKT3. Even without Cytoxan the anti-OKT3 antibody levels are not sufficiently high to inhibit treatment during the 2-week course of antibody administration. Using Cytoxan at the same time might keep reactive antibody levels down so that OKT3 therapy could be used for treatment of a second rejection episode. OKT3 is currently being used only for the single treatment of first rejection episodes and, without control of reactive antibody production, would not be useful for a second treatment.

A third area in which treatment with native antibody may be successful even with current limitations, is in the treatment of autoimmune diseases with native monoclonal antibodies. To date this treatment has been demonstrated only in animal models (11). It looks promising as a treatment because the treatment itself prevents the production of antibodies which react with the therapeutic monoclonal antibody. To understand why this treatment works, it is necessary to review some background immunology. An autoimmune disease is characterized by production of antibodies that react with self-proteins. These antibodies contribute in a number of ways to the pathology of autoimmune diseases. Examples of these diseases are multiple sclerosis, systemic lupus erythematosus, and myasthenia gravis. Blocking autoantibody production in these diseases would alleviate many of the debilitating symptoms.

To block antibody production, it is necessary to take into account the process involved in triggering a B cell to make antibody (Fig. 6). The B cell is activated by a signal from a helper T cell, which in turn uses several receptors to recognize antigen bound to receptors on an antigen-presenting cell. It has been found that this helper cell–presenting cell interaction can be blocked with monoclonal anti-

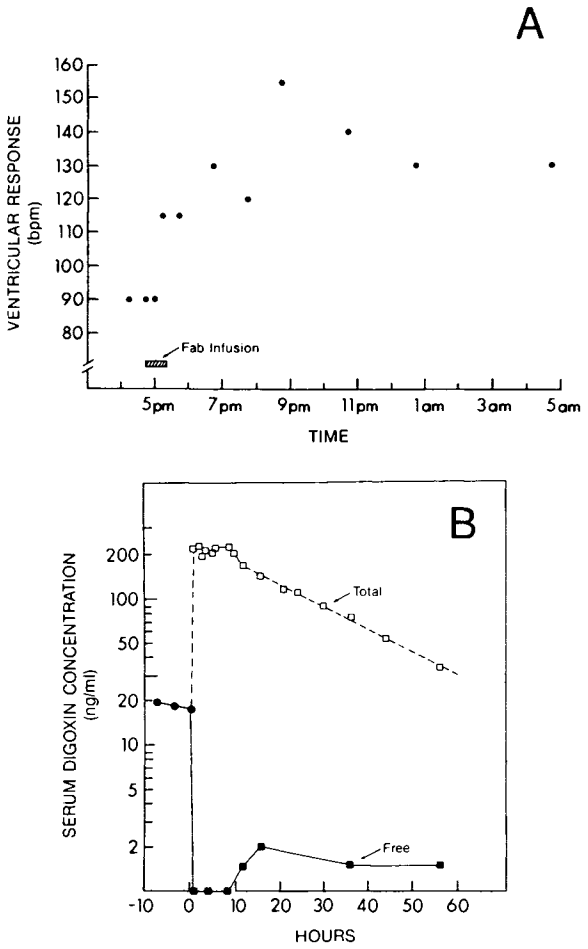

Fig. 4. Digitalis intoxication reversed with antibody therapy (22). (A) An adult patient with atrial fibrillation and a serum digoxin concentration of 4.7 ng/ml was given 520 mg of digoxin-specific Fab fragments, purified from sheep immunoglobulin. The antibody fragments were administered by infusion between 4:45 and 5:15 PM. The ventricular response returned to normal within 3.5 hr. (B) The serum digoxin concentrations in a similar patient are shown. Digoxin-specific Fab fragments were administered at time 0. (●) The serum digoxin concentration before Fab administration. (■) The free digoxin (active) concentration after treatment. (□) The total serum digoxin concentration after treatment (free plus bound). [Figures 4A and B are reprinted with permission from the American College of Cardiology from Ref. 22 with the permission of the authors (Wenger *et al.*) and the publisher (Elsevier). Figure 4B shows data reproduced by permission of the *New England Journal of Medicine* (Vol. 294, p. 799, 1976) and the author (Smith).]

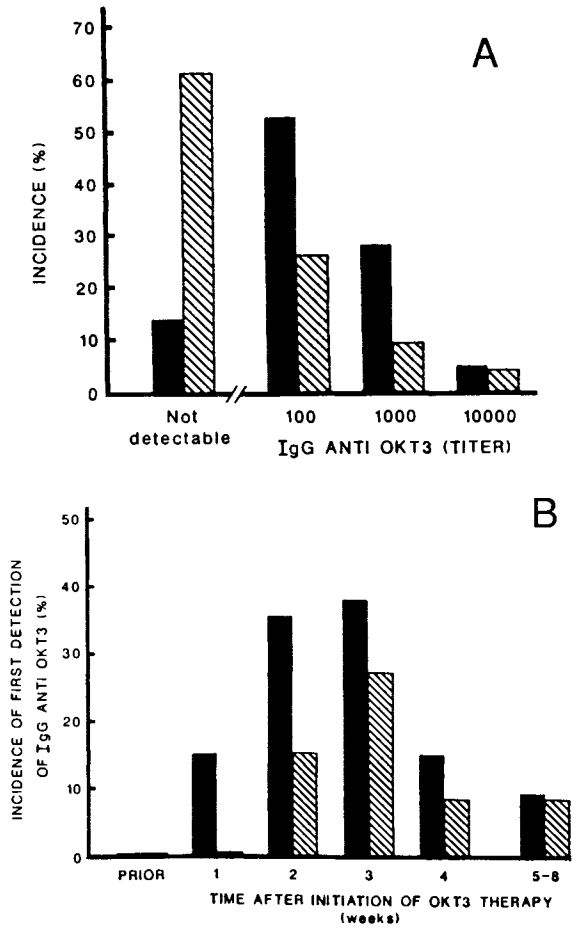

Fig. 5. Control of anti-immunoglobulin response during monoclonal antibody therapy (24). Kidney transplant patients (43 total) were treated for acute renal allograft rejection with OKT3 monoclonal antibody (5 mg intravenous daily for 10–14 days with reduced steroids). An additional 23 kidney transplant patients were treated with the same regimen of OKT3 antibody-steroids plus Cytoxan (400 mg intravenous on days 0, 7, 10, and 13). OKT3 reacts with T cells which are destructive to transplants. Cytoxan is a B cell inhibitor, administered to prevent anti-immunoglobulin response. (A) Anti-immunoglobulin response to OKT3 was measured by titration to determine the strength of the response. The titer indicates the dilution at which the anti-immunoglobulin is active. The percentage of patients developing anti-immunoglobulin of a given titer is shown. The solid bars indicate patients receiving OKT3 alone. The shaded bars indicate patients receiving OKT3 plus Cytoxan. (B) The time when anti-immunoglobulin activity was first detected after initiation of OKT3 treatment in these patients is shown. The percentage of patients developing anti-immunoglobulin each week is indicated by solid bars for those receiving OKT3 alone and by shaded bars for those receiving OKT3 plus Cytoxan. [Figures 5A and B are reproduced from Ref. 24 with permission from Ortho Pharmaceutical Corporation and the publisher (Williams and Wilkins).]

bodies that bind to the CD4 receptor on the T cell or to the major histocompatibility Class II antigen on the presenting cell (11,25,26).

Studies by McDevitt and colleagues have indicated that anti-Class II antibodies injected into mice can reverse the autoimmune symptoms in mouse models of multiple sclerosis, myasthenia gravis, and lupus (27–29). Studies on *in vivo* administration of anti-CD4 monoclonal antibody have been shown by Wofsy and Steinman to be equally effective in reversing symptoms in mouse models of lupus and multiple sclerosis, respectively (30,31). The anti-CD4 antibodies have an additional benefit. With appropriate doses these therapeutic antibodies not only inhibit autoantibody production but also inhibit production of antibodies reacting to the therapeutic antibody, thus preventing the usual side effects. While this therapy has great promise, it is not yet clear

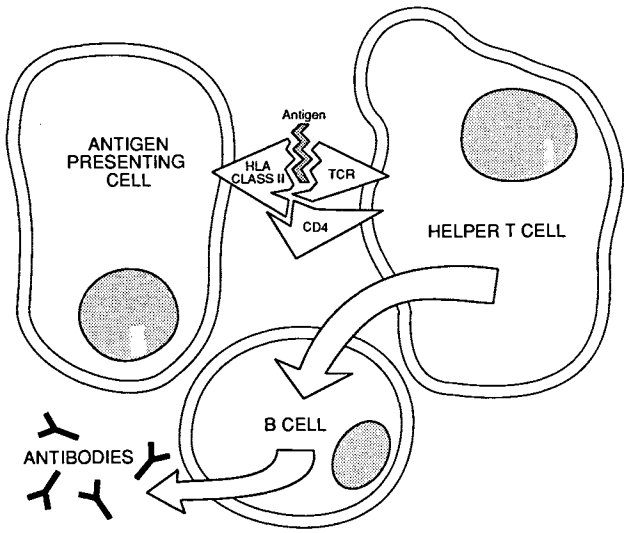

Fig. 6. Molecular interactions between the helper T cell and the antigen-presenting cell which stimulate antibody production. The T cell receptor (TCR) on the helper T cell recognizes a fragment of antigen bound to the Class II histocompatibility antigen (HLA Class II) on the presenting cell (25,42,43). The CD4 molecule is an adhesion molecule which enhances this recognition event by binding to the HLA Class II antigen (26,44). These interactions influence the B cell to produce antibody (7,11,25). It has been shown that monoclonal antibodies to HLA Class II molecules or monoclonal antibodies to the CD4 molecule will block this interaction and prevent antibody production (11,27–31). [Adapted from Ref. 11 with the permission of the author (Wofsy).]

whether the anti-CD4 suppression of antibody production might have serious effects on the production of needed antibodies.

Finally, bone marrow transplantation is one area where monoclonal antibodies can be used safely in a therapeutic procedure (12). In this situation, antibodies are used to remove cells from the bone marrow *in vitro,* prior to transplantation, and the transplant recipient is never exposed to the antibody. Patients receive bone marrow transplants as an attempt to reconstitute their immune system. Generally, these are leukemia patients whose immune system must be destroyed to eliminate the tumor. They receive either their own bone marrow (autologous) or bone marrow from a donor (heterologous) (32). In the autologous transplant, the bone marrow is removed prior to destruction of the malignant cells and monoclonal antibodies are used to destroy the malignant cells *in vitro,* leaving behind the normal stem cells which are capable of repopulating the immune system. In the heterologous transplant, certain donor cells may attack the cells they encounter in the recipient (graft-versus-host reaction). These reactive cells can be removed by antibody treatment prior to transplantation, again leaving behind the functional cells needed for repopulation. Monoclonal antibodies are ideal for bone marrow "clean-out" because of their ability to recognize unique receptors characteristic of one cell type but not present on another. Trials with monoclonal antibodies have used antibodies plus complement to eliminate target cells (12). Complement is a natural serum component which lyses cells to which antibody is bound. Monoclonal antibody–toxin conjugates are also being used in bone marrow clean-out trials (16). The success of this application of monoclonal antibodies will depend primarily on which method of elimination, if any, proves to be most efficient without destroying the ability of the bone marrow to repopulate the immune system. A further exciting application of monoclonal antibody therapy is suggested by the experimental mouse model of Waldmann and colleagues (33). They have found that treatment of the recipient animal with anti-T cell monoclonal antibodies at the time of bone marrow transplantation tolerizes the recipient to a second graft from the donor animal, even if the donor has an incompatible tissue type. This approach may make it feasible to carry out organ transplantation in humans with greater success.

FUTURE POTENTIAL

Most of the examples of successful therapy using monoclonal antibodies have involved native monoclonal antibodies. Although few antibody conjugates have been used in human trials, some progress has been made in establishing strategies for application of antibody conjugates to human therapy. The specificity problem of identifying tumor-specific and tissue-specific markers is gradually being solved. Although it has been more difficult to produce monoclonal antibodies reacting with such markers than was initially expected, a large number of characteristic markers have been defined over the past 10 years. In a review of the applications of immunotoxins to cancer therapy, Frankel *et al.* list 21 types of human tumors for which specific markers have been found and which could therefore be targets for tumor-specific therapy (16).

Anti-tumor antibodies conjugated to radioisotopes have been used in humans to image tumors (20). These trials have allowed evaluation of different isotopes for this purpose. Most importantly they have shown that it takes several days for sufficient antibody to accumulate specifically in the tumor for a clear image to be visible. This information has been useful in tumor diagnosis and will also be extremely useful in predicting whether a monoclonal antibody–drug conjugate will actually localize to the tumor and how much would be distributed throughout the body nonspecifically. This is an important consideration because many of the agents which have been conjugated to antibodies are highly cytotoxic. These include radioisotopes, protein toxins, chemotherapeutic drugs, and liposomes carrying drugs or toxins. Such conjugates are valuable only if they can be efficiently localized to the target tissue, tumor, or organ. They are harmful to tissues mediating nonspecific uptake. This can be true even for biological conjugates such as enzymes or growth factors conjugated to antibodies.

The properties of cytotoxic agents raise another important consideration for strategic design of antibody conjugates. Many drugs and toxins are not cytotoxic unless they can be delivered into the cytoplasm of the target cell. Many of the plant toxins that have been conjugated to antibodies for experimental therapy are composed of two active domains. One domain binds a cell and causes toxin internalization and the other domain mediates toxicity by inactivation of vital cellular factors and organelles, involved in protein biosynthesis (16). One strategy that has been used to reduce nonspecific toxicity has involved inactivation of the cell-binding domain of the toxin and replacing it with an anti-

body. Then it is important that once the antibody reaches its target cell, it is internalized. This can be achieved by choosing an antibody directed against a receptor that is known to enter a cell in a defined pathway called receptor-mediated endocytosis (Fig. 7). This pathway is used by cells to internalize receptors with ligands such as hormones and nutrients bound to them (34). In this case, the immunotoxin serves as a ligand and triggers its internalization with the receptor along the pathway of receptor-mediated endocytosis. This pathway involves invagination of the surface membrane into the cell, triggered by polymerization of a protein called clathrin on the cytoplasmic side (35). Because of the presence of clathrin this structure is called a coated pit and, when it completely pinches off into the cell, a coated vesicle. Clathrin then depolymerizes from the vesicle, allowing fusion of several vesicles, forming an endosome (34). This structure has a lower pH than the surrounding cytoplasm or extracellular fluid. For some types of conjugates, this change in environment is sufficient to cleave the toxin–antibody bond, allowing the toxin to diffuse across the endosome membrane and inactivate protein synthesis. Eventually, the material in the endosome winds up in lysosomes which have an even lower pH and many proteolytic enzymes. This is another site where toxin might be cleaved off antibody and diffuse into the cytoplasm to become active.

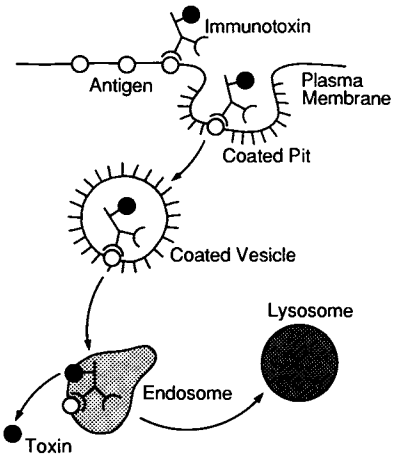

Fig. 7. Internalization of immunotoxin by receptor-mediated endocytosis. The immunotoxin is an antibody with a drug or toxin conjugated to it by an acid-labile linkage. The immunotoxin binds to its antigen, a cell surface receptor. It is internalized by the normal pathway of receptor uptake via a clathrin-coated pit, forming a clathrin-coated vesicle (35). After the clathrin depolymerizes the vesicle fuses into an endosome with a lower pH than the cytoplasm. Ultimately, the contents of the endosome fuse into the more acidic lysosome, where they are degraded (34). The shading indicates the progressive decrease in pH of these organelles. Because of the acid-labile linkage, the toxin dissociates from the antibody in an environment with a sufficiently low pH. It can then diffuse across the organelle membrane into the cytoplasm, where it exerts its toxic effects. [Adapted from Ref. 16 with the permission of the authors (Frankel *et al.*).]

A similar strategy has been developed by Shen and Ryser (36) for drug delivery and its efficacy has been demonstrated by Diener *et al.* (37). They have prepared *cis*-aconityl daunomycin, which can in turn be coupled to a protein substrate. The *cis*-aconityl linkage is acid sensitive so that daunomycin is released from the conjugate at pH 5 or below. Daunomycin coupled to an antibody cannot diffuse across a cell membrane and is effectively inactive. If the antibody induces internalization of the conjugate, the daunomycin will be released in acidic intracellular compartments (endosomal pH 5–6) and diffuse across the compartment membrane, where it will be active in the cytoplasm. This type of strategic approach to drug delivery may circumvent some of the problems of nonspecific toxicity.

In summary, the moderate successes of monoclonal antibody therapy are extremely encouraging. However, they include therapies that work in a very limited set of circumstances. The therapy must be exogenous, as in bone marrow transplantation, or administered once, as in the treatment of blood poisoning and transplant rejection. To overcome these limitations it is clear that more basic research is required before antibody therapy can be used safely. In particular, further research in immunology will be critical for learning how to control tolerance to the immunogenic effects of antibodies and how to avoid uptake of antibodies by the reticuloendothelial system. Both of these goals will be assisted by genetic engineering on antibody molecules which might make them less immunogenic and remove their recognition sequences for reticuloendothelial cell uptake. For example, several research groups have developed the technology for producing "chimeric" antibodies. These are composed of the antigen combining site and variable region of a mouse monoclonal antibody, genetically engineered to be attached to a human immunoglobulin constant region (38,39). In addition, more basic research is needed to understand the cell biology of receptor internalization and signaling. The effects of monoclonal antibodies do not stop at surface receptors. Their binding can trigger or block activity within the cell and their uptake can alter the metabolism of conjugated drugs and toxins. Fortunately, research in both immunology and receptor biology has entered a revolutionary age and it is likely that new insights applicable to antibody therapy will soon be discovered.

ACKNOWLEDGMENTS

Preparation of this paper was supported in part by a Pharmaceutical Manufacturers Association Foundation Research Starter Grant and by NIH Grant GM26691-09. The author thanks J. Schwartz for helpful comments, A. Frankel, D. Wofsy, T. Wenger, T. Smith, and Ortho Pharmaceutical Corporation for permission to reproduce or adapt previously published material. Reproduction permission from Chapman and Hall, the *Journal of Experimental Medicine*, the American College of Cardiology, Elsevier, Williams and Wilkins, and McGraw–Hill Book Company is also appreciated.

REFERENCES

1. A. N. Houghton and D. A. Scheinberg. *Semin. Oncol.* 13:165–179 (1986).
2. P. Ehrlich. *Collected Studies on Immunity,* John Wiley and Sons, New York, 1906.
3. M. Marquardt. *Paul Ehrlich,* Schuman, New York, 1951.
4. P. de Kruif. *The Microbe Hunters,* Harcourt, Brace, Jovanovich, New York, 1926.
5. A. M. Amzel and R. J. Poljak. *Annu. Rev. Biochem.* 48:961–997 (1979).
6. D. R. Davies and H. Metzger. *Annu. Rev. Immunol.* 1:87 (1983).
7. J. Klein. *Immunology: The Science of Self-Nonself Discrimination,* John Wiley and Sons, 1982.
8. G. Köhler and C. Milstein. *Nature* 256:495–497 (1975).
9. E. Haber. *Annu. Rev. Med.* 37:249–261 (1986).
10. G. Goldstein *et al.* (Ortho Multicenter Transplant Study Group). *N. Engl. J. Med.* 313(6):337–342 (1985).
11. D. Wofsy. *West. J. Med.* 143:804–809.
12. G. Hale, S. P. Cobbold, and H. Waldmann *Transplantation,* in press.
13. J. N. Lowder and R. Levy. *West. J. Med.* 143(6):810–818 (1985).
14. R. A. Miller, D. G. Maloney, R. Warnke, and R. Levy. *N. Engl. J. Med.* 306:517–522 (1982).
15. D. C. Edwards. *Pharm. Ther.* 23:147–177 (1983).
16. A. E. Frankel, L. L. Houston, B. F. Issell, and G. Fathman. *Annu. Rev. Med.* 39:125–142 (1986).
17. I. Pastan, M. C. Willingham, and D. J. P. Fitzgerald. *Cell* 47:641–648 (1986).
18. E. S. Vitetta and J. Uhr. *Annu. Rev. Immunol.* 3:197–212 (1985).
19. C. Bode, G. R. Matsueda, K. Y. Hui, and E. Haber. *Science* 229:765–767 (1985).
20. A. R. Bradwell, D. S. Fairweather, and P. W. Dykes. In R. W. Baldwin and V. S. Byers (eds.), *Monoclonal Antibodies for Cancer Detection and Therapy,* Academic Press, New York, 1985.
21. D. A. Vallera, R. C. Ash, E. D. Zanjani, J. H. Kersey, T. W. LeBien, P. C. L. Beverly, D. M. Neville, and R. L. Youle. *Science* 222:512–515 (1983).
22. T. L. Wenger, V. P. Butler, E. Haber, and T. W. Smith. *J. Am. Coll. Cardiol.* 5(5):118A–123A (1985).
23. P. Lechat, M. Mudgett-Hunter, M. N. Margolies, E. Haber, and T. W. Smith. *J. Pharmacol. Expt. Ther.* 229(1):210–213 (1984).
24. G. Goldstein, A. J. Fuccello, D. J. Norman, C. F. Shield, R. B. Colvin, and A. B. Cosimi. *Transplantation* 42(5):507–511 (1986).
25. S. L. Swain. *Immunol. Rev.* 74:129–142 (1983).
26. D. R. Littman. *Annu. Rev. Immunol.* 5:561–584 (1987).
27. L. Steinman, J. T. Rosenbaum, S. Sriram, and H. O. McDevitt. *Proc. Natl. Acad. Sci. USA* 78:7111–7114 (1981).
28. M. K. Waldor, S. Sriram, H. O. McDevitt, and L. Steinman. *Proc. Natl. Acad. Sci. USA* 80:2713–2719 (1983).
29. N. E. Adelman, D. L. Watling, and H. O. McDevitt. *J. Exp. Med.* 158:1350–1355 (1983).
30. D. Wofsy and W. E. Seaman. *J. Exp. Med.* 161:378–391 (1985).
31. M. K. Waldor, S. Sriram, R. Hardy, L. A. Herzenberg, L. A. Herzenberg, L. Lanier, M. Lim, and L. Steinman. *Science* 227:415–417 (1985).
32. R. A. Clift and R. Storb. *Annu. Rev. Immunol.* 5:43–64 (1987).
33. S. P. Cobbold, G. Martin, S. Qin, and H. Waldmann. *Nature* 323(6084):164–166 (1986).
34. R. M. Steinman, I. S. Mellman, W. A. Muller, and Z. A. Cohn. *J. Cell. Biol.* 96:1–27 (1983).
35. B. M. F. Pearse and M. S. Bretscher. *Annu. Rev. Biochem.* 50:85–101 (1981).
36. W.-C. Shen and H. J.-P. Ryser. *Biochem. Biophys. Res. Comm.* 102(3):1048–1054 (1981).
37. E. Diener, U. E. Diner, A. Sinha, S. Xie, and R. Vergidis. *Science* 231:148–150 (1986).
38. S. L. Morrison, M. J. Johnson, L. A. Herzenberg, and V. T. Oi. *Proc. Natl. Acad. Sci. USA* 81:6851–6855 (1984).
39. M. S. Neuberger, G. T. Williams, E. B. Mitchell, S. S. Jouhal, J. G. Flanagan, and T. H. Rabbitts. *Nature* 314:268–270 (1985).
40. F. M. Brodsky. In *Encyclopedia of Science and Technology, Vol. 11,* McGraw–Hill, New York, 1987, pp. 366–368.
41. C. Milstein. *Sci. Am.* 243(4):66–74 (1980).
42. B. P. Babbitt, P. M. Allen, G. Matsueda, E. Haber, and H. M. Grey. *Nature* 317:359–361 (1985).
43. A. Sette, S. Buus, S. Colon, J. A. Smith, C. Miles, and H. M. Grey. *Nature* 328:395–399 (1987).
44. D. Gray, P. Maddon, R. Sekaly, M. A. Talle, M. Godfrey, E. Long, G. Goldstein, K. Chess, R. Axel, J. Kappler, and P. Marrack. *Nature* 328:626–629 (1987).