# HUMAN MONOCLONAL ANTIBODIES IN CLINICAL MEDICINE

MEDICAL GRAND ROUNDS

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## INTRODUCTION

In 1975 I presented my first of now twelve Medical Grand Rounds. I had just returned from a meeting where I learned of the production of mouse monoclonal antibodies by Kohler and Milstein and chose it as the topic of my first presentation at Southwestern. I predicted wide applicability of monoclonal antibodies in human medicine and advances in both diagnosis and therapy of human illness because of these new reagents.

Sixteen years later, it is safe to say that as diagnostic reagents, monoclonal antibodies have been more useful and practical than any of us could ever have predicted. However, at the level of clinical intervention, the promise of these highly specific protein reagents is yet to be fulfilled. Indeed, to date, they have been a disappointment. The reasons for this are complex. While it has been fifteen years since the discovery of monoclonal antibodies, fifteen years is not a long time when one thinks of introducing an entirely new set of biological reagents into the therapeutic armamentarium. Additionally, and perhaps more importantly, these molecules (until recently) have been of foreign origin, *e.g.*, they have been made in mice; and as such, except in desperate situations, they are not routinely used in clinical medicine. Indeed, while hundreds of researchers in universities, medical centers, and biotech companies have been working steadily on MAbs as therapeutics for diseases as diverse as arthritis and cancer, to date only one MAb has been fully approved by the Food and Drug Administration (FDA). Only two have received preliminary approval. The FDA approval process for drugs is long and complex. All told, the approval time for a new drug takes, on average, twelve years. Thus, Mabs has not been exceptional in this regard.

The purpose of this Medical Grand Rounds is to provide an update on murine monoclonal antibodies and their genetically engineered relatives. Additionally, I would like to address the emerging technologies being used to produce human monoclonal antibodies. With FDA approval on the horizon for several human monoclonal antibodies, I believe the promise of these powerful reagents will be appreciated in medical practice in the very near future.

## PASSIVE IMMUNOTHERAPY

Intramuscular injections of human gammaglobulin have long been used in clinical medicine primarily for the treatment of immunodeficiency syndromes and in certain immunosuppressed patients. About a decade ago, several pharmaceutical companies introduced intravenous gammaglobulin for the treatment of immunodeficiency diseases. I do not believe anyone on the scene at the time could have predicted the extent to which this reagent would be used in virtually all aspects of clinical medicine. The intravenous administration of human gammaglobulin is fairly convenient. Generally, patients with full-blown immunodefiency syndromes are given a two to four hour infusion every three weeks. This has proven to be enormously safe and free of almost all side effects. Spectacular results have been reported in pediatric immunodeficiency syndromes (Buckley and Schiff,

1991) and intravenous immunoglobulin has been found useful in bone marrow transplantation (Sullivan, et al., 1990), patients immunosuppressed for a variety of reasons, and treatments of such a wide variety of disorders as idiopathic thrombocytopenic purpura and rheumatoid arthritis. Rather recently, an National Institute of Health Consensus Conference was convened to discuss the uses of intravenous gammaglobulin. While there remains some controversy as to its usefulness in a whole host of autoimmune diseases, there is no question that this reagent has taken its place in the medical pharmacopoeia (Eibl and Wedgwood, 1989; Pocecco, et al., 1987; Beriman, et al., 1988; Imbach, et al., 1990; and Schwartz, 1990).

Not so with mouse monoclonal antibodies. These reagents, while highly specific, are foreign to the human immune system; and as such, vigorous immune responses are made that are not unlike those that generations of physicians saw with the administration of horse serum for snake bites, and in tetanus and diphtheria prophylaxis and treatment. While the amounts of foreign protein that are required with murine antibodies are miniscule as compared to the previously used doses of horse serum, nonetheless, for benign diseases, this form of therapy has barely gained a foothold in medicine.

One of the purposes of today's review is to illustrate how intravenous immunoglobulin and mouse monoclonal antibodies, through advances in cell and molecular biology, are now becoming relatively safe and easy to produce. As such, chimeric, humanized and indeed human monoclonal antibodies are increasingly reaching the end of the biotechnology pipeline and into clinical practice (Waldmann, 1991; Winter and Milstein, 1991).

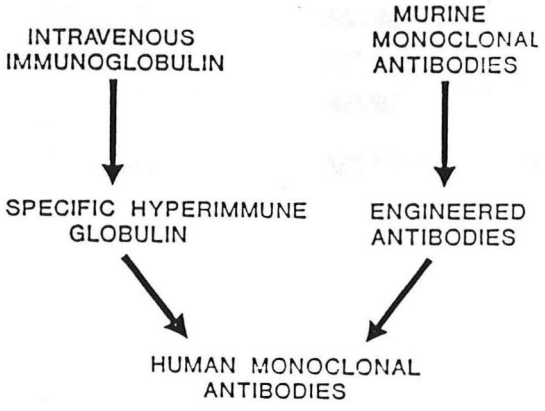


**Figure 1.** Use of IVIG and murine Mabs leads logically to human monoclonal antibodies.

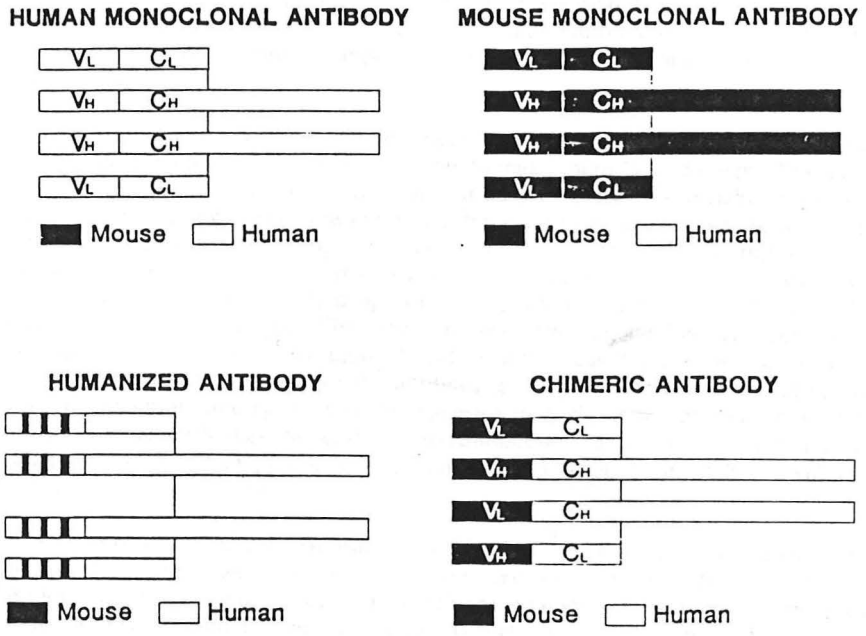


Figure 2. Various forms of human, mouse and "engineered" antibodies.

## TERMINOLOGY

Mouse monoclonal antibodies should be familiar to you. Mice are immunized with almost any antigen and antibodies can be generated by fusion of murine spleen cells with certain "fusion partners" to produce monoclonal antibodies of almost any specificity. As mentioned already, these molecules are highly immunogenic in humans; and although in most instances, only milligram amounts are given to patients, over the course of most therapeutic interventions this becomes a problem and immune responses are elicited. Specific examples of these include the OKT3 antibody that has been shown to be so effective in the prevention of renal allograft rejection and antibodies to CD4 and CD8--the molecules that distinguish human helper and cytotoxic T cells. I will come back to these examples a little later.

Because mouse monoclonal antibodies are foreign, various biotechnological approaches have been applied to them in order to make them "less" foreign. The simplest approach is the production of a "chimeric antibody". Here the variable regions (the specificity determing regions) of the mouse antibody are grafted onto the constant regions of human antibodies. As such, approximately a third of the monoclonal antibody remains "mouse" or "foreign", and about two-thirds of the molecule is "human". Such chimeric antibodies retain all the specificity properties of the original mouse monoclonal antibody, but have two major advantages. First, they are less immunogenic because they are "two-thirds human" and only "one-third mouse"; and second, because the Fc region is "human", the so-called "biological function" of the antibody is better preserved. Thus, complement fixation and receptor binding is generally superior in a chimeric antibody than in most mouse monoclonal antibodies. Several chimeric antibodies are close to or into clinical trials, including antibodies to CD3, CD4, and CD25 and HIV-1 (Liou, et al., 1989). I will discuss each of these in turn a bit later.

The next most logical extension of this principal of genetic engineering is the production of a "humanized antibody". Here, only the true specificity determining regions of the mouse antibody are dissected by molecular techniques out of the mouse monoclonal antibody and inserted into a human antibody. As such, over 95% of the antibody is human and less than 5% is murine. Grafting the complementarity determining regions of a mouse antibody into a human immunoglobulin "backbone" is almost trivial as a molecular biological procedure, but it has enormous ramifications for our discussion today. Several of these humanized antibodies have reached a point of clinical trial (An IND for humanized anti-Tac was filed very recently), and the consensus at the present time is that these humanized antibodies are only slightly more immunogenic than human monoclonal antibodies themselves.

The promise of "humanized" antibodies is that one can fundamentally immunize a mouse with any molecule that one pleases and have an almost limitless array of possibilities for building such specific reagents. This circumvents one of the major problems faced in

the production of human monoclonal antibodies: we are not at liberty to immunize humans with any molecule we wish in order to develop human monoclonal antibodies. For example, a reagent that would be of tremendous value in clinical medicine would be a human antibody to the human IL-2 receptor. There are two problems with developing such an antibody. First, the human IL-2 receptor is not likely to be immunogenic in a normal person because of the phenomenon of immunologic tolerance, *e.g.*, we normally do not make antibodies against our tissues or receptors. Second, while it is known that there are many mechanisms whereby one can break tolerance, for ethical reasons we are not at liberty to immunize humans with human IL-2 receptors (no matter how modified) for the purpose of generating antibodies that might be useful to other people.

Thus, for a variety of reasons the chimeric antibody and humanized antibody approach is quite attractive and is being explored in academic laboratories and biotechnology companies today. For example, within the last month "humanized" antibodies to Hemophilus influenza B have been reported.

## SOME EXAMPLES OF CLINICAL TRAILS UTILIZING MURINE ANTIBODIES AND THEIR DERIVATIVES

There are several approaches to suppressing immune responses. Drugs that impact lymphocytes or other effector cells of the immune system obviously suppress immune responses. A whole host of drugs from cyclosporin to steroids have been effectively used to alter a wide variety of immunologic phenomenon from transplantation to autoimmune disease. X-radiation has a profound impact on lymphocytes and, it, as well as the external depletion of lymphocytes, has been used. The difficulty with all of these approaches is their lack of specificity. In general, drugs, X-irradiation and external depletion alter far more than just lymphocytes and even those that are specific for lymphocytes influence all lymphocytes. Antibodies hold the promise of specifically targeting particular lymphocytes; and, for example, in the case of lymphocytes, there are specific target molecules that are present on all B cells or present on all T cells. Thus, anti-CD3 or OKT3 is specific for a molecule present on all peripheral T cells, whereas the CD4 and CD8 antigens are present on specific subsets of T cells. It is also possible using antibodies to T cell receptors to target not only all T cell receptors but even specific T cell receptors. At the present time, many laboratories are producing monoclonal antibodies for the treatment of a wide variety of immunologic diseases by this means.

### APPROACHES TO SUPPRESSING IMMUNE RESPONSES

- Drugs
- X-irradiation
- External Depletion of Lymphocytes
- Antibodies
  1) Anti-CD3 (All T cells)
  2) Anti-CD4 (T Helper cells)
  3) Anti-CD8 (T Cytotoxic cells)
  4) Anti-TCR
     pan TCR
     specific

The OKT3 monoclonal antibody was the first murine monoclonal antibody to be widely used in clinical medicine. This monoclonal antibody is directed against a cell surface antigen present on all peripheral T cells. As such, it will react with effectively all peripheral T cells. Its major usefulness in clinical medicine is in the treatment of acute renal allograft rejection. At the present time, in an extensive compilation of various studies, it is approximately 93% effective in reversing acute renal allograft rejection episodes. Prior to the use of this monoclonal antibody, the very best reported results were approximately 75% reversibility. Despite its utility, there are some difficulties with its use. There is obviously a broad immunosuppression associated with the use of OKT3 because all peripheral T cells are affected. There is an increased incidence of infection and several reports of the development of B cell neoplasias. However, the most difficult problem in dealing with OKT3 is that as a murine monoclonal antibody it is immunogenic, which limits the amount we can use and its longterm administration. Additionally, because it does not fix human complement very well *in vitro*, it is probably not cytotoxic.

When one considers that the majority of diseases for which monoclonal antibodies might be useful are diseases that are not in and of themselves fatal, the usefulness of murine antibodies becomes problematic. Thus, there has been a drive to generate more and more specific antibodies and those directed against the CD4 and CD8 subsets of T cells are now reaching the stage of clinical trial. CD4 is a molecule that is present on all human T helper cells. Since T helper cells are central cells in immunologic effector systems, a wide variety of monoclonal antibodies have been directed toward that antigen; and indeed, two recent clinical trials were published using antibodies to CD4 to treat patients with rheumatoid arthritis. Two groups in Germany (Reiter, et al., 1991; Horneft, et al., 1991) have treated several patients with active rheumatoid arthritis for seven days with a daily dose of twenty milligrams of CD4 monoclonal antibody administered intravenously over 30 minutes. They describe no negative side effects and considerable improvement in several therapeutic indices. For example, they were able to demonstrate a 31% improvement in grip strength in patients that were treated with antibody. The impact of this antibody on peripheral T cells is shown in Figure 3. Here in 56 days follow up of patients that were treated in the manner described above, the CD3, CD4 and CD8 cells are enumerated. You will see that CD3 which measures all T cells is very dramatically altered by the treatment, CD4 as well; whereas the CD8 subset is not affected at all. The reason that the CD3 cells show such a dramatic drop is that the majority of peripheral T cells are CD4 positive, and an antibody to CD4 would have an impact on the total T cells in the peripheral blood. However, notice that the CD8 subset is hardly altered by the treatment. It's clear that after the seven day course of therapy, the cells return to nearly their pretreatment level. Longer term administration of the antibody would probably be useful. Similarly, in another study with a different antibody to CD4, improvements were noted in the number of swollen joints, grip strength, morning stiffness and the Richie index. Both of these studies while documenting efficacy ran into the difficulty of the generation of antibodies to mouse globulin or "HAMA"--human anti-mouse antibody-responses. This would severely limit the usefulness of this approach even if it were extremely effective. Thus, most likely, further approaches to using such specific antibodies in clinical practice will use chimeric or humanized antibodies and such molecules already exist in several laboratories.

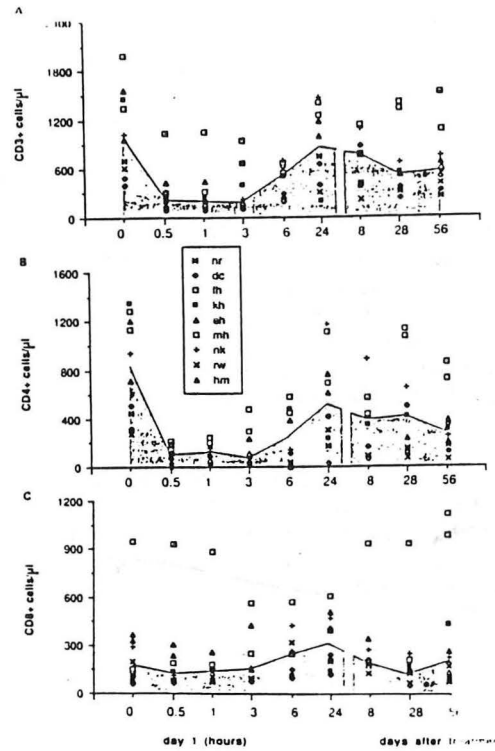


**Figure 3.** Kinetics of the CD3+, CD4+, and CD8+ cells from 10 patients with intractable rheumatoid arthritis (day 1) who were treated with a 7-day course of an anti-CD4 monoclonal antibody (Horneff et al., 1991).

One of the murine monoclonal antibodies that has been subjected to perhaps the widest clinical application in different "engineered" forms is anti-Tac, a murine monoclonal antibody with specificity for the human IL-2 receptor (specifically for the 55kd subunit of the human IL-2 receptor). IL-2 is central in most immune responses and a critical feature of the IL-2 receptor is its presence on "activated" T cells and on T cells in several patients with acute T cell leukemia. This monoclonal antibody has been administered to patients in its "murine form", and in its humanized form so that we have more experience with this

engineered antibody than any other. Waldmann group's at the NIH has spearheaded this work and phase 1 clinical trials have been reported using these antibodies in the therapy of patients with human T cell lymphotrophic virus 1 induced adult T-cell leukemia and in clinical renal transplantation.

The results in adult T-cell leukemia were encouraging with the murine antibody. About one third of the patients had partial or complete remissions with this treatment alone (Waldmann, et al., 1988). More encouraging is its use in renal transplantation. As you know, at the present time, renal allografts are dealt with utilizing a combination of immunosuppressive drugs (particularly cyclosporine) and the murine monoclonal antibody OKT3 (directed against the CD3 universal peripheral T-cell marker). Anti-Tac (T activated), as mentioned above, is directed against the IL-2 receptor which is upregulated in activated T cells, precisely the T cells that would be involved in transplant rejection. After work demonstrating that murine anti-Tac as a single agent would significantly delay rejection of renal allografts in cynomologus monkeys, the Boston group (T.B. Strom) in collaboration with Waldmann initiated a randomized trial of prophylactic therapy with anti-Tac in clinical renal transplantation (Kirkman, et al., 1989). The studies were particularly encouraging as some of the patients that were treated with anti-Tac subsequently required treatment with OKT3 and responded well to the second murine monoclonal. The hope of this particular kind of therapy (particularly if it can be generated in its "humanized" form) would be that a group of monoclonal antibodies could potentially be targeted at T cells with increasing or decreasing specificity (*i.e.*, all T cells versus activated T cells) depending upon the severity of the circumstances. Most encouraging also was the decreased requirement for other immunosuppressive therapy during these treatment protocols. Others have reported similar findings with other anti IL-2R antibodies (Soulillou, et al., 1990).

I will not further discuss the use of these murine monoclonal antibodies other than to mention that in neoplasia some of these issues are not as critical, *e.g.*, patients with terminal illness or where all other treatment modalities have failed are candidates for straight murine monoclonal antibodies despite the drawbacks described above. One such clinical trial that is just getting under way involves highly specific murine antibodies to the c-erbB-2 oncogene. c-erbB-2 is overexpressed in a number of human neoplasms particularly of the breast, ovary and colon. Approximately 30-40% of all such tumors (particularly those that are metastatic) overexpress c-erbB-2 at both the DNA, mRNA and protein level. Since this molecule is hardly expressed on any other tissue of the body, it is an attractive candidate for specific immunotherapy. Several groups have generated such monoclonal antibodies and either as mouse monoclonals, chimerics or humanized antibodies are now entering clinical trials. Since this molecule is overexpressed on so many human tumors, it offers considerable promise for these diseases.

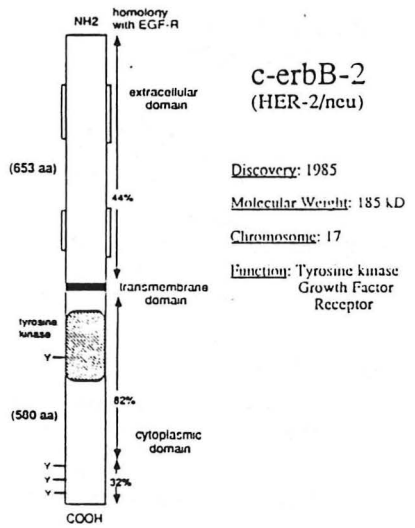


Figure 4. Structure of c-erbB-2. Courtesy of Dr. Beatrice Langton.

## HUMAN MONOCLONAL ANTIBODIES

The advantages and disadvantages of the use of human monoclonal antibodies have been briefly mentioned earlier. Obviously, the critical advantage of human monoclonal antibodies is that they lack any significant antigenicity; and except for the possibility of an

anti-idiotype response, human monoclonal antibodies should not be perceived by patients as foreign. (There is the possibility of anti-allotype responses but these have not been seen to date and are very rare in whole blood transfusions). There are, however, some inherent disadvantages. Human monoclonal antibodies are difficult to produce. Either one has to find patients that have made antibodies of particular specificities or one has to develop immunization protocols that are ethical. Both of these routes have been used. Patients that are recovering from a wide variety of infectious diseases have antibodies to a host of both protective and nonprotective bacterial and viral antigens. As such, human monoclonal antibodies have been produced to cytomegalovirus, respiratory syncytial virus, endotoxin, human immunodeficiency virus and the like. It is likely that these types of antibodies will replace, in specific situations, the nonspecific intravenous immunoglobulin that I mentioned in the Introduction. However, the vast majority of specificities that one would desire in a human monoclonal antibody are beyond the reach of ethical approaches; and as such, the specificity range of these antibodies is limited.

## PRODUCTION OF HUMAN MONOCLONAL ANTIBODIES

- Fusion (human-human, mouse-human)
  - preferential loss of human chromosomes
  - instability of hybrids
- EBV
  - low transformation efficiency (<4%)
  - mainly IgM
  - low secretory rate
- Anti-CD3/T cell
  - requires additional immortalization step
- Anti-CD40/transfected cell
  - requires additional immortalization step
- SCID-hu mice
  - primary immunization still controversial
- Repertoire cloning

Beyond the specificity problem, there are significant problems in production of human monoclonal antibodies. While there was initial excitement concerning the possibility of making human-human fusions, or human-mouse fusions the loss of human chromosomes, the instability of the hibrids and the very low secretory rate of most lines has made this approach highly problematic. While there are a few human monoclonal antibodies that have reached the clinical trial state utilizing these techniques, all in the field are

disappointed that more efficient means of immortalizing human cells have not been developed. Alternative strategies include Epstein Barr virus transformation. However, the low transformation efficiency, the fact that the secretory rate in this circumstance is very low and the vast majority of such antibodies are of the IgM class have limited the use of EBV transformation. There are a few newer techniques. Dr. Lipsky has pioneered the use of anti-CD3 stimulated T cells to stimulate virtually all human B cells; and in this regard after an immortalization step probably with Epstein Barr Virus, one could generate antibodies of many specificities. Jacques Banchereau in Lyon, France, has used an alternative procedure, using a fibroblast transfected cell line and anti-human CD40; but this procedure again requires an additional transformation step (Banchereau and Rousset, 1991). The SCID-human mouse provides yet another approach. As you know, mice with subacute combined immunodeficiency (SCID) are now available. These mice can have their lymphoid systems "repopulated" with human lymphocytes and, as such, these mice produce human antibodies. Cells from these mice could be immortalized by the techniques described above. Such mice could be immunized with any antigen circumventing the issues described earlier concerning the specificity range of most human monoclonal antibodies. However, this procedure still remains somewhat controversial. Primary immunizations are not easy, *e.g.*, if one puts lymphocytes into SCID mice that come from a person who has been previously immunized, there is no question one can get a "boost" response; however, immunization of the SCID mice with a new antigen has not been widely successful. There has also been some success producing human monoclonals from in vitro-primed human splenocytes (Boerner, et al., 1991). Some other techniques to get around this problem will be discussed later.

HALF-LIFE OF VARIOUS ANTIBODIES
IN HUMAN PLASMA

| PREPARATION | HALF-LIFE IN DAYS |
|---|---|
| Mouse Monoclonal Antibody | 1 |
| Chimeric Mouse/Human Antibody | 4 |
| Human Myeloma IgG1 | 21 |
| Human Mab Anti CMV (IgG1) | 24 |
| Human Mab Anti Endotoxin (IgM) | 5 |

Sources: Lobuglio et al. 1989, Morel et al. 1970, Masuho et al. 1990, Ziegler et al. 1991

In order to emphasize the importance of the human monoclonal antibody, it is useful to reflect on the levels of such antibodies in human plasma. In many studies, it has been demonstrated that murine monoclonal antibodies reside in human plasma for less than a single day. Chimeric antibodies last longer. We have very little information on the humanized antibodies, but much of the evidence suggests that they will have a half life in the neighborhood of 15 days. Human myeloma proteins (from work done 25 years ago)

have a half life of about 21 days, and the human monoclonal antibodies that have been tested including one antibody to cytomegalovirus lasts for approximately the same time. The IgM Centoxin antibody that I will describe later has a half life less than a day, but this is because it is an IgM antibody rather than an IgG antibody.

### Human Anti-Endotoxin

There is a large body of evidence that suggests that endotoxin from the surface of circulating bacteria plays a central role in gram negative shock, a disease that continues to occur at high frequency often resulting in death. While antibiotic resistance and the severity of underlying disease play a major role in outcome, it has long been thought that if antibody could be used to specifically interact with the circulating endotoxin in this disease that it would be therapeutically beneficial. Almost ten years ago, in a randomized controlled trial, patients were given either human antiserum to endotoxin vs. control albumin and it was concluded that the human antiserum to the lipopolysaccharide core substantially reduced deaths from gram negative bacteremia (Ziegler, et al., 1982). This vaccine was prepared by isolating mutant endotoxin from *E. coli,* immunizing male volunteers, and preparing immunglobulin from the sera of these volunteers. More recently, two biotech firms have developed monoclonal antibodies for use in gram negative sepsis. Xoma developed an IgG mouse anti-endotoxin monoclonal antibody that has been used in a few clinical trials. Centocor produced a human anti-endotoxin monoclonal antibody (of the IgM type) which served as a basis for a very large clinical study that was recently published in the *New England Journal of Medicine*. The same authors who performed the clinical trial with the polyclonal human anti-endotoxin antiserum undertook an extensive study of gram-negative bacteremia and septic shock and its treatment with the human monoclonal antibody against

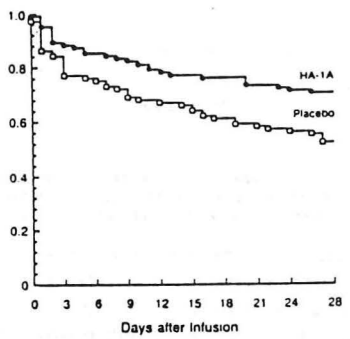


Figure 5. Probability of survival in patients with gram-negative bacteremia. Comparison of the cumulative survival estimates over a 28-day period for patients who received HA-1A (n = 105) and those who received placebo (n = 95) showed a 39 percent reduction in mortality with HA-1A treatment (P = 0.014) From Ziegler, et al., 1991.

endotoxin. Five hundred and forty-three patients with suspected gram negative sepsis based on a number of criteria were randomized and either given a single dose of 100 milligrams of the human monoclonal antibody or a placebo intravenously. The results in the entire group were not particularly impressive. However, retrospectively, in the group that subsequently proved to have gram negative sepsis (200 of the 543 patients--a commentary on the effectiveness of the clinical diagnosis!) there was a 40% reduction in mortality. This is illustrated in Figure 6 below, where four weeks after infusion the antibody treated group fared considerably better than the placebo treated group. Broken down into the probability of survival in people in different APACHE groups (this represents a score indicating the severity of gram negative shock, etc.), the results are even more impressive. There was no benefit of treatment with the monoclonal antibody in the patients who did not prove to have gram negative bacteremia. All patients tolerated the antibody and no anti-idiotypic antibodies were detected in any patients.

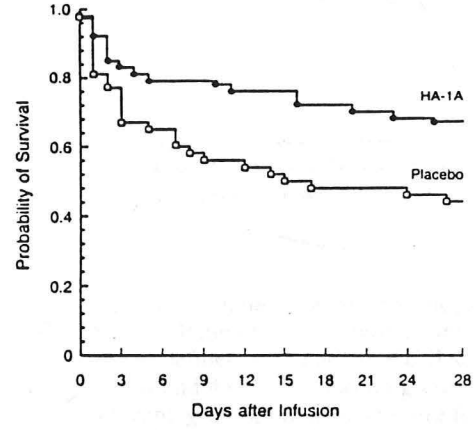


Figure 6. Probability of survival in patients with gram-negative bacteremia and shock at entry. Comparison of the cumulative survival estimates for patients who received HA-1A (n=54) and those who received placebo (n=48) showed a 42 percent reduction in mortality with HA-1A treatment (P=0.017), from Ziegler, et al., 1991..

This study evoked a storm of criticism; the difficulty is clear. While the benefits of the drug are obvious, they are only obvious in the patients with proven gram negative bacteremia. Since the clinician is often faced with a situation of having to treat the patient before the bacteriologically proven diagnosis is evident, there is considerable controversy as to whether the drug should be approved for the use in gram negative sepsis. For example, in letters to the editor that followed this paper (and in other commentaries) the argument was made that considering that there are between 100 and 300,000 patients in the United States each year that develop gram negative sepsis, if the clinical diagnosis is only effective in approximately a third of the cases, up to 800,000 patients would be eligible for such

treatments each year. The annual costs of this would be in the neighborhood of two billion dollars based on the current market price of human monoclonal antibodies (approximately $4,000 per patient). However, when one considers that the average patient with sepsis spends 21 days in the hospital, much of it in an ICU bed, the cost of this form of therapy, if eventually proven to be effective, would be less than 10% of an average hospital stay.

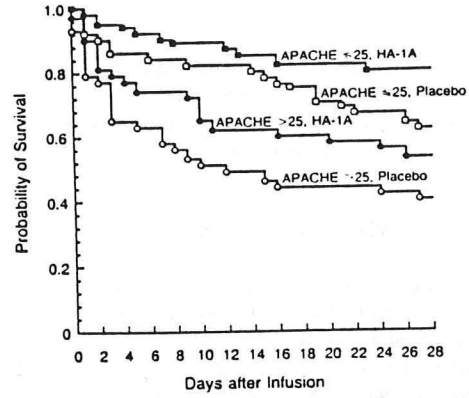


**Figure 7.** Probability of survival in patients with gram-negative bacteremia stratified according to APACHE II Score. Patients were stratified into two groups according to whether their severity of illness as defined by the APACHE II score at enrollment was >25 (43 patients in the HA-1A group and 43 in the placebo group) or less than 25 (62 in the HA-1A group and 52 in the placebo group), the median score for the population. Analysis in a Cox proportional-hazards model showed a reduction in mortality with HA-1A treatment in both severly ill and less severely ill patients (P=0.017), from Ziegler, et al., 1991.

The study provides interesting dilemmas for modern medical practice and, in particular, for the use of monoclonal antibodies. The major difficulty, of course, is the problematic diagnosis of gram negative bacteremia. Since the study was only able to demonstrate efficacy when bacteremia was documented (almost always posthoc) there is considerable reluctance to add an expensive treatment modality to an already expensive regimen. However, in defense of the investigators, the trial was blinded. It was large and it is not the problem of the antibody or the investigators that the diagnosis of gram negative sepsis is so problematic (Gershon, 1991).

HA-1A (Centoxin) has been used in gram negative sepsis in Dallas at both Parkland and Baylor under a compassionate use protocol in about 10 patients at this time.

Infectious agents remain the prime target for the production of human monoclonal antibodies (Silverman and Lucas, 1991). As mentioned earlier, patients either recovering from disease or individuals after immunization can have specific lymphocytes immortalized and several groups have generated high affinity human monoclonal antibodies (generally of the IgG class) to a number of infectious agents. I will mention three that are in clinical trials at this stage.

Sandoz Pharmaceuticals has two monoclonal antibodies of considerable interest in clinical trials--an antibody to the hepatitis B virus surface antigen and another to cytomegalovirus. This latter monoclonal antibody has just completed phase 1 studies and is in a large trial in allogenic bone marrow transplant recipients. Initial studies are encouraging.

**Human Anti-CMV**

Recall that cytomegalovirus infection is a major cause of morbidity and mortality in patients undergoing allogenic marrow transplantation. It is also a serious problem in patients undergoing renal and liver transplantation. There is currently no vaccine available to prevent CMV infection. The immune response to cytomegalovirus infection involves both cell mediated and humoral components. After marrow transplantation, both because of the procedure itself as well as the immunosuppressive regimens that are generally employed, immune responses are poor and various strategies have been employed to improve these responses by passive immunoprophylaxis. Intravenous gammaglobulin was used and continues to be used in many centers. High titer specific anti-CMV human polyclonal immunoglobulin from screened donors has proven to be more efficacious than pooled IVIG. The logical extension of this would be a panel of human anti-CMV monoclonal antibodies. The hope would be that using the specific combination of a monoclonal anti-CMV antibody and for example, gancyclovir the dreaded CMV complications of marrow transplantation including pneumonitis could be blunted. The phase 1 study of the Sandoz anti-CMV antibody was recently published in *Transplantation* (Drobyski, et al., 1991), and the phase 2 study has been submitted for publication. It seems very likely that this IgG antibody will gain wide acceptance because the current therapy is inadequate and the human monoclonal antibody should be entirely safe. Incidentally, in the published phase 1 study (and to my knowledge in the unpublished phase 2 study), no anti-idiotypic antibodies were generated that led to any significant difficulties. Other groups have also developed anti CMV antibodies (Masuho, et al., 1990).

**Human Anti-HBV**

Sandoz has also developed a number of human monoclonal antibodies to the hepatitis B virus surface proteins. The bulk of the work in humans with these antibodies

has been in compassionate clinical trials in patients undergoing liver transplants--particularly those in whom the transplanted liver has become reinfected with hepatitis B virus. Infusion of the human monoclonal antibody (an IgG1) has been followed by the dramatic reduction of circulating antigen and has resulted in the suppression of disease in several patients. A few patients have "escaped therapy" with viral variants and have been treated with a second anti-hepatitis B monoclonal antibody with success. Another aspect of these studies that is most interesting is that some of these patients were treated with anti-hepatitis B monoclonal antibodies "in preparation for transplantation" with a dramatic loss of antigenemia. When transplantation was then performed, the patients remained antigen free for periods as long as one year. These results suggest that reagents of this type could play a significant role in the treatment of chronic active hepatitis in an earlier stage, specifically prior to the phase of the disease in which liver transplantation becomes imperative.

Both the anti-CMV and anti-hepatitis B monoclonal antibodies have been administered to nearly 100 patients and 40 rhesus monkeys on a long term basis. While almost all of the patients receiving these antibodies have been immunosuppressed in one form or another, the rhesus monkeys were healthy. In no instance was an anti-idiotypic response generated; and indeed, in the rhesus monkeys even though there are slight species differences between rhesus and human immunoglobulin, anti-human responses were detected in only 1 of 40 rhesus monkeys. These data suggest that antibodies of this type are minimally immunogenic in humans although their long term use in individuals not on immunosuppressive regimens remains to be explored. However, I believe it is safe to assume that this toxicity will be minimal.

**Human Anti-HIV**

There is another group of human monoclonal antibodies that are on the brink of human clinical trials which are worth mentioning before we leave this subject. These are the antibodies to the human immunodeficiency virus. The vast bulk of contemporary opinion teaches that cell-mediated immune responses are critical in the recovery from viral infections, and, in addition, that many patients develop AIDS and succumb having apparently adequate levels of circulating antibodies to the HIV virus (Fauci, 1991), because of the extent of the epidemic and the obvious difficulty in generating vaccines that will generate cell mediated immunity. Yet there has been a resurgence of interest in the possibility of providing passive immunoprophylaxis in this disease (Tomiyama, et al., 1991; Gorny, et al., 1991; Putkonen, et al.; Posner, et al., 1991; and NICH & HD Study Group, 1991). Several groups have produced human monoclonal antibodies and biotech companies and major pharmaceutical firms are major players in bringing these reagents to market (Robinson et al., 1990). Mouse monoclonal antibodies, chimeric antibodies, humanized antibodies and particularly human monoclonal antibodies are being developed in several locations. Our own laboratory has studied the structure of several such antibodies to the human immunodeficiency virus in collaboration with Susan Zolla-Pazner of New York University. Jennifer Andris, a graduate student in my laboratory, has spearheaded this work locally. These antibodies have been inserted into various expression systems in our

laboratory and in collaboration with Evan Hersh in Tucson we anticipate the production of human monoclonal antibodies in a baculovirus expression system for human consumption in the near future. There have been some encouraging reports of passive immunotherapy regarding plasma from AIDS patients into other AIDS patients, but these need far more rigorous control at the present time. The standardization provided by human monoclonal antibodies, and in particular, the opportunity to select those monoclonal antibodies that will be "neutralizing" and of specific isotype should improve the chances of success in this regard. I would predict that we will hear much more about these reagents in the near future.

## NEWER TECHNIQUES

I have not spent time discussing the numerous toxin and toxin-like molecules that can be coupled to antibodies to target them to specific cells. Ricin A chain, which has been used extensively in this institution by Drs. Vitetta and Uhr, falls into this category. Our focus today is on the specificity end of the molecule. Whether the antibody variable regions deliver an Fc region (to effect complement binding cytotoxicity or Fc receptor mediated cytotoxicity) as opposed to a toxin molecule is not the issue. The question is: "how does one generate the specificity of the delivery system that will not induce an immune response?" Clearly human monoclonal antibodies are the better approach. There are, however, some newer techniques that should be mentioned.

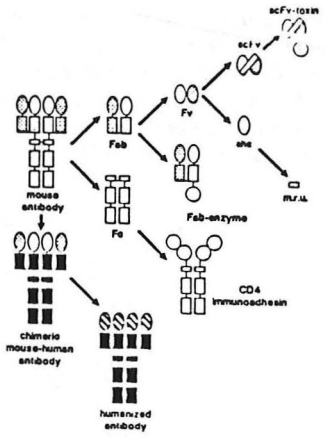


**Figure 8.** Diagrammatic representation of various engineered forms of antibodies and their fragments (adapted from Winter and Milstein, 1991).

## Single Chain Antibodies

Twenty-five years ago, immunologists argued whether it was the heavy or the light chain of antibody that conferred the major specificity. In general, today most believe that both chains, contribute significantly. However, undoubtedly, single chain antibodies do have specificity and offer attractive advantages. Specifically, single chains particularly light chains, have no carbohydrate moieties, and therefore can be easily produced in *E. coli* systems (Laroche, et al., 1991). This would circumvent one of the major industrial problems in scale-up. Bacterial fermentors represent two orders of magnitude less complexity than the development of myeloma cell lines in tissue culture. Additionally, the production of antibodies in bacteria allows for easier manipulation at the molecular level for the production of particular specificities and higher affinities. Work in this institution by Sally Ward is directed in this direction (Ward, et al., 1991). Other workers are attempting de novo synthesis of combining regions (Uri, et al., 1991).

## Bifunctional Antibodies

Bifunctional or bispecific antibodies have also reached the stage of clinical trials. These are used to direct cytotoxic cells to targets and lyse cells that normally are not lysed (Berg, et al., 1991). A good example of such an antibody is illustrated in Figure 9. Antibodies to Tac (activated T cell associated antigen--the IL-2 receptor) are useful but do not participate in ADCC. However, when coupled to molecules to CD16 (the Fc gamma receptor 3 which is present on NK cells for example), these antibodies are capable of binding two cells, an IL-2 receptor bearing cell and an NK cell. This leads to cell death since the NK cell kills the IL-2 receptor-bearing cell. Thus, the bifunctional antibody brings two cells together, one of which kills the other. This is an effective means of using the body's own defenses to effect a better outcome. These can be generated either by chemically cross linking two sets of antibodies, or by a fusing two hybridomas to produce what is called a "quadroma". The final product has the specificity of both original antibodies.

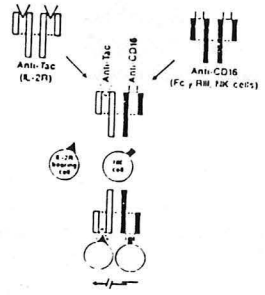

Figure 9. Example of how a bifunctional antibody might work.

## Catalytic Antibodies

A second new direction that is worth mentioning concerns the generation of catalytic antibodies. Several investigators have described that antibodies, like conventional enzymes, can perform catalytic functions. One of the best described is an antibody that cleaves vasoactive intestinal peptide into two inactive peptide fragments. Others have been shown to cleave viral coat proteins and indeed participate in any number of catalytic reactions. Richard Lerner in a University Lecture last year reviewed that process for us. Such catalytic antibodies or "abzymes" work rather slowly, but the prediction for the future is that investigators will be able to increase their catalytic rates and combine a number of strategies simultaneously in the design of a catalytic antibody. One nice feature of catalytic antibodies is that they release their antigens after processing them and can therefore continue to attack additional substrates (Shokat, et al., 1989; Mei, et al., 1991; Paul, et al., 1991; Tang, et al., 1991; Bowdish, et al., 1991 and Gibbs, et al., 1991).

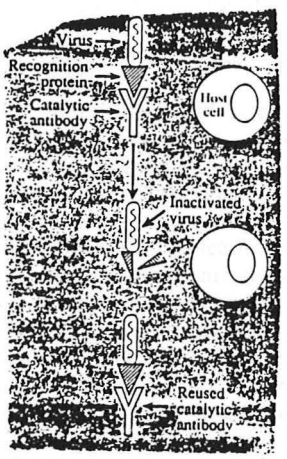


**Figure 10.** Catalytic antibodies release their antigens after processing them; standard antibodies do not. Reusability makes catalytic antibodies potentially more valuable. Here, for example, a hypothetical therapeutic catalytic antibody breaks down and inactivates one virus after another, preventing infection of cells (Courtesy Sudhir Paul, University of Nebraska Medical Center).

## Antibodies With Higher Affinities

Increasing the affinity of antibodies has long been a goal of immunochemists. The immune system itself is obviously geared to producing higher and higher affinity antibodies as antigen becomes limiting in a secondary immune response. However, often we are faced with an antibody of exquisite specificity but relatively low affinity without the opportunity of returning to the immune system for the generation of additional antibodies. Such antibodies have been expressed in any number of expression systems available to modern cell and molecular biologists. Then, by site directed mutagenesis, these antibodies have had specific amino acids in the hypervariable regions replaced. Changing such aminoacids has lead to remarkably different specificities and often to higher affinities. There are two practical implications of such approaches. First, antibodies could potentially be made even more specific than originally designed. More but more importantly, by raising the affinity of antibody, one or two logs, the likelihood that less antibody would be required for a specific therapeutic result is enhanced. While I have not stressed this it should be obvious that the production of such antibodies in large scale, even in industrial operations. And as mentioned above, the cost of the monoclonal antibody used in the gram negative sepsis trial would undoubtedly be prohibitively expensive for routine clinical practice.

## Repertoire Cloning

Finally, a procedure called "repertoire cloning" has the potential of circumventing almost all of the issues that have been addressed so far in this presentation. In this procedure, monoclonal antibodies can be isolated without the intermediate of cell fusion technology (Burton, 1990 and Burton, 1991). As illustrated in Figure 11, either an animal or a human is immunized and then lymphocytes are isolated. Typically, if these lymphocytes can be "panned" or "sorted" in such a way as to enrich for lymphocytes that bind specifically with antigens, the heavy and light chain genes within these lymphocytes can be amplified, inserted into bacterial viruses and expressed within bacteria. By having appropriate screening techniques, one can isolate the relatively few combinations of heavy and light chain genes that result in the desired specificity. These genes can then be cloned and sequenced and introduced into more efficient expression systems for widespread production (Hasemann and Capra, 1990). If this procedure is carried out with humans, for example, human monoclonal antibodies can be generated with almost any specificity. However, this procedure has yet to prove itself in terms of "unimmunized" specificities, *e.g.*, the original hope of this technology was that antibodies could be isolated to almost any antigen regardless of whether immunization had occurred (Barbas, et al., 1991; Kang, et al., 1991; Kang, personal communication; and Persson, et al., 1991). That is, a single library could be readdressed with several different antigenic specificities thereby obviating the immunization procedures. This clearly has not happened and at the present time the only antibodies that have been isolated from these libraries are from individuals who have been infected or immunized with particular agents.

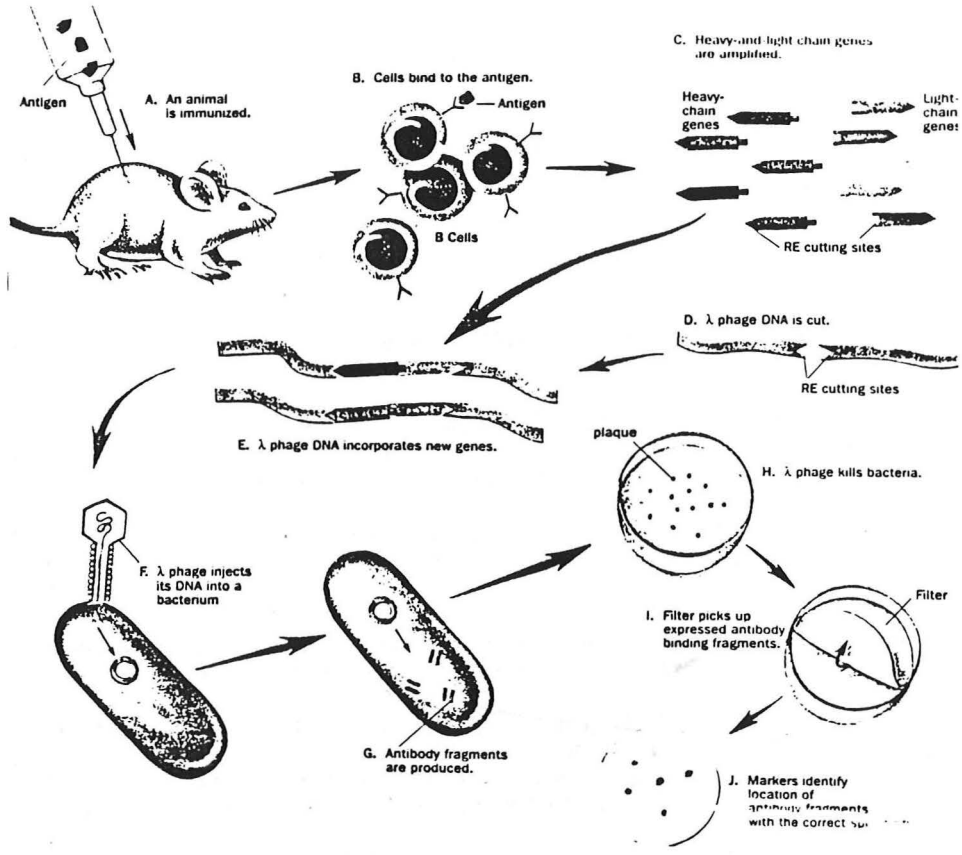


**Figure 11.** Concept of repertoire cloning (from J. NIH Research, July, 1990).

## CONCLUSIONS

Monoclonal antibodies have gradually worked their way into clinical medicine. At the present time, while only a single monoclonal antibody has obtained the imprimatur of FDA approval (and it is a murine antibody), genetically engineered murine antibodies and human monoclonal antibodies are closer to widespread use. I believe the future is bright for these exquisitely specific delivery systems particularly as they demonstrate both specificity and lack of immunogenicity. In my judgment, once this barrier is breached, these antibodies will become acceptable therapies not only devastating complications of transplantation and for the treatment of malignancies but also in the general therapeutic armamentarium against dreaded increasingly though not generally fatal diseases such as the bulk of autoimmune diseases, chronic infectious diseases, and the like.

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