Therapeutic Monoclonal Antibodies: Attractions and Challenges

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Due to the technological advances made during the past decade, therapeutic monoclonal antibodies (MAbs) now represent an important and growing class of bio-therapeutics. Sales of MAbs are increasing rapidly from virtually nil in 1996 to potentially over $1 billion in 2001. With the potential new targets resulting from genomics and with methods now in place to make fully human antibodies, the potential of antibodies as valuable therapeutics in oncology, inflammation and cardiovascular disease is being fully realised. There are 235 MAbs under different stages of development. More than 100 clinical trials are in progress, 14 of them in phase III (mostly for cancer diagnosis and therapy). To meet the increased demand of the monoclonals, worldwide manufacturing capacity for MAbs must increase dramatically, during the next few years, if the industry segment is to avoid a manufacturing bottleneck. Many novel expression strategies are being evaluated to increase the production capacity. Factors including molecular fidelity and cost of products will be critical in the selection of the expression systems.

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Introduction

The ability to generate antibodies of a given specificity is one of the most powerful inventions of the last 20 years. These Monoclonal antibodies (MAbs) are now an important tool for the identification and purification of cells and molecules in basic research (Abbas et al., 1991). In contrast, the clinical application of MAbs for therapeutic use is just emerging. Antibody therapeutics, labelled as “magic bullets” can potentially treat diseases ranging from autoimmune disorders to cancer and viral or bacterial infections. The number of MAbs currently in development is higher than in any other therapeutic category except vaccines (Davis et al., 1999).

Magic Bullets History

Antibody-based therapy is not new. A hundred years ago, physicians began using human serum from patients exposed to particular diseases (and therefore rich in antibodies) to treat a variety of infectious diseases, including diphtheria, influenza, and pneumonia. Serum therapy had problems with lot-to-lot variations in effectiveness, and therapy was accompanied by numerous side effects such as "serum sickness"—a syndrome characterized by rash, proteinuria, and arthralgia occurring in up to 50% of patients and probably the result of an immune reaction. Despite these drawbacks, serum therapy became standard treatment for lobar pneumonia by the 1930s, reducing the death rate in patients with pneumococcal pneumonia by 50%. Serum therapy was quickly abandoned with the introduction of antibiotics that were far more effective and had fewer side effects.

Interest in antibody therapy was reawakened in the mid-1970s when cultured myeloma cells could be made to fuse with normal B-cells from the spleen of an immunized mouse (Kohler & Milstein, 1975). When a myeloma cell that had lost the ability to secrete immunoglobulin was fused with a B-cell known to secrete a particular antibody, the result was a hybrid cell, called a hybridoma, that could multiply indefinitely like the myeloma cell, and produce enormous quantities of the particular antibody.

The potential applications for MAbs appeared almost limitless (Goding, 1996). Apart from their obvious use as therapeutic agents for bacterial and viral infections, researchers envisioned them as carriers for anti-neoplastic and radio-therapeutic agents and as diagnostic tools of heretofore-undreamed-of specificity. But MAb-based therapy almost immediately began to demonstrate limitations. MAbs are pathogen-specific. This limited their effectiveness in mixed infections and made them somewhat less attractive to pharmaceutical manufacturers, since their range of potential uses would be considerably smaller than, for example, that for a broad-spectrum antibiotic. Also, antibodies are
more effective in preventing infection than in eradicating existing disease and, because they are proteins, they usually require systemic rather than oral administration. Then there are the cost challenges.

**Murine Monoclonals as Therapeutics**

The utility of the murine antibodies as human therapeutics was limited by their immunogenicity in humans (Richards et al., 1999; Kipriyanov & Little, 1999). The main difficulty is that mouse antibodies are "seen" by the human immune system as foreign, and the human patient mounts an immune response against them, producing human anti-mouse antibodies (HAMA). The HAMA not only causes the therapeutic antibodies to be quickly eliminated from the host, but also form immune complexes that cause damage to the kidneys.

**Attractions**

**New Generation of MAb Technologies**

Recombinant DNA technology makes it possible to genetically engineer a chimeric antibody to reduce the risk of a host immune response (Goding, 1996). The following approaches have been used in an attempt to reduce the problem of HAMA:

1. **Chimeric antibodies**: The antigen-binding part (variable regions) of the mouse antibody is fused to the effector part (constant region) of a human antibody using genetic engineering.
2. **Humanized antibodies**: The amino acids responsible for making the antigen-binding site (the hyper variable regions) of the mouse antibody are inserted into a human antibody molecule replacing its own hyper variable regions.

The resulting mouse/human chimeric antibody minimizes the development of HAMA (Goding, 1996). The human Fe portion also allows for lysis of tumour cells *in vitro*, mediated by human complement and ADCC (Males et al., 1999) *In vitro* studies demonstrate that chimeric antibodies are more effective at lysing tumour cells than murine antibodies (Jurcic et al., 1996).

Thus the emergence of antibodies as an attractive therapy, is the result of the evolution of MAb technology over the past 25 years, from 100% mouse protein through chimeric and humanised proteins to fully human antibodies (Fig. 1). In 1984, chimeric MAbs containing 66% human and 34% mouse sequences were engineered (Morrison et al., 1984). Approximately half of the therapeutic antibodies currently approved by the FDA are of this design. Between 1988 and 1991, techniques to successfully humanise murine antibodies using complementarity-determining region (CDR) grafting and veneering techniques were established (Riechmann et al., 1988; Padlan, 1991) reducing the mouse proportion of the sequence to only 5–10%. An alternative approach for producing fully human antibodies is phage display (Huls et al., 1999). The initial product is either a single-chain Fv fragment (scFv), in which a short polypeptide is used to directly link the variable heavy and variable light chains, or an Fab antibody fragment (Fig. 2) from which a full-length antibody must then be constructed and produced using suitable expression systems (Green, 1999). The mid 1990s saw the emergence of transgenic mice that had been genetically engineered to contain a human antibody repertoire (Green et al., 1994; Mendez et al., 1997; Taylor et al., 1994). This technology allows the use of standard hybridoma techniques to generate fully human antibodies, obviating the need for humanisation altogether. The generation of fully human antibodies has now raised the standards for antibody manufacturing because the protein structure itself should not be immunogenic or have a short half-life.

**Uses for MAbs**

MAbs are widely used as diagnostic and research reagents. Their introduction into human therapy has been much slower. In some *in vivo* applications, the antibody itself is sufficient. Once bound to its target, it triggers the normal effector mechanisms of the body. In other cases, the MAb is coupled to another molecule, for example, a fluorescent molecule to aid in imaging the target or a strongly radioactive atom, such as Iodine-131 to aid in killing the target. Some MAbs, which have been introduced into human medicine, are as follows:

- **OKT3**. Binds to a molecule on the surface of T cells. Used to prevent acute rejection of organ, e.g., kidney, transplants.
- **Lympho Cide**. Binds to CD22, a molecule found on some B-cell leukaemias.
- **Rituximab** (*trade name*-Rituxan). Binds to the CD 20 molecule found on most B-cells and is used to treat B-cell lymphomas.
- **Lym-1** (*trade name*-Oncolym). Binds to the HLA-DR-encoded histocompatibility antigen that can be expressed at high levels on lymphoma cells.

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1 Monoclonal antibodies for clinical and research use: http://www.ultranet/-/kimball/Biology/Pages?/M/Monoclonals.html
Fig. 1—Evolution of monoclonal antibodies from (a) mouse, through (b) chimeric (67% human), (c) humanized (90-95% human) and (d) human (100% human). Mouse-derived sequences (light shading) and human-derived sequences (dark shading) are shown., indicates glycosylation

**Daclizumab** (*trade name: Zenopax*). Binds to part of the IL-2 receptor produced at the surface of activated T cells. Used to prevent acute rejection of transplanted kidneys.

**Infliximab**. Binds to tumour necrosis factor-α (TNF-α). Shows promise against some inflammatory diseases such as rheumatoid arthritis.

**Herceptin**. Binds HER-2/neu, a growth factor receptor found on some tumour cells (some breast cancers, lymphomas). The only monoclonal so far that seems to be effective against solid tumours.

**CMA-676**. A conjugate of a MAb that binds CD33, a cell-surface molecule expressed by the cancerous cells in acute myelogenous leukaemia (AML) but not found on the normal stem cells needed to repopulate the bone marrow. The toxin calicheamicin, is an oligosaccharide that blocks the binding of transcription factors (proteins) to DNA and thus inhibits transcription. CMA-676 is the first immunotoxin that shows promise in the fight against cancer.

**Vitaxin**. Binds to a vascular integrin (αβ3) found on the blood vessels of tumours but not on the blood vessels supplying normal tissues. In Phase II clinical trials, Vitaxin has shown promise in shrinking solid tumours without harmful side effects.

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Fig. 2—Structural summary of antibodies and antibody-derived clinically useful proteins that are. (a) A full-length antibody of the IgG subclass contains both heavy (VH, CH1, CH2 and CH3 domains) and light (VL and CL domains) chains. (b) An Fab fragment contains VH, VL, CL and CH1 domains, along with a portion of the hinge region. (c) A scFv contains VH and VL domains connected via a polypeptide linker.

**Abciximab** (*trade name: Reopro*). Inhibits the clumping of platelets by binding the receptors on their surface that normally are linked by fibrinogen. Helpful in preventing re-occlusion of the coronary arteries in patients who have undergone angioplasty.

**Palivizumab**. Directed against an antigenic site of the respiratory syncytial virus (RSV). Palivizumab is used as a prophylactic in infants at high risk of RSV infection.

**Enbrel**. Antibody directed against soluble TNF receptor and is useful for rheumatoid arthritis.

**HumaSPECT**. Totally human Mab labelled with technecium Te99m is used for monitoring of recurrence of colorectal cancer. Clinical trials are underway for ovarian and prostate cancer patients.

**HumaRAD**. Totally human Mab for the treatment (radio-immunotherapy) of compartmentalized solid tumours.

**Xolair**. Directed against IgE and is useful for the treatment of respiratory disorders. This has been approved by FDA in July 2001 and should soon hit the market.
This list will soon become bigger as more than 14 antibodies are in final stages of clinical trials\textsuperscript{2}.

**Challenges**

**Production Bottlenecks**

In recent years the volume of commercialised antibodies has increased from 257 kg in 1999 to an anticipated 558 kg in 2002 (Dutton, 2001). Within the next year or two, average volume of MAbs made by an individual manufacturer is expected to increase by 20\% per year, from today's average of about 75-100 Kg/year. To complicate that, approximately 235 MAbs are in development now. By 2010, total capital cost outlay. At present there are approximately six large-scale mammalian cells antibody production plants in the whole world (Morrow, 2000). These include Glaxo-Wellcome, Biogen, American Home Products and three Genentech plants. The recent Genentech plant has antibody production capacity of about 1000 kg. The facility has more than 300,000 sq ft of space on 100 acres, with eight 12,000-litre fermenters, three seed trains, and one recovery train. Since it takes about 5 years to construct an approved facility it will be essential to plan ahead to meet the future demand of recombinant antibodies.

**High Cost of MAbs**

The MAb therapies currently on the market are expensive. Abciximab and infliximab cost $450 per shot. To put this in perspective, the average patient requires three to four shots of Infliximab per treatment. Trials estimated that the cost of Abciximab use was $1407 per patient. Palivizumab is marketed in 100 mg vials at a cost of $948.93 per vial; Rituximab costs $9438 for a course of four infusions given over 22 days; and Herceptin costs $718 per weekly infusion\textsuperscript{3}.

The high prices of MAbs reflect not only R & D costs, but also the expense of sophisticated and highly complex manufacturing systems needed to produce MAbs in volume. With time and advances in the antibody expression systems, these costs should come down. Another way to reduce costs is to enhance the effectiveness of the antibodies, thus reducing the amount required per dose. Developing mutants of antibodies with higher affinities and neutralising activity than those had by the native antibody can do this. In laboratory experiments at the Scripps Research Institute in La Jolla, California, for example, researchers enhanced the affinity of a prototype of an anti-HIV antibody by 420-fold\textsuperscript{3}.

**Choice of the Expression Systems**

There are various expression systems that can be used for the production of whole antibodies and antibody fragments (Chadd & Chamow, 2001). These include bacterial or mammalian cell cultures and transgenic animals or plants. The expression system of choice is partially dependent upon the intended use of the antibody, as well as the antibody yield derived from each system.

**Bacterial, yeast and insect cell culture.** Bacterial antibody production systems can generate multi-ton yields, but can not perform proper glycosylation, making them more suitable for the expression of antibody fragments like scFvs and Fabs (Fig. 2). In this system, product recovery and refolding may be variable. Recombinant fragments can be localised intracellular or within the periplasm. The yield of scFvs in bacterial expression systems is usually low, primarily as a result of incorrectly folded protein retained either at the inner cell membrane or as an insoluble aggregate in the periplasm. Sánchez et al (1999) described the generation of 27-30 \( \mu \)g/ml of active scFv expressed in bacterial cytosol. Although the insoluble periplasmic protein can be extracted and refolded, high periplasmic expression can cause toxicity and cell death (Sánchez et al, 1999; Cupit et al, 1999).

ScFvS have also been expressed in insect cells (Sánchez et al, 1999; Cupit et al, 1999; Grant et al, 1999; Reavy et al, 2000; Stoger et al, 2000; Freyre et al, 2000). In contrast to bacterial and yeast cells, there is some debate on the ability of yeast cells to express functional antibodies; indeed, the glycosylation pattern in yeast is very different from that in mammalian systems (Cupit et al, 1999; Freyre et al, 2000).

**Bacterial expression systems are cost-effective for the production of antibody fragments where no effector function or extended pharmacokinetic activity is required.** To take advantage of bacterial expression, pegylation of the antibody fragment can be performed to increase the half-life of the antibody fragments in humans (Chapman et al, 1999). Because the assembly and glycosylation of full-length

antibodies is not possible in bacterial systems, mammalian cell culture or transgenic organisms are better suited for production of these molecules.

**Mammalian cell culture.** Any human antibody IgG sub-class can be generated in mammalian cell culture systems using either CHO cells, requiring methotrexate amplification, or NS0 cells, requiring methionine sulfoximine amplification (using glutamine synthetase selection in NS0 cells). In general, productivity in mammalian cell culture continues to increase because of improved recombinant expression vectors, the identification of 'hot integration spots' within the genome and the enhancement of biomass accumulation. This has resulted in cell-culture processes achieving yields of 1–2 g/L of unpurified antibody (Zhou et al, 1997). Further increases in the productivity of mammalian cell culture could be achieved by enhancing cell growth and the inherent specific productivity of the manufacturing cell line. Increasing the yield of antibody from a manufacturing process is particularly important where the product is to be used chronically and therapeutic doses are high. For some high-dose indications >200 kg of purified bulk material may be required annually at a cost of goods of >$500/kg. Assuming a productivity of 2 g/L with a 50% process yield, at least twenty 10 kL bioreactor runs per year would be required to meet this output. For the industry to maintain an acceptable margin on future production, the cost of goods must be reduced by 1–2 orders of magnitude to tens of dollars per gram.

**Transgenic organisms.** An alternative method for the large-scale production (e.g. hundreds of kg) of antibody is expression in transgenic animals or plants.

**Goats.** The generation of a transgenic goat herd able to produce antibody for phase I clinical trials takes up to 24 months. Antibody DNA fused to a milk-specific regulatory element is inserted into a single cell embryo by micro-injection. At this time, several MAbs expressed in transgenic goat milk are in early-to-late stage of development and clinical testing. There is, as yet, limited clinical data on the efficacy and safety of antibodies generated in goat milk (Pollock et al, 1999). Current figures suggest that, at 1000 kg/year, antibodies could be produced in goat milk for a cost of approximately $40/g (Fulton, 1999), compared with mammalian cell culture in which the cost is $300–1000/g (at 100 kg/year assuming a titer of 0.5g of antibody/L) (Russell, 1999).

**Chicken.** The transgenic production of antibodies in egg white has been reported to take approximately 18 months. This expression system has the potential to supply large quantities of material for clinical trials relatively inexpensively. The commercial cost of chicken eggs [produced under conditions that do not comply with current good manufacturing practice (cGMP)] is currently $0.05/egg (Morrow, 2000). Hence, the cost of generating unpurified material from transgenic chickens is calculated at $0.5/g. Although this cost appears low, it does not account for the production of material under cGMP conditions. There are, as yet, no clinical data available to support the production of therapeutic antibodies using this system.

Transgenic animals offer the advantage of lower material and product costs. Concerns over prions and viruses require careful evaluation of hygiene standards.

**Plants.** Transgenic plants represent the final alternative, again with advantages and disadvantages. Costs are potentially the lowest of any method, and plants have the capacity to meet extremely high demand, greater than 500kg/ear. The generation of transgenic plants for preclinical and phase I studies takes approximately 20 months. Antibody DNA is introduced into the plant using either Agrobacterium infection or gene bombardment in the presence of a carrier molecule and gold particles. In the case of corn, antibody is produced in the seed; in tobacco plants, antibody is synthesised in the leaf. Recent advances with inducible promoters allow controlled expression only in harvested plant tissue so that antibody is generated in a GMP facility overnight and not out in the field (Doran, 2000). Although expression levels can be quite high, the carbohydrate structures generated in plants are quite distinct in composition and structure from human lipoproteins. To overcome this problem, aglycosyl-antibodies are typically produced in transgenic plants (Baez et al, 2000). For IgG1 subclasses requiring effector function, this type of expression has obvious limitations; however, an aglycosyl form of IgG2 or IgG4, both of which lack significant effector function, might be successful.

The antibody huNR-LU-10 mAb, for example, generated in corn, has been genetically engineered to knockout the glycosylation site. The aglycosyl molecule was comparable in function to its glycosylated counterpart, although in vitro antibody-dependent cell cytotoxicity was reduced (Morrow,
In addition, a humanised aglycosyl IgG1 to treat herpes simplex virus 2, produced in soybean, was compared with the glycosylated molecule produced in murine cells. Both antibodies were similar in affinity, neutralizing activity and stability. Also, a monoclonal IgA for the oral treatment of tooth decay, produced in transgenic tobacco, demonstrated higher functional activity and longer survival times than the murine IgG equivalent.

A rudimentary cost analysis conducted for plant-produced antibodies gave a figure of $100/g (Russell, 1999). Growth in the field versus the greenhouse makes a difference in production cost. For field crops, production costs of only $43/g have been reported, whereas the generation of the same material in plants cultivated in greenhouses rises to $500–600/g (Doran, 2000).

**Constraints of Antibody Glycosylation**

Alterations in glycosylation patterns can change the properties of an antibody (Raju et al., 2000). Since it is absolutely essential that antibodies generated through different expression systems demonstrate the same patterns of glycosylation, this is a major stumbling block to switching systems, for instance from mammalian cells to plants, regardless of the economic advantages.

Recent developments in glycobiology have increased the ability to control and understand glycosylation (Raju et al., 2000; Umanna et al., 1999). These include more sensitive and accurate assays, more thorough understanding of expression systems, cloning of glycosylation genes, and extensive study of cell culture influences on glycosylation patterns. It is known that most anti-cancer MAbs do not function when used as Fab or F(\(\alpha\)b)2 fragments. Thus, glycosylation is highly relevant to proper functioning of therapeutic antibodies, and aglycosylation can abolish Fc receptor binding and other critical effector functions. Thus, the oligosaccharide can function as a structural ‘rheostat’ modulating effector function.

These critical functions of glycosylation in recombinant antibodies have been a motivating force for selecting the mammalian cells as an expression system by the major manufacturers of antibodies. Recently Dr. Krummen’s group has engineered human B1,4 galactosyl transferase over-expression in mammalian cells in order to maximise levels of antibody glycosylation (Weikert et al., 1999). With the over-expression, there was a profound effect on the degree of galactosylation of Fc carbohydrates. Structures with two galactosylated branches increased from 5%-95%; demonstrating that molecular engineering methods can affect the cell’s ability to regulate glycon structure.

The direct therapeutic benefit lies in the ability of Fc structure to provide enhanced antibody-dependent cell cytotoxicity (ADCC). Increase in ADCC through enhanced glycosylation is believed to be a simple and cost-effective method to improve MAb function.

**The Future of MAb Therapy**

MAbs, despite the high costs and limited therapeutic range of specific MAbs, are proving to be one of the most exciting fields of current clinical research. MAbs are seen as a means of treating patients who develop infections by drug-resistant strains of pathogens, for combating newly emergent pathogens for which there is no effective treatment, and for use in immunocompromized patients in whom conventional antimicrobial therapy is frequently ineffective (e.g., patients with AIDS). The time and cost of travelling from the laboratory to the clinic are shorter for MAbs than for many conventional drugs. Antibodies are relatively easy to detect, manipulate, and test. The FDA now regards MAbs as "biotechnology-derived pharmaceuticals," which has created a straightforward review process, and there have been relatively few reports of serious side effects or safety problems. A recently published longterm followup study reported almost half of 166 patients with relapsed or refractory lowgrade non-Hodgkin’s lymphoma responded to treatment with Rituximab, Phase III trials of Herceptin have shown that adding this MAb to chemotherapy in women with breast tumours expressing the target antigen increased the positive response rate by 53% (Baselga et al., 1998).

The public health possibilities of MAbs are striking. Additional MAbs are being investigated most actively in treatment of a variety of cancers, including B-cell lymphomas, multiple myeloma, and colorectal cancer, besides allergic asthma, rheumatoid arthritis, and other ailments characterised by chronic inflammation (Dutton, 2001; Morrow, 2000). The route of entry of many diseases is through the mucosal membranes, and a MAb delivered to a mucosal surface can provide immediate protection against infection.

Recent advances in the expression of antibodies in transgenic goats, chicken and plants with respect to productivity, competitive timeliness and cost of goods suggest the need to seriously evaluate these recombinant systems as alternative platforms. Comparing cost of goods analyses between cell
culture and the various transgenic production systems, expression in transgenic chicken appears to show great promise when compared with goats and plants. With a growing number of antibodies moving into clinical evaluation, the utility of transgenic production will be evaluated alongside mammalian cell culture for the production of therapeutic antibodies. However, genetic engineers will be pressed in the coming years to develop transgenic plants, yeast and animal cells that can execute the identical glycosylation steps performed by mammals.

MAbs have significant impact in medical practices. If current research efforts continue, MAbs, the "magic bullets" of modern medicine, may indeed become the shots heard 'round the world.

References
Russell D A, 1999. Feasibility of antibody production in plants for human therapeutic use. in Current Topics in Microbiology


