Monoclonal antibodies: Pharmacological relevance

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ABSTRACT

Monoclonal antibodies (MAbs), a new class of biological agents, are used these days in therapeutics and diagnosis. MAbs also labeled as ‘magic bullets’, are highly specific antibodies produced by a clone of single hybrid cells formed in the laboratory by fusion of B cell with the tumor cell. The hybridoma formed yields higher amount of MAbs. MAbs can be produced in vitro and in vivo. Animals are utilized to produce MAbs, but these antibodies are associated with immunogenic and ethical problems. Of late, recombinant DNA technology, genetic engineering, phage display and transgenic animals are used to produce humanized MAbs or pure human MAbs, which have fewer adverse effects. MAbs alone or conjugated with drugs, toxins, or radioactive atoms are used for treatment of cancer, autoimmune disorders, graft rejections, infectious diseases, asthma, and various cardiovascular disorders. New MAbs are being developed which are more specific and less toxic.

KEY WORDS: Antibodies, biological response modifiers, cancer chemotherapy, immunosuppressant, immunotherapeutics.

Introduction

Humans and animals have the ability to make antibodies (Abs) to recognize any antigenic determinant (epitope) and to even discriminate between similar epitopes. These antibodies provide the basis for protection against various diseases. The use of vaccine for immunization is an excellent example of preventive use of Abs. Abs usage has now extended to treatment; hence, these are promoted as potential candidates for the management of various diseases. The Abs synthesized against a particular antigen (Ag) are known as monoclonal antibodies (MAbs). By definition, MAbs are a class of highly specific Abs produced by the clones of a single hybrid cell formed in the laboratory by the fusion of B-lymphocytes with a tumor cell.

MAbs are also known as ‘magic bullet’. The idea of ‘magic bullet’ was first proposed by Paul Ehrlich who at the beginning of the 20th century figured ‘if a compound could be made to selectively target a disease causing organism, then a toxin for that organism could be delivered along with the agent of selectivity’. The market for therapeutic MAbs is a most potential sector within the pharmaceutical industry. These antibodies are forecast to drive the market towards the $30 billion mark by the year 2011 due to a high level of innovation. Several MAbs are set to be launched in the next 5 years.

Experimental cancer studies have used various substances attached to MAbs such as radioactive material, drugs, immune killer cells, and so on, when injected into patients, home in on Ags that grow on the surface of killer cells. Therefore, MAbs are also labeled as biological response modifiers. Since they affect the immune system, they are also called immunotherapeutics as opposed to chemotherapeutics, which are drugs used to interfere cell growth (cancer). MAb therapy is a form of passive immune therapy because the antibodies are made in large quantities outside the body (in the laboratory) rather than by a person's immune system. Therefore, these MAbs do not require the person's immune system to take an ‘active’ role in fighting the cancer.

The development of the immortal hybridoma requires the use of animals. No method of generating a hybridoma that avoids the use of animals has been found. Recent in vitro techniques allow the intracellular production of antigen-binding antibody fragments, but such techniques are still experimental and have an uncertain yield, efficacy and antibody function. There are two methods for growing these cells: injecting them into the peritoneal cavity of a mouse or using in vitro cell-culture techniques.

Production of MAbs

The process of producing MAbs was invented by Kohler and Milstein in 1975. They shared the Nobel Prize in Physiology/Medicine in 1984 for the discovery of MAb. The key idea was to use a line of myeloma cells that had lost the ability to secrete antibodies. They came up with a technique
to fuse these cells with healthy antibody producing B cells. This fusion resulted in a clone of cells that retained the myeloma cell line and ability to live indefinitely in tissue culture. The procedure yielded a cell line capable of producing one type of antibody protein for a long period. The fused cell was called a hybridoma and produced large quantities of MAbs.

**Production in animals** [Figure 1]

When injected into a mouse, the hybridoma cells multiply and produce fluid (ascites) in its abdomen. This fluid contains a high concentration of antibody. The mouse ascites method is inexpensive and easy to use. However, if too much fluid accumulates or if the hybridoma is an aggressive cancer, the mouse will likely experience pain or distress. If a procedure produces pain or distress in animals, regulations call for a search for alternatives. The mouse ascites method usually produces very high MAbs concentration that often does not require further concentration procedures that can denature antibody and decrease effectiveness. It avoids the effects of contaminants as in *in vitro* batch-culture fluid method and no expert guidance is required to teach the method. However, it involves the continued use of mice requiring daily observation and the MAbs produced contains various mouse proteins and other contaminants that might require purification.[9]

**Production in cell-culture**

One alternative is to grow hybridoma cells in a tissue culture medium, but this technique requires some expertise, special media and can be expensive and time-consuming. There has been considerable research on *in vitro* methods for growing hybridomas and these newer methods are less expensive, faster, and produce antibodies in higher concentration than has been the case in the past. Following are the *in vitro* production methods that are available.

**Batch tissue-culture methods.** The simplest approach for producing MAb *in vitro* is to grow the hybridoma cultures in batches and purify the MAbs from the culture medium. Fetal bovine serum is used in most tissue culture media. The MAb concentration achieved is low (around a few micrograms per milliliter) and some MAbs are denatured during concentration or purification process.

**Semipermeable-membrane-based system.** A barrier, either a hollow fiber or a membrane, with a low-molecular-weight cutoff (10,000-30,000 kD), called semipermeable-membrane-based system permits cells to grow at high densities in culture. The objective of this system is to isolate the cells and MAbs produced in a small chamber separated by a barrier from a larger compartment that contains the culture media. Culture can be supplemented with numerous factors that help optimize growth of the hybridoma. These methods produce MAbs in concentrations often as high as those found in ascitic fluid and are free of mouse ascitic fluid contaminants. These methods reduce the use of animals and are the methods of choice for large-scale production by the pharmaceutical industry because of the ease of culture.[7,8]

Although *in vitro* techniques can be used for more than 90% of MAb production, it must be recognized that there are situations in which *in vitro* methods will be ineffective, as some hybridomas do not grow well in culture or are lost in culture. Because hybridoma characteristics vary and MAb production needs are diverse, *in vitro* techniques are not suitable in all situations. These techniques might impede research, especially if large numbers of MAbs are to be screened for efficacy or specificity in the treatment of diseases. *In vitro* methods generally require the use of fetal calf serum, which limits some antibody uses and which is a concern from the animal-welfare perspective. The loss of proper glycosylation of the antibody (in contrast with *in vivo* production) might make the antibody product unsuitable for *in vivo* experiments because of increased immunogenicity, reduced binding affinity, changes in biologic functions or accelerated clearance in *in vivo*. MAbs produced by membrane-based *in vitro* methods are contaminated with dead hybridoma cells and their products, thus require early and expensive purification. *In vitro* culture methods are generally more expensive than the ascites method for small or medium-scale production of MAbs.

**Evolution of MAbs**

The significance of MAbs lies in their specificity and immortality. Whereas hybridoma development of murine MAbs was the requisite for the development of MAbs as drugs, the inherent immunogenicity of rodent sequences in humans has presented obstacles to the clinical application of MAbs. Sensitization to MAb therapeutics poses significant risk to the patient and may blunt the efficacy of these therapies. The advent of chimeric antibodies lessened but did not eliminate the rodent content of MAbs. Thus, immunogenicity remained a concern. Further, elimination of rodent sequences enabled the production of humanized MAbs. This was followed by current technology using phage display and finally, transgenic mice technology, which allows for the generation of fully human therapeutic MAbs. The reduced immunogenicity of this new generation of MAbs is expected to enhance efficacy, safety, and ease of use.[9] The MAbs are also classified into generations as per their evolution and immunogenicity as follows:

1. **First generation MAbs.** Majority of the earlier MAbs available were murine, rabbit, or rat proteins purified following immunization of the animal with an antigen.
preparation. These were labeled as first generation antibodies. Patient often generated Abs to these foreign antigens. These host antibodies are referred to as human anti-mouse antibody (HAMA) or human anti-rat antibodies or human anti-rabbit antibodies (HARA). The host antibody blocked the effectiveness of therapy by prematurely clearing the treatment antibodies and limiting possibilities for future immunotherapy.\textsuperscript{[10]} HAMA or HARA responses could be associated with immune-complex related adverse events, such as serum sickness or anaphylaxis. In addition, these first generation MAbs are not able to recruit human effector functions such as antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), which cause destruction of a malignant cell.

2. Second generation MAbs. To overcome the obstacles of first generation antibodies, Winter et al. pioneered the techniques to humanize MAbs, by removing the reactions that many earlier MAbs caused in some patients.\textsuperscript{[11]} Nowadays, DNA technology or genetic engineering is used to construct hybrids composed of human antibody regions linked with a murine or primate backbone. These are labeled as second generation MAbs and are referred to as chimeric, humanized, primatized, or pure human MAbs.\textsuperscript{[12,13]}

(i) Chimeric Abs. These are composite of antibodies from two different species. The Ab combines the Ag binding parts (variable region) of the mouse Ab with the effector parts (constant region) of a human Ab, e.g., infliximab, rituximab, abiciximab.\textsuperscript{[12,13]}

(ii) Humanized Abs. Human antibody containing the complementarity-determining region from a non-human source. The Ab combines only the amino acids responsible for making the Ag binding site (the hypervariable region) of a mouse Ab with the rest of human Ab molecule, thus replacing its own hypervariable regions, e.g., daclizumab, herceptin, vitamins.\textsuperscript{[12,13]}

(iii) Primatized Abs. It is a composite of primate variable regions and human constant regions.\textsuperscript{[12,13]}

(iv) Genetically engineered Abs. Fab portion from rodent Abs is attached toFc portion of human Abs. Human MAbs have been produced by immortalizing human B lymphocytes with the Epstein-Barr virus and using special immunodeficient mice immunologically reconstituted with immunocompetent cells of tissue. Using genetic engineering it is possible to make mouse-human hybrid antibodies.

(v) Human MAbs can be produced by the following techniques:

(a) Recombinant DNA technology. By inserting random human genes coding for variable Fab portion of human Abs into the genome of filamentous bacteriophages. As the bacteriophage replicate they display Fab portion Ab on their surface. The bacteriophages are subsequently mixed with an antigen to select those producing complementary Fabs. Those bacteriophage genomes are then converted into plasmids that can subsequently produce specific Fabs in bacteria.\textsuperscript{[9]}

(b) Transgenic mice. Transgenic technology has been exploited to make a transgenic mice that have human Ab gene loci inserted into their bodies (using embryo stem cell method) and their own genes for making antibodies ‘knocked out’. Therefore, mouse can be immunized with the desired antigen and produces human Abs.\textsuperscript{[9]}

(c) Phage display. This is another technique for making human MAbs. It is used when MAbs do not directly recognize antigen or when antigen is undetectable normally and expressed only in disease. Phage display libraries are available.\textsuperscript{[9]}

Plant genetic engineering has also led to the production of plant-derived MAbs (MAbs\textsuperscript{*}), which provides a safe and economically feasible alternative to the current methods of antibody production in animal systems. Transgenic plants have proven to be an efficient production system for the expression of functional therapeutic proteins. Plant-derived MAbs\textsuperscript{*} have the same advantages, namely, lack of animal pathogenic contaminants, low production cost and ease of agricultural scale-up compared with the conventional fermentation methods. Since the initial report of functional MAbs expressed in transgenic plants, therapeutic and diagnostic MAbs\textsuperscript{*} have been successfully produced in transgenic tobacco, soybean, alfalfa, and other plants. Two MAbs\textsuperscript{*} have recently been used for topical passive immunization against Streptococcus mutans and herpes simplex virus in animals. Till date, no study has reported the use of systemic administration of MAbs\textsuperscript{*} to provide immunoprotection.\textsuperscript{[11,13]}

Large scale production of MAbs

The development of a commercial monoclonal antibody production process involves much more than just scaling-up the laboratory process and making it cost-effective. It involves establishing the hybridoma cell bank with cells that are free of adventitious agents such as viruses and mycoplasma, that have stability in continuous culture for antibody-production rate and cell viability and that do not have unusual or expensive media requirements. The style and mode of operation of the bioreactor used to produce the antibody must be explored. The antibody-based product must be processed to high levels of purity and specific contaminants such as DNA and endotoxin must be reduced to extremely low levels. Appropriate labeling or drug conjugation methods must also be developed. The product must be formulated, so that it has performance characteristics that are stable over a reasonable period of time. Adequate test procedures must be developed to assure product purity, activity, stability, and safety on a lot-to-lot-basis. Compliance with drug regulations, guidelines, and procedures must be guaranteed. In the coming decade, it is likely that the two arms of biotechnology, hybridoma technology and recombinant DNA technology, will be used together to generate unique protein molecules.\textsuperscript{[16]}

Nomenclature

The United States adopted name (USAN) Council has
outlined specific guidelines for the nomenclature of MAbs. These guidelines provide a foundation of knowledge about a specific MAb just by looking at the generic name.

- All MAbs end in the suffix - mab. It is used to identify a class of medicines.
- The infix preceding - mab denotes the source of the product.
  e.g., u = human i = primate
  o = mouse e = hamster
  a = rat xi = chimera
  zu = humanized
- The infix preceding the source of the Ab refers to medicine target.
  e.g., vi(r) - viral ci(r) - cardiovascular
  ba(c) - bacterial co(l) - colonic tumor
  li(m) - immune le(s) - infectious lesions
  me(l) - melanoma ma(r) - mammary tumor
  go(t) - testicular tumor go(y) - ovarian tumor
  pr(o) - prostate tumor tu(m) - miscellaneous tumor

When combining a target or disease infix stem with the source stem for chimeric or humanized MAbs the last consonant of this target syllable is often dropped to make the name more pronounceable (e.g., -tum- changed to -tu-).

Target source MAb stem
e.g., ci(r) - xi -mab Abciximab
tu(m) -zu -mab Trastuzumab

- The starting prefix is a distinct syllable carrying no special meaning and unique for each drug. If the product is radiolabeled or conjugated to another chemical such as a toxin, a separate word is used to identify the conjugate.

Types of MAbs that are used in treatment

Naked MAbs

These are those without any drug or radioactive material attached to them. They attach themselves to specific Ag on cells, e.g. cancer cells. They mark the cancer cells for the immune system to destroy it. Others attach to certain Ag sites called receptors where other molecules that stimulate the cancer cell growth might otherwise attach. By blocking other molecules from attaching there, MAbs prevent cancer cells from growing rapidly. Some examples of naked MAbs available for use are:

1. Trastuzumab - For advanced breast cancer
2. Rituximab - For B cell non-Hodgkin’s lymphoma
3. Cetuximab - For advanced colorectal cancer
4. Bevacizumab - For metastatic colorectal cancer
5. Alemtuzumab - For B cell chronic lymphocytic leukemia

Conjugated MAbs

MAbs, because of their inherent specificity, are ideal targeting agents. They can be used to deliver radionuclides, toxins, or cytotoxic drugs to a specific tissue or malignant cell population. These are attached to drugs toxins or radioactive atoms. They are also referred to ‘tagged’ ‘labeled’ or ‘loaded’ antibodies. MAbs with radioactive particles attached are referred to as radiolabeled and this type of therapy is known as radioimmunotherapy. MAbs attached to toxins are known as immunotoxins. The MAb acts as a homing device, circulating in the body until it finds a cancer cell with a matching antigen.

It delivers the toxic substance to where it is needed most, minimizing damage to normal cell. Examples are:

1. Ibritumomab tiuxetan- Radiolabeled MAb to treat B cell non-Hodgkin’s lymphoma.
2. Tositumomab- Radiolabeled MAb for non-Hodgkin’s lymphoma
3. Gemtuzumab ozogamicin (Mylotarg) - Immuno toxin MAb for AML. It is the only immunotoxin to receive FDA approval. It contains a toxin called calicheamicin. It is attached to an antibody directed against CD33 antigen present on leukemia cells.

Mechanism of action of MAbs

The mechanism by which MAbs achieve therapeutic effect is not very clear. Potential mechanisms include:

1. Blocking or steric hindrance of the function of target antigen i.e., T-lymphocytes. B lymphocytes, tumour necrosis factor-α (TNFα) and interleukin (IL) which are capable of transducing intracellular signals.
2. Cytotoxicity to the cell expressing target Ag by ADCC or CDC.
3. Inhibition of growth factors: Epidermal growth factor receptor (EGFR) is a cell surface receptor involved in regulation of cell proliferation and survival. Also new vessels grow to feed the cancer cells through this factor. These factors can be inhibited to arrest growth of cancer cells e.g., cetuximab act as EGFR inhibitor.

Pharmacokinetics

MAbs are used by intravascular route and remain essentially intravascular. Intravenous injection may not always be appropriate for long-term treatment for a variety of reasons. Hour-long infusions require a hospital environment and are often associated with mild to very severe side effects. Continuous and sustained delivery of antibodies can lead to induction of neutralizing anti-idiotypic immune responses, which sometimes develop when massive doses of purified immunoglobulins are repeatedly injected into patients. Additionally, the bioavailability of therapeutic antibodies is often detrimental to the treatment efficacy. They have small volume of distribution and limited tissue penetration. They remain in circulation for 2 days to 2 weeks. Another limitation is the high cost of recombinant proteins certified for human use.

Antibodies can have exquisite specificity of target recognition and thus generate highly selective outcomes following their systemic administration. While antibodies can have high specificity, the doses required to treat patients, particularly for a chronic condition, are typically large. Fortunately, advances in production and purification capacities have allowed for the exceptionally large amounts of highly purified MAbs to be produced. Additionally, genetic engineering of antibodies has provided a stable of antibody-like proteins that can be easier to prepare.

Genetic manipulations of the immunoglobulin molecules are effective means of altering stability, functional affinity, pharmacokinetics, and biodistribution of the antibodies required for the generation of the ‘magic bullet.’

Adverse effects

Adverse effects with MAbs are related to one of three mechanisms.
1. Xenogenetic nature of MAb used
2. Suppression of physiological function
3. Activation of inflammatory cells or mediators after binding of MAb to its target

Adverse effects with naked MAbs are usually mild and often related to an 'allergic' reaction and occur while the drug is being first infused. This reaction is attributed to massive cytokine release resulting from transient activation of T lymphocytes. Reactions may include fever, chills, weakness, headache, nausea, vomiting, diarrhea, low blood pressure, and rashes. Some MAbs cause leucopenia, thrombocytopenia, and anemia. Conjugated MAbs cause more side effects and the actual effects depend to which substance it is attached. Sometimes it also causes suppression of physiological function depending on specificity of tissue targets. Thus anti-lymphocytes MAb cause immunosupression. There is also an increased risk of infection and cancer development. AntiTNF-α MAb treatment has been reported to increase the reactivation of tuberculosis because it interferes with the cellular response against mycobacteria and this adverse effect is more in areas with high incidence of tuberculosis. AntiTNF-α MAb treatment also leads to development of lymphomas.

Therapeutic Potentials of MAbs

MAbs were being used in laboratory research and in medical tests since the mid 1970s, but their effectiveness in disease treatment was limited. MAbs created much excitement in the medical world and in the financial world in 1980s especially as a potential cure for cancer. Although this resulted in great optimism that a therapeutic ‘magic bullet’ could be engineered, success with MAbs was many years away. By early 21st century, several drugs based on MAbs were introduced for a wide variety of therapeutic uses. Herceptin, a humanized MAb for breast cancer treatment, became the first drug designed by biomolecular engineering approach to be approved by the FDA. A recent survey suggested that 1 of all biotech drugs in development are MAb based. At least an additional 400 MAbs are under clinical trials to treat cancer, transplant rejection or to combat autoimmune or infectious diseases. It is now possible to obtain engineered antibodies, chimeric, or humanized or fully human MAbs via the use of phage display technology or of transgenic mice. Important therapeutic implications of MAbs are given in the preceding chapter.

Immunosupression - inhibition of alloimmune reactivity

In 1985, Muromonab CD-3 (OKT3) a murine MAb, was the first to be approved by the US FDA for clinical use in humans, for prevention of graft rejection in renal transplant patient. As first line or in steroid resistance rejection therapy, OKT3 has proved efficacious and improved graft survival. It specifically reacts with the T-cell receptor-CD3 complex on the surface of circulating human T cells. OKT3 binds to a glycoprotein (the 20-kd epsilon chain) on the CD3 complex to activate circulating T cells, resulting in transient activation of T cells, release of cytokines and blocking of T-cell proliferation and differentiation. Nearly all functional T cells are transiently eliminated from the peripheral circulation.

Although T cells reappear in the circulation during the course of treatment, these cells are CD3-negative and are not capable of T-cell activation. However, the use of OKT-3 was hampered due to production of Ags and rapid clearance from circulation. This led to development of humanized OKT-3 which is under investigation.

Acute graft rejection is a T-cell mediated immune response and depends on presence of IL-2. IL-2 binds to IL-2 receptor. In the search for more specific immunosupression with MAb L-2 Receptor (IL-2R), that is expressed on T-cells, which were chosen as target. Chimeric and humanized MAbs, basiliximab, and daclizumab were developed to bind to IL-2R. They competitively antagonized IL-2 or caused elimination of activated T-cells. They have been efficacious in preventing acute rejection episode after renal transplant. Table 1 lists the MAbs used in immunosupression.

Autoimmune diseases - inhibition of autoimmune reactivity

For the treatment of autoimmune disease, MAbs need to target immune response cells, i.e., B or T-cells. MAbs may function as an immunosuppressant by removing activated cells, blocking their function or normalizing elevated levels of proinflammatory cytokine. Therapeutic targets in this condition include T-cell surface Ags, T-cells activation Ags, molecules involved in T/B cell interaction, adhesion molecules, and cytokines.

The most promising result emerged from TNF-α blocking therapy in rheumatoid arthritis (RA) and Crohn's disease. TNF is a cytokine produced by activated monocytes and macrophages. The cytokine is actively produced at the synovial and mucosal sites of inflammation in RA and Crohn's disease. It is involved in vasodilation, increased vascular permeability, and activation of platelets and regulation of production of acute phase proteins involved in inflammation. TNF is also actively produced in various infectious diseases such as sepsis, malaria, adult respiratory disease syndrome, and AIDS.

TNF is considered to have an important role in autoimmune inflammatory disease. This led to the discovery of infliximab which is a chimeric MAb and found to be effective in various animal models and clinical trail for RA and Crohn's disease. It is clinically beneficial in Crohn's disease, reduces the response duration and also reduces fistula formation. The first trial of usefulness of infliximab was shown in 1994. Infliximab halts radiographic progression of RA and also clinically cures the disease. Adalimumab, a humanized IgG1 MAb is also approved for the treatment of RA. It binds to soluble and cell membrane-bound TNF-α. It is proved to be efficacious and halts radiological progression of the disease. Anti TNF-α MAb treatment has shown promise in patients with seronegative spondyloarthopathies and psoriatic arthritis. Anti-IL-6 and anti-IL-6 receptor MAbs have also been found to be useful in RA as IL-6 is elevated in patients with RA and levels of RA correlates with disease activity and extent of joint erosion.

Cancer

Classical therapeutic modalities such as surgery, radiation, and chemotherapy not only fail to cure the great majority of...
malignant tumors, but also their employment often leads to severe and debilitating side effects. Immunotherapy as a fourth modality of cancer therapy has already been developed and proven to be quite effective. Strategies for the employment of antibodies for anti-cancer immunotherapy include: (1) Immune reaction directed destruction of cancer cell, (2) interference with the growth and differentiation of malignant cells, (3) antigen epitope directed transport of anti-cancer agents to malignant cells, (4) anti-idiotype vaccines, and (5) development of engineered (humanized) mouse monoclonals for anti-cancer therapy. In addition, a variety of different agents (e.g., toxins, radionuclides, chemotherapeutic drugs, etc.) have been conjugated to mouse and human MABS for selective delivery to cancer cells.[45]

Unconjugated antibodies show significant efficacy in the treatment of breast cancer, non-Hodgkin's lymphoma, and chronic lymphocytic leukemia. Promising new targets for unconjugated antibody therapy include cellular growth factor receptors, receptors, or mediators of tumor-driven angiogenesis and B cell surface antigens other than CD20. One immunoconjugate containing an antibody and a chemotherapy agent exhibits clinically meaningful anti-tumor activity in acute myeloid leukemia.[46] Clinical trials of MAB therapy are in progress for almost every type of cancer. Rituximab was the first MAB used for treatment of cancer [Table 2]. It is chimeric IgG1 MAB directed against CD20, which is a transmembrane protein on mature B-lymphocytes. Its efficacy has been demonstrated against low grade and follicular non-Hodgkin's lymphoma relapse.[47] Rituximab has also been useful in

### Table 1

<table>
<thead>
<tr>
<th><strong>Generic name</strong></th>
<th><strong>Trade name</strong></th>
<th><strong>Type of MAB</strong></th>
<th><strong>Indication</strong></th>
<th><strong>Mechanism of action</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Muromonab CD-3</td>
<td>Orthoclone OKT3</td>
<td>Murine monoclonal</td>
<td>Renal graft rejection</td>
<td>Binds to a molecule of T cell to form receptor-CD3 complex and prevents acute rejection of organ.</td>
</tr>
<tr>
<td>Basiliximab</td>
<td>Simulet</td>
<td>Human–mouse chimaera IgG1K</td>
<td>Renal graft rejection</td>
<td>Renders it unable to stick to VLA-4.</td>
</tr>
<tr>
<td>Daclizumab</td>
<td>Zenapax</td>
<td>Humanized</td>
<td>Renal graft rejection</td>
<td>Binds to the IL-2 receptor on activated T cell surface.</td>
</tr>
<tr>
<td>Ranibizumab</td>
<td>Lucentis</td>
<td>IgG1 recombinant humanized IgG1 kappa isotype monoclonal antibodies</td>
<td>Neovascular (wet) age-related macular degeneration</td>
<td>Inhibits vascular endothelial growth factor activity.</td>
</tr>
<tr>
<td>Infliximab</td>
<td>Remicade</td>
<td>Human–mouse chimaera IgG1 monoclonal antibodies</td>
<td>Rheumatoid arthritis</td>
<td>Binds to and blocks TNF-α.</td>
</tr>
<tr>
<td>Other uses</td>
<td></td>
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</tr>
<tr>
<td>Abciximab</td>
<td>Reopro</td>
<td>Human–mouse chimaera</td>
<td>Antiplatelet</td>
<td>Inhibits platelet clumping by binding to surface receptors that are normally linked by fibrinogen.</td>
</tr>
<tr>
<td>Omalizumab</td>
<td>Xolair</td>
<td>Recombinant DNA-derived humanized IgG1</td>
<td>Allergic asthma</td>
<td>Binds to and prevent IgE binding to mast cells.</td>
</tr>
<tr>
<td>Natalizumab</td>
<td>Tysabri</td>
<td>Humanized monoclonal antibodies</td>
<td>Multiple Sclerosis</td>
<td>Attaches to alpha-4-integrin and renders it unable to stick to VLA-4. This interferes with the process by which lymphocytes are able to enter the CNS and thus reduce the damage that they cause in MS.</td>
</tr>
<tr>
<td>Efalizumab</td>
<td>Raptiva</td>
<td>Recombinant humanized IgG1</td>
<td>Plaque psoriasis</td>
<td>Interrupts the CD11A and lymphocyte function-associated antigen-1 (LFA-1) interaction.</td>
</tr>
<tr>
<td>Adalimumab</td>
<td>Humira</td>
<td>Humanized monoclonal antibodies</td>
<td>Rheumatoid arthritis, psoriasis</td>
<td>It binds specifically to TNF-α and blocks its interaction with the p55 and p75 cell surface TNF receptors.</td>
</tr>
<tr>
<td>Palivizumab</td>
<td>Synagis</td>
<td>Humanized IgG1 monoclonal antibodies</td>
<td>Antiviral</td>
<td>For prophylaxis in premature infants against Respiratory Syncytial virus infection and bronchopulmonary dysplasia.</td>
</tr>
</tbody>
</table>
Waldenstrom’s macroglobulinemia, posttransplantation lymphoma, and multiple myeloma[48-49] CD52 MAb (Campath-1H) has also been studied to lyse malignant hemopoetic cells.[50] CD52 MAb provides an effective therapy for chronic leukemia of T-cell or B-cell origin that is resistant to conventional chemotherapy. Anti-tumor therapy with MAbs targets growth factor receptor too.

Angiogenesis also plays a central role in the growth and metastasis of cancers. Antibodies directed against EGFR directly inhibit the growth of tumors bearing such receptors. Trastuzumab, a humanized Ab targets HER2 receptor found in breast cancer. It is the first MAb approved by US FDA for the treatment of solid tumors.[51] Trastuzumab blocks HER2 receptor action and this receptor is expressed on the surface of tumor cells in 30% patients with breast cancer and signifies poor prognosis. Strategies aimed at interfering with tumor blood supply offer promise for new cancer therapies. Vitaxin (an anti-alpha-v/beta-3 antibody) interferes with blood vessel formation by inducing apoptosis in newly generated endothelial cells.[52] In Phase II clinical trial it has shown promise in shrinking solid tumors. Bevacizumab which blocks the vascular endothelial growth factor (VEGF) receptor has been approved by US FDA for the treatment of colorectal cancer.[53] Bevacizumab has shown promising results in clear-cell renal cancer in various clinical trials.[54] The promising result of naked and conjugated type MAb in cancer may make MAb therapy significant in the management of these patients. It is expected that MAb-based immunotherapy may be accepted as a conventional form of therapy and employed not only in terminal cancer patients but also, in other instances like, during and following surgical resection.

**Antiplatelet therapy**

Table 2 shows various MAbs used in therapeutics. Acute coronary syndromes and percutaneous coronary intervention share a common physiological mechanism of intimal disruption and platelet aggregation. Glycoprotein IIb/IIIa receptor antagonist which interrupt the final common pathway of platelet activation and aggregation are used for

<table>
<thead>
<tr>
<th>Generic name</th>
<th>Trade name</th>
<th>Monoclonal antibodies type</th>
<th>Indication</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rituximab</td>
<td>Rituxan</td>
<td>Human–mouse chimaera IgG1</td>
<td>Lymphoma</td>
<td>Binds to CD20 molecule found on most B-cells. Binds to HER2, a receptor for epidermal cancer factor (EGF) receptor found on tumour cells cancer, lymphoma. Stops cell division and growth.</td>
</tr>
<tr>
<td>Transtuzumab</td>
<td>Herceptin</td>
<td>Humanized monoclonal antibodies</td>
<td>Metastatic breast</td>
<td>Conjugate of MAb to either Radiosotope&lt;br&gt;• Indium-111&lt;br&gt;• Yttrium-90 Binds to CD20 molecule on B cells and lymphoma</td>
</tr>
<tr>
<td>Gemtuzumab ozogamicin</td>
<td>Mylotarg</td>
<td>Recombinant humanized IgG4 kappa antibody</td>
<td>AML</td>
<td>Conjugate of MAb with I$^{131}$&lt;br&gt;Binds to CD-20 expressed on normal B-cells Blocks HER1, epidermal growth factor receptor</td>
</tr>
<tr>
<td>Alemtuzumab</td>
<td>Campath</td>
<td>Recombinant DNA-derived humanized MAb</td>
<td>CLL</td>
<td>Conjugate of MAb with calicheamicin. Calicheamicin is an oligosaccharide which causes double strand break in DNA. First immunotoxin to fight against cancer. MAb binds to CD33 expressed by cancerous cells in AML. Binds to CD52 molecule on WBC</td>
</tr>
<tr>
<td>Ibritumomab tiuxetan</td>
<td>Zevalin</td>
<td>Conjugated MAb</td>
<td>Non-Hodgkin’s Lymphoma</td>
<td>Conjugate of MAb with I$^{131}$&lt;br&gt;Binds to CD-20 expressed on normal B-cells Blocks HER1, epidermal growth factor receptor</td>
</tr>
<tr>
<td>Tositumomab</td>
<td>Bexxar</td>
<td>Conjugated MAb</td>
<td>Non-Hodgkin lymphoma</td>
<td>Conjugate of MAb with I$^{131}$&lt;br&gt;Binds to CD-20 expressed on normal B-cells Blocks HER1, epidermal growth factor receptor</td>
</tr>
<tr>
<td>Cetuximab</td>
<td>Erbitux</td>
<td>Recombinant, human/mouse chimeric MAb</td>
<td>Colorectal Head and Neck cancer</td>
<td>Blocks vascular endothelial growth factor receptor.</td>
</tr>
<tr>
<td>Becavizumab</td>
<td>Avastin</td>
<td>Humanized MAb</td>
<td>Colorectal cancer</td>
<td>Blocks vascular endothelial growth factor receptor.</td>
</tr>
<tr>
<td>Epratuizumab</td>
<td>Lymphoscope</td>
<td>Humanized MAb. Completed Phase II study</td>
<td>Indolent and aggressive NHLs, autoimmune diseases like SLE and Sjogren’s syndrome B-cell non-Hodgkin’s lymphoma. Currently in Phase III trial.</td>
<td>Binds to CD22 on B cell.</td>
</tr>
<tr>
<td>131ILym-1</td>
<td>Oncoly</td>
<td>Mouse MAb</td>
<td>B-cell non-Hodgkin’s lymphoma. Currently in Phase III trial.</td>
<td>Binds to HLA-DR encoded histocompatibility Ag expressed at high levels on lymphoma cells</td>
</tr>
<tr>
<td>Vitaxin</td>
<td></td>
<td>Humanized monoclonal antibody</td>
<td>Solid tumours</td>
<td>Blocks the vascular integrin (alpha-v/beta-3) found on blood vessels of tumour Binds to glycoprotein 17-1 A.</td>
</tr>
<tr>
<td>Edrecolomab</td>
<td>Panorex</td>
<td>Murine MAb</td>
<td>Colorectal cancer</td>
<td>Binds to glycoprotein 17-1 A.</td>
</tr>
</tbody>
</table>

MAb - Monoclonal antibodies
acute therapy. Abciximab was the first antagonist to be evaluated [Table 1]. It inhibits the clumping of platelets by binding to surface receptors that normally are linked by fibrinogen. It is helpful in preventing the reclogging of the coronary arteries.[35-37]

**Infectious disease**

Palivizumab, a humanized MAb directed against Respiratory Syncytial virus is used for the treatment of premature infants and infants with bronchopulmonary dysplasia [Table 1]. A MAB was also found to be useful to cure West Nile fever in mice.[39]

**Ophthalmological disorders**

Daclizumab has shown to be efficacious for noninfectious uveitis.[68] Ranibizumab (Lucentis) is a recombinant humanized IgG1 kappa isotype monoclonal antibody fragment designed for intraocular use, which competitively binds and inhibits VEGF. Therefore, indicated for the treatment of neovascular (wet) age related macular degeneration.[61]

**Multiple sclerosis**

Natalizumab, a humanized MAB was approved by FDA in November, 2004 for relapsing form of multiple sclerosis, but was withdrawn in February 2005 after three patients in the drug’s developed progressive multifocal leukoencephalopathy (PML) during clinical trial.[62,63] On 24th March, 2006 the FDA lifted the hold on clinical trials of natalizumab after confirming that there were no additional PML cases. In March, 2006, FDA consulted its advisory committee on drugs for peripheral and central nervous systems about the possibility of making natalizumab available to appropriate MS patients. The committee recommended a risk-minimization program with mandatory patient registration and periodic follow-up.[67]

**Psoriasis**

Data suggest that MAbs directed against T-cell mediated inflammation are clinically effective in the treatment of psoriasis. MAbs directed against key components of inflammatory process have been studied for safer, selective, and effective immunosuppressant agent as psoriasis is a T-cell mediated autoimmune disease in which proinflammatory Th-1 cytokine play an essential role. Efalizumab is the agent that is near the market launch [Table 1]. It is a humanized MAb that interrupts the interaction between the T-cell surface molecule lymphocyte function associated antigen LFA1 (composed of 2 subunits CD11a and CD18) and intercellular adhesion molecule1, which is found on the surface of antigen-presenting cells. It is used as a once weekly self-administered subcutaneous injection.[65,66] It is administered every other week and carries a risk of tuberculosis reactivation, serious infections, and demyelinating disease.

**Juvenile diabetes**

Anti-CD3 MAB is in phase II trial for type I juvenile DM. This MAB targets an antigen expressed on T lymphocytes that is responsible for destruction of islet cells of pancreas and thus could slow the disease progression.[67]

**Refractory Wegener’s granulomatosis**

Humanized antilymphocyte MAbs may provide an effective treatment in patients with systemic vasculitis which is refractory or intolerant to steroids or cytotoxic agents.[66]

**Systemic lupus erythematosus (SLE)**

IL-6 levels are elevated in human and murine SLE. Blocking the action of IL-6 ameliorates disease activity in murine model of SLE. A humanized MAB is in Phase I of clinical trial. T cells, B cells and monocytes with SLE expressess CD40 L on their surface which have been found to produce autoantibodies in vitro.[69] Therefore, humanized anti-CD40L IDEC-131 was tried for SLE but not found to be successful.[70] Another humanized anti-CD40L Mab, ruplizumab was effective in SLE, but increased the incidence of myocardial infarction. Therefore the trials were discontinued.[71]

Rituximab has also been shown to be effective in patients of SLE with glomeronephritis,[72] by causing B-cell depletion. B-cells in SLE display abnormal signaling, express aberrant cell surface markers and finally produce autoantibody and present auto antigen to T cells at increased rates.[73]

**Active immunotherapy**

Direct evidence of the importance of gangliosides as potential targets for active immunotherapy has been suggested by the observation that human MAbs against these glycolipids induce shrinkage of human cutaneous melanoma metastasis. Thus, the cellular over-expression and shedding of gangliosides into the interstitial space may play a central role in cell growth regulation, immune tolerance and tumor-angiogenesis, thereby representing a new target for anticaner therapy.[74] Anti-idiotype vaccine has been developed from proteins derived from the outer membrane proteins of Neisseria meningitidis. B, 1E10 vaccine, is an anti-idiotype vaccine designed to mimic the N-Glycolyl-GM3 gangliosides. This monoclonal antibody is an Ab2-type-antibody which recognizes the Ab1 antibody called P3, the latter is a MAB that specifically recognize gangliosides as antigens. Results of the phase I clinical trials proved that the three vaccines were safe and able to elicit specific antibody responses. Phase II trials are being undertaken in several neoplastic diseases, with these vaccines.[75] Vaccination of immunologically responding metastatic colorectal carcinoma patients with SCV 106 leads to slowing of disease progression, tumor dissemination and significantly prolongs survival time.[76] Increased CMI responses to HIV-1 envelope glycoprotein measured by lymphocyte proliferation were associated with HIV-1 recombinant envelope glycoprotein vaccines.[77] HIV-1 specific T helper cell responses can be successfully increased by therapeutic immunization in individuals with chronic infection on suppressive antiretroviral therapy.[78] Further studies will be needed to determine whether the augmentation of these responses correlate with long-term clinical benefits.

**Diagnistics**

Generally, MAbs are being used as invaluable reagents in diagnostics. In fact, they have played a major role in deciphering the functions of various bio-molecules in cryptic biosynthetic pathways. These have also become the reagents of choice for identification and characterization of tumor specific antigens and have become a valuable tool in the classification of
cancer.[79] The ability of MAb to accumulate at tumor sites, led to its approval for localization of cancer, for example, igoryvomab for ovarian cancer, teeenamab K-1 for melanoma, votumab, and acrololumab for colorectal cancer and sulemba for detection of infection.[59] MABs will be useful agents for diagnostic imaging of prostate cancer.[79] MABs are used commercially in pregnancy tests where they are directed against proteins in urine, determine glucose level in diabetes, detect antibody residues in milk, and to detect salmonella too.

Conclusion

MABs are new biological agents that have good clinical effects and an extended choice in the treatment spectrum to the patients who were not responding to the existent treatments. The use of MABs for the treatment of autoimmune diseases, infections, and malignancies is an evolving field. New therapeutic approaches are rapidly emerging and further studies may help in designing more specific MABs that would spare the normal tissue, have less adverse effects and improve the patient’s quality of life. Soon, new therapeutic drugs and high-value biomolecules will be designed and produced by biomolecular engineering for the treatment and prevention of not-so-easily cured diseases such as cancers, genetic diseases, age-related diseases, and other metabolic diseases.

References

Kaur et al.: Monoclonal antibodies