# 11 Monoclonal Antibody Production and Applications

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**Abstract**

Antiserum is a heterogeneous mixture of antibodies produced against an antigen. Antigens usually have multiple epitopes and separate antibodies are produced against each by an individual clone of B cell. Monoclonal antibody produced by hybridoma technique is a single homogeneous antibody population which is specific for one epitope and exhibits no cross-reaction. The technique involves fusion of B cell with the myeloma cell and cultivation of the fused clone for indefinite production of the desired antibody. Myeloma cells are the tumor cells and impart immortality to the clone while B cells contribute to the antibody production ability. Selection of the fused cell is carried out in hypoxanthine-aminopterin-thymidine (HAT) containing medium so that unfused myeloma cells are unable to grow by virtue of their HGPRT negative character. Hybridomas once produced can be cryopreserved in liquid nitrogen for indefinite storage. It is possible to antibodies in culture medium or as ascitic fluid in mice. Both procedures allow sizeable harvest of the antibody. Monoclonal antibodies have proven to be valuable tools in immunodiagnostic, immunotherapy, and in biological and biochemical research. Monoclonal antibody-based immunodiagnostic kits are available for detection of pregnancy, for diagnosing numerous pathogenic microorganisms, measuring level of drug in blood or urine, matching histocompatibility antigens, and detecting antigens shed by various tumors. A sizable number of monoclonal antibodies are available as therapeutic agents.

## 11.1 Introduction

Antibodies play an important role in body defence against infectious diseases. Antibodies are glycoprotein and are produced by B lymphocytes with the help of helper T lymphocytes and antigen presenting cells. An antigen, when

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**Table 11.1** Comparison between conventional serum and monoclonal antibodies

| S. N. | Property | Conventional antiserum | Monoclonal antibody |
|---|---|---|---|
| 1. | Determinant | Several | Single |
| 2. | Specificity | Variable with animal and bleed | Standard |
| | | Partial cross-reactions with common determinants | Unexpected cross-reactions may occur |
| | | Seldom too specific | May be too specific for requirements |
| 3. | Affinity | Variable with bleed | May be selected during cloning |
| 4. | Yield of useful antibody | Up to 1 mg/ml | Up to 100 μg/ml in tissue culture, Up to 20 mg/ml in ascitic fluid |
| 5. | Contaminating Immunoglobulin | Up to 100 % | None in culture, 10 % in ascetic fluid |
| 6. | Purity of antigen | Either pure antigen or serum absorption | Some degree of antigen purification desirable but not essential. |
| 7. | Approx. Minimum cost | Usually below £100 | Capital cost £10,000, Running cost £10,000 p.a. |

administered in an appropriate vertebrate host, results in eliciting an immune response. Basically there are two types of immune responses, humoral and cell mediated. In humoral response, B lymphocytes are activated by antigen presenting cells and helper T lymphocytes. On the other hand, cell-mediated immune response requires activation of cytotoxic T lymphocytes. An antigen molecule possesses a large number of antigenic determinants which are called epitopes. Each antibody is specific for a particular epitope. Thus the antisera raised against a given antigen are a mixture of antibodies, each specific for an epitope. Such antisrea are known as polyclonal antisera. As all antibody molecules are similar in their physical and chemical properties, it is impossible to separate epitope specific antibodies from each other. Antibodies produced from a single clone of B lymphocyte are called as monoclonal antibody (MAb).

The theory of monoclonal antibody production is based on the clonal selection hypothesis of F. Macfarlane Burnet (1959). Each mammalian B lymphocyte has the potential to make a monospecific antibody. The constant region of the antibody chain may alter during the differentiation of the lymphocyte clone but the variable region retains this singular specificity. The first report of Hybridoma production was in fact in 1970 (Sinkovics et al. 1970). Kohler and Milstein in 1975 developed a technique that made it possible to raise epitope specific antibodies; this technique was christened as Hybridoma Technology and the antibodies produced by this method are called Monoclonal antibodies. Table 11.1 gives a comparison between conventional serum and monoclonal antibodies.

## 11.2 Production of Monoclonal Antibodies

Antibodies are secreted by plasma cells which are programmed in lymph glands for producing antibodies against a particular epitope. The plasma cells can be cultured and made to secrete antibodies but they have a very short life span and die soon. The technique developed by Kohler and Milstein (1975) immortalizes the plasma cells which can thus produce monoclonal antibodies indefinitely. For this purpose they fused the plasma cells with cancerous cells called myeloma cells. Myeloma cells can be obtained from animals suffering from myeloma tumors or tumors can be induced in experimental animals by injecting mineral oil. They used an inbred strain of mice known as Balb/c mice for this purpose. A schematic illustration for monoclonal antibody production is given in Fig. 11.1. All the steps are also detailed subsequently.

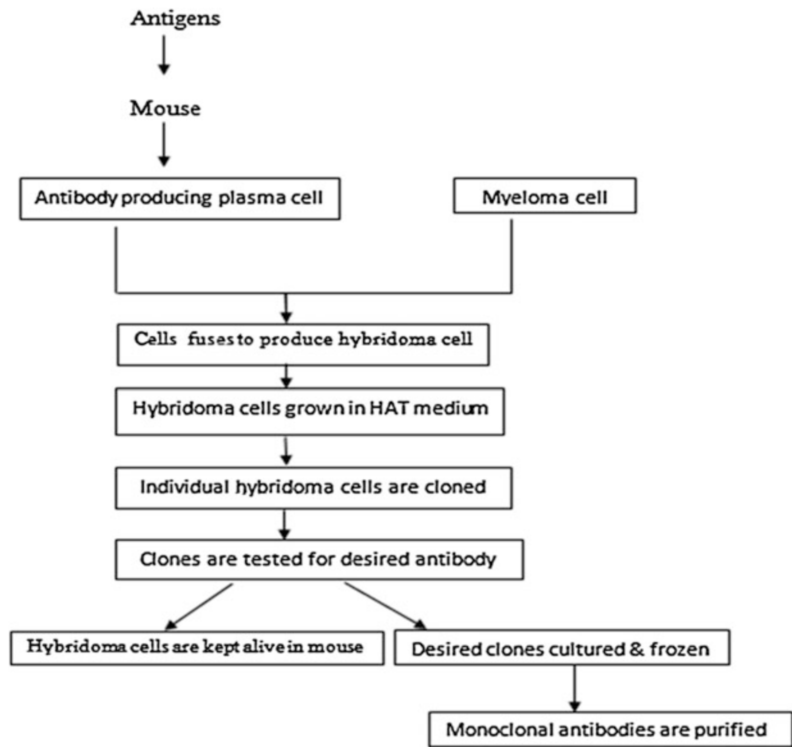



**Fig. 11.1** Schematic representation of monoclonal antibody production

## 11.3 Monoclonal Antibody Assay Requirements

Assay is the most critical factor in production of good hybridomas and is very sensitive. Understanding of the theoretical background is essential for the production of a large number of hybridomas secreting antibody of the required characteristics.

a. **Rate of association**

The initial rate of association of an antibody with an antigen is described by the equation:

$$\text{Rate of formation of product} = K_1[\text{antibody}][\text{antigen}]$$

The number of epitopes on the antigen is much reduced and may be one in case of small protein. Thus the effective concentration of the antigen is quite low. The antibody will still be bivalent or decavalent for IgM but its concentration may be very low. The association rate is usually the parameter which determines the final equilibrium constant and the concentration of antigen in most assays remains constant.

b. **Rate of dissociation**

The rate of dissociation of an antibody-antigen complex is represented by the following equation:

$$\text{Initial rate of dissociation} = K_2([\text{antibody} - \text{antigen}])$$

The dissociation rate is nearly always very much lower than the association rate. With most antibody-antigen reactions, it is the dissociation rate rather than the association rate that determines the affinity as the dissociation rate can vary over 8–9 orders of magnitude. The dissociation rate is not only the most variable among different antibodies under a defined set of conditions but also the most variable in a single antibody with respect to environmental conditions such as pH, temperature, etc.

c. **Equilibrium concentration of reactants**

The equilibrium constant for an antibody-antigen reaction is the ratio of the forward to the backward rates i.e.,

$$Keq = K_1/K_2 \text{ or}$$

$$Keq = (Ab - Ag)/(Ab)(Ag)$$

The concentration of the reactants and the assay conditions may influence the possibility of detection of a suitable hybridoma. With polyclonal sera it can be assumed that an optimal equilibrium condition will be achieved by incubation for one or two hours at room temperature, biological pH, and ionic strength, etc. but for monoclonal antibodies several of the parameters will have to be varied and screening procedures will have to be extended if antibodies to more interesting or relevant epitopes are to be detected.

d. **Effect of multivalence**

All antibodies are at least divalent and IgA and IgM antibodies may have several idiotypes on the same molecule. Multivalent antibodies are helpful to screening procedures since the effective dissociation rate can be reduced if enough antigens are present. Antigens having multiple epitopes, mostly found in bacterial systems with symmetrical cell wall structures, can affect the reaction in many ways.

e. **Specificity and affinity**

Monoclonal antibodies against polymorphic antigens could exhibit extreme specificity in one assay and considerable epitope overlap in a second higher affinity assay. However, it is assumed that at least in the initial stages, an assay detecting the maximum numbers of positive clones is required. Suitable conditions for obtaining specific responses can then be achieved later.

f. **Number of assays**

In a typical fusion using 4 × 96 well plates some 400 samples must be assayed in a short time. Subcloning usually involves a similar number although a valuable clone may be subcloned with more. Further, a comprehensive screen of hybridomas should ideally involve the use of several plates under different sets of conditions. If the final application of the antibody is not considered then positive samples detected by the first screening should be assayed by a second directed to the final application, since the number of positive clones is much lower than the total number of clones.

g. **Time of assay**

The time of assay, in terms of clonal growth, should be soon after clones are microscopically visible and again a few days after when the clones are visible to the eye. Screening should continue for several weeks. The actual assay however, is influenced by the antibody concentration.

h. **pH of assay**

It is expected that the most antibodies have their optimal reaction with antigen at physiological pH. It is quite possible to fail to detect a good hybridoma by screening at a single pH. The pH of tissue culture fluid in which cells are growing can vary almost a whole pH unit. If a defined pH is required for the final application then the assay should be buffered accordingly during the incubation of antibody with antigen, particularly for *in vivo* uses. Where pH adjustments are necessary the nature of the buffer should be considered as the buffer components themselves may affect the assay.

i. **Temperature of assay**

Dissociation constant is the variable which is most sensitive to temperature. The best assay conditions for hybridoma favor incubation at 4 °C rather than at room temperature or higher but ideally both should be tried unless the final use precludes certain temperature.

j. **Ionic strength of assay**

There is no detailed information on the ionic strength variations in hybridoma assays. Nonspecific binding is more likely to occur at low ionic strengths. If different pH buffers are used in the assay then all buffers used should have the same ionic strength.

## 11.4 Cell Culture Requirements for Hybridomas

1. **Media**

Two main types of media used for hybridoma production are Dulbecco's Modification of Eagles Medium (DMEM) and Rosewell Park Memorial Institute (RPMI) medium. Media are prepared in double distilled water, sterilized by filtration through 0.2 μm membranes, and usually stored in 500–1,000 ml aliquots for up to 6 weeks. Both the above media are bicarbonate buffered with phenol red indicator. The correct color for medium is bright orange indicating a pH of 7.2.

DMEM or RPMI 1640 supplemented with high glucose (4.58 g/l) are popularly used for culturing myeloma cells. Glutamine (2 mM final concentration), antibiotics such as penicillin (100 U/ml) or streptomycin (10 μg/ml) and 10 % fetal calf serum is also added. Myeloma cells should be grown in the presence of 8-azaguanine prior to fusion to ensure HGPRT negative character. For fusion, cells should be in logarithmic phase ($3–8 \times 10^6$/ml).

2. **Sera**

Fetal calf serum (FCS) is used in nearly all hybridoma work because of the low level of contaminating immunoglobulin. It is kept frozen at −20 °C. Horse and rabbit serum is also sometimes used.

3. **Antibiotics for prevention of contamination**

The main antibiotics used in hybridoma production are penicillin and streptomycin. Penicillin inhibits the growth of most gram-positive bacteria, whereas streptomycin inhibits the growth of most gram-negative bacteria. The antibiotic preparations are usually made up in 100 × stock solutions containing $10^7$ units of sodium benzyl penicillin and 10 g of streptomycin sulfate per liter. This is filter sterilized through 0.2 μm membranes and stored in 20 ml aliquots at −20 °C. Some labs use fungizone (Amphotericin B) at 2.5 μg/ml medium.

Mycoplasma contamination may be prevented by kanamycin (100 μg/ml), tylocine (50 μg/ml) or lincomycin, and vanomycin to some extent. Exposure of cells at elevated temperatures has also been reported to curb mycoplasma contamination.

Viral contamination with Epstein Barr (EB) virus in a latent form is usually present if human lymphocytes are used. The virus transforms culture especially in the absence of cytotoxic T lymphocytes. Lymphocyte donors are usually tested for antibody to the viral capsid antigen by use of cell line P3HR1which secretes non-transforming EB.

Yeast contamination is rare. The use of fungizone or nystatin (50 μg/ml) is usually helpful in such situations.

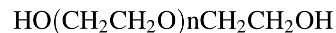
4. **Feeder cells**

Feeder cells are absolutely essential for cloning of hybridomas. They feed or nourish the emerging hybridomas but may have a limited lifespan. If phagocytic cells such as macrophages and monocytes are used they can also be helpful in cleaning debris of dead cells found after aminopterin treatment. Spleen cells are convenient to use as feeders.

5. **Important chemicals**

**Dimethyl sulphoxide (DMSO)**: DMSO is required for freezing and thawing of cells and some use it in fusion procedures. It is sterilized by autoclaving or filtration.

**Polyethylene glycol (PEG)**: All fusions designed to produce hybridomas are performed with chemical fusogens and PEG is the main chemical used for this.



$$HO(CH_2CH_2O)nCH_2CH_2OH$$

Structure of polyethylene glycol (PEG)

PEG has a molecular weight range from 200 to 20,000 Daltons. It is toxic to cells; low molecular weight PEG is more toxic than high molecular weight PEG. Most successful fusions are performed with PEG of molecular weight 600–6000 Daltons. Most protocols use PEG at 37 °C. It has been also shown that room temperature is superior to 37 °C and that the optimum pH is 7.5.

The exact mechanism of fusion is not fully understood, but it is thought that hydrophilic PEG occupies the 'physical free space' leading

to agglutination of the cells. This occurs at concentrations of PEG in the range of 40–50 % with some variations. Lower PEG concentrations such as 35 % can, however, be used with a longer exposure time. Mostly fusions are done using PEG in 15 % DMSO.

6. **Myeloma cells**

Myeloma cells are cancerous cells and impart immortality to hybridoma clones. Spleen cells are antibody-producing cells; hence provide the ability to generate the antibody. Most of the murine myeloma cell lines are available from American Type Culture Collection (ATCC), Maryland 20852 USA. A few commonly used murine myelomas are Sp 2/0 Ag.14, NS1/1, Ag 4.1 NSO, and P3X63 Ag8.

Rational for using HAT media for selecting hybridoma clones is that, myeloma cells are deficient in HGPRT and thymidine kinase (TK), hence lack the salvage pathway for nucleotide synthesis. Aminopterin is added to inhibit the de novo pathway of nucleotide synthesis. Thus myeloma cells do not grow in HAT medium. The spleen cells are primary cells and cease to grow after a few divisions. Only hybridoma cells which have acquired HGPRT and TK genes from spleen cells multiply and form clones. These cells secrete very high level of antibodies in the media.

7. **Subcloning of hybridoma clones**

To ensure monoclonality of the hybridoma clones, the cells should be re-cloned under limiting dilution conditions for a few cycles. Culture is diluted so that 1–2 cells per well are distributed. Limited dilution cloning technique requires presence of feeder cells in the cloning wells. This process eliminates non-secretors and contaminating hybridomas ensuring monoclonality of the antibody.

8. **Enzyme Linked Immunosorbent Assay for antibody detection**

The wells of the ELISA plate are coated with the antigen and the supernatant from the hybridoma wells is added. The unbound antibody is washed away. A second antibody conjugated to an enzyme which is specific to the Fc (fragment crystallizable) region of the first antibody is added and allowed to bind to the first antibody. After washing, the substrate for the enzyme is added and the reaction is allowed to proceed. If the first antibody binds to the antigen the second antibody will also bind (to the first antibody) and the enzyme will react with the substrate and colored product is formed which can be easily visualized.

## 11.5 Steps in Monoclonal Antibody Production

1. Balb/c mice are immunized with the antigen *via* suitable route. For most protein antigens, 2–3 immunizations are sufficient to evoke a strong immune response. Usually a group of 4–5 mice are immunized and the one exhibiting highest titer is used as the source of plasma cells. This mouse is immunized by IV (intravenous) route and after 3 days the mouse is sacrificed.
2. Spleen is removed from the mouse and single cell suspension is prepared.
3. Exponentially growing myeloma cells are mixed with splenocytes in the proportion of 1:10.
4. Fusion between these cells is accomplished in the presence of a fusion agent, usually poly ethylene glycol (MW 4,000). Both cells are centrifuged and the supernatant media is removed. The cell bottom is loosened by gentle tapping and 50 % PEG is slowly added drop-wise. The cell suspension is held at 37 °C for 2–3 min. After that, PEG is diluted with the serum free media and cells are centrifuged to remove the PEG.
5. The cell pellet is suspended in about 100 ml hypoxanthine, guanine, and aminopterin (HAT) medium. The HAT medium consists of RPMI 1,640 medium supplemented with HAT.
6. The suspension is distributed in ten 96-well microtiter plates.
7. The plates are incubated at 37 °C in a carbon dioxide incubator overnight (the incubator is operated at 37 °C, 95:5 air and $CO_2$ environment and 95 % humidity)

8. Next day, 0.1 ml HAT media is added to each well.
9. Plates are examined regularly for appearance of growth.
10. Between 10 and 14 days of incubation, some wells exhibit growth and medium turns slightly yellowish in color in the wells.
11. The supernatant from wells is screened for the presence of antibodies by Enzyme-Linked Immunosorbent Assay (ELISA) technique.
12. Hybridoma cells from positive wells are expanded by growing in 24-well plates followed by growth in 25 $cm^3$ culture flask.
13. Desirable hybridoma clones are preserved by cryopreservation in liquid nitrogen.

## 11.6 Large-Scale Production of Antibodies

Antibodies can be produced by two different techniques. In the first method the hybridoma cells are grown in serum free media. Growing cells secrete antibodies which can be purified by affinity chromatography technique. The antibody yield usually varies between 10 and 50 µg/ml. In exceptional cases one may get a yield of up to 100 µg/ml as well. The second method which involves production of ascitic fluid can produce up to 20 mg/ml antibody yield. For ascitic fluid production, the hybridoma cells are injected in the peritoneal cavity of pristine primed-immunocompromised Balb/c mice. The ascitic fluid develops in the peritoneal cavity and can be easily aspirated with the help of 18 gauge hypodermic needle. From ascitic fluid also antibodies can be purified by affinity chromatography using Protein A coupled Sepharose beads. Lately, due to objection from organizations for prevention of cruelty to animals, ascites production is becoming problematic. Antibodies can also be directly obtained from the fluid by ammonium sulfate precipitation.

The rapid progress being made in the commercialization of monoclonal antibodies has led to large-scale production of MAb. Commercial interests consider production scales of 0.1–10 g as small, 10–100 g as medium and over 100 g as large. Commercial-scale production is generally performed to produce MAb for three purposes: diagnosis, therapy and research, and development of new therapeutic agents. Monoclonal antibodies are being manufactured for clinical trials in large-scale suspension culture in fermenters. A completely automated pilot plant used for fermentation has been employed with direct digital control (DDC) technology for monitoring and regulating growth of human cells. A human hybridoma cell line (3D6) producing anti-human immunodeficiency virus (HIV)-1 antibodies was used as a model for large-scale production (300-liter airlift fermentor) in continuous culture. The production of anti-$\alpha$-fetoprotein monoclonal antibodies for diagnostic use was carried out in a stirred tank fermenter equipped with a double membrane stirrer for bubble free aeration and continuous medium perfusion.

## 11.7 Applications of Monoclonal Antibodies

Monoclonal antibodies are widely used as diagnostic, research, and therapeutic agents for treatment of numerous human ailments. The applications can be divided in mainly three major groups:

1. Immunodiagnostic reagent
2. Research tools in molecular localization of antigens in intact cells and epitope analysis.
3. Immunotherapeutic and Immunoprophylactic agents.

### 11.7.1 Immunodiagnostic Reagent

Monoclonal antibodies are useful tools for detection of antigens in various body fluids and tissues. MAb-based commercial kits are available for detection of viral, bacterial, fungal, and parasitic pathogens. MAbs attached to latex beads or erythrocytes can be added to a body fluid. Presence of antigen causes agglutination. This is easily visible. It should be kept in mind

that some antigens may not cause agglutination. Detection of antigens in intact tissue can be made with use of either a fluorochrome-labeled or enzyme-labeled or isotope-labeled antibody. Pathogens can be visualized by fixing a tissue slice on microscope slide or slide culture of the pathogen. Detection is made either with the help of a fluorescent microscope, use of an enzyme substrate which provide an insoluble colored product in case of fluorochrome-labeled or enzyme-labeled MAbs, respectively. A radioisotope can be detected by autoradiography. Many animal viruses (Koprowski and Wiktor 1980; Sonza et al. 1983), bacteria (Gustafson et al. 1982), and parasites (Wrisht et al. 1983; Scott 1983) have been diagnosed using MAbs.

A very large number of monoclonal antibodies have been produced to a wide range of viruses such as influenza, hepatitis, polio, Epstein Barr virus, and rabies. Their high specificity has led to accurate identification between similar stains of virus such as Herpes simplex Types I and II. They may also be used for early diagnosis of the IgM production in affected patients.

Antibodies have been designed for bacterial diseases as well though they are less common. They are helpful in analysis of bacterial spores which tend to show extensive cross-reactivity in conventional immunological analysis. Monoclonal antibodies to bacterial toxins have also been generated.

The potential of monoclonal antibodies in the study of parasitic diseases has already been widely exploited for diseases such as malaria, schistosomiasis, and leishmania. The advantage here is that the disease may be studied not only for diagnostic purpose but also in greater depth since many different surface antigens may be expressed at varying stages of the life cycle of the parasite.

Competitive or noncompetitive ELISA procedures have been developed for detecting antigens in body fluids. The MAb is usually attached to a microtiter well and body fluid (containing the antigen) is allowed to bind to the antibody. Detection can be made by using a different MAb coupled to an enzyme or radioisotope. Two MAbs used should be either for different epitopes or against a repetitive epitope. In competitive ELISA, the test sample is mixed with the known amount of labeled antigen and added to the antibody-coated microtiter well. Less labeled antigen binds to MAb with increasing concentrations of the test antigen.

Some precautions are required for diagnostic utility of the MAbs. Since there are multiple strains of the particular organism which can cause infection, a MAb may detect a single strain only. These strains may differ in their antigenic epitopes. Therefore, a monoclonal antibody against a common epitope must be selected for immunodiagnosis. MAbs are extremely useful for unambiguous diagnosis of diseases which produce closely related symptoms. As an example, MAb against Marek's disease does not bind to lymphoid leucosis virus though both cause similar symptoms. Similarly, MAbs can distinguish canine parvovirus infection from panleukopenia virus.

MAb are being used for cancer diagnosis too. MAbs to certain tumor markers are being used for detection in tissue fluid, tissue explants, or in intact organism through imaging. Precise information about the type of cancer is very helpful in its therapy. Tumor markers against prostate cancer, colorectal cancer, and ovarian tumors have been utilized for monitoring the tumor regression or progression as the marker levels fluctuate with its status. MAbs are also being extensively used for diagnosis of hematopoietic malignancies such as primary or acquired immunodeficiency syndrome (AIDS).

Immunoscintigraphy procedures have been developed for scanning human body for the location of cancerous tissue. The MAbs are labeled with radioisotopes such as $^{131}$I (Iodine) or $^{99}$Tc (Technetium) and injected intravenously into the patient. It binds to cancer cells in different locations which can be detected by imaging.

### 11.7.2 Basic Research

The potential of monoclonal antibody in basic research is substantial. In principle they can resolve a single protein from a complex mixture or a single epitope responsible for a specific function of a complex macromolecule. They have been widely used in basic enzymology, in nucleic acid structural studies, and in the analysis of hormone receptors. Another field in which monoclonal antibodies may prove of particular value is in the study of chromosomal proteins which are responsible for determining cell phenotype. For this they are ideal tools for the dissection of the complex mixture of proteins.

### 11.7.3 Therapeutic Applications

One of the earliest applications of MAb for humans came in the form of murine MAb OKT3 (orthoclone) which was used for preventing renal-transplant rejection. Human allografts are often rejected by the host and to sustain the allograft the patients are subjected to administration of highly toxic immunosuppressive drugs or high doses of glucocorticoids. OKT3 is an antibody which prevents the proliferation of cytotoxic T cells, hence prevents graft rejection.

Murine MAbs are not preferred for human therapeutic application, as this initiate immune response in human patients. In order to prevent or minimize deleterious immune responses murine MAbs have been humanized. Using genetic engineering techniques human Fc region is grafted onto the variable region of murine MAb. This reduces the immune response as the Fc region is strongly immunogenic. With further improvement in the technology, now even the framework regions of the MAbs have been grafted along with the Fc region from human immunoglobulins. Humanized murine MAbs which have only complementary determining region (CDR) from the murine MAb and rest of the molecule is of human immunoglobulin, are finding application mainly in cancer therapy.

HIV infects Th (T helper cell) lymphocytes *via* gp 120 envelope protein of the virus. Virus binds to Th lymphocytes on $CD4^+$ (Cluster of differentiation) receptors *via* gp 120 glycoprotein. Using genetic engineering techniques, Fc region of the antibody has been conjugated to a CD4 glycoprotein molecule. When this conjugate is administered to HIV patients, the HIV infected cells of the patient exhibiting gp 120 bind to $CD4^+$ Fc conjugate. This event triggers cell mediated immune response and the infected cell is killed. This strategy may prove as an effective therapy for HIV patients.

MAbs can also be used for targeting drugs to cancer cells or virally infected cells. The drug is conjugated onto the MAb which binds to only affected cell and it is selectively delivered to the cancer cell or virally infected cell. Not only drugs but also deadly toxins such as diphtheria toxin or ricin could be coupled to MAb molecules and can be used to selectively kill targeted cells. The normal cells are not killed by the drug or the toxin and prevent the side effects of chemotherapy.

Anti-idiotype MAbs can replace the antigen in detection assays. Anti-idiotype antibody (Ab2) has binding ability to antigen binding amino acid sequence (epitope) of the hypervariable region of the antibody (Ab1). The resulting Ab2 is capable of competing with the original antigen. Thus if the antigen availability is limiting or toxic, the MAb can easily be substituted for the antigen.

A substantial number of MAbs have been approved by U.S. Food and Drug Administration for use in humans. A few of these are listed here.

RiuxiMAb and Zevalin conjugated with isotopes of indium or yttrium have been approved for treatment of B cell lymphomas. Both these antibodies are targeted to CD20, a marker present on B cells. Administration of $^{131}I$ labeled TositumoMAb in B cell lymphoma patients have kept them free from disease for a substantial number of years. These antibodies also bind to CD40 receptors on B cells. CetruxiMAb and Herceptin have been used for successful treatment of breast cancer. CetruxiMAb is specific for HER1 (human epidermal growth factor)

receptor present on breast cancer cells. Herceptin blocks the growth factor receptor HER2 which is also found in some breast cancers. MAb against CD30 conjugated with Vedotin blocks the proliferation of lymphomas. The preparation is called Adcetris. A number of MAb preparations are also available for treatment of leukemia. These are Lymphocide and AlemtuzuMAb. Lymphocide is against CD22 receptor and is used for treatment of B cell leukemias. AlemtuzuMAb is against CD52 receptor which is found on B as well as on T cells. Thus this antibody is useful for treatment of both B and T cell lymphomas. An antibody called Lym-1 has been shown to bind to HLA-DR which is expressed heavily on lymphoma cells. An antibody LipimuMAb has shown promise in suppressing all types of tumors acting as tumor suppressor agent.

Vitaxin is an antibody against integrin found on the blood vessels of tumor cells and has shown promise to be an inhibitor of angiogenesis selectively in tumor cells. This results in regression of solid tumors. BevaxizuMAb prevents clumping of platelets and prevents re-clogging of arteries after angioplasty.

Autoimmune disorders are generally difficult to treat by using conventional drugs. Two MAbs namely InflixiMAb and AdalimuMAb have been used for treatment of rheumatoid arthritis and Chrohan's disease. These antibodies bind to TNF-alpha (Tumor necrosis factor) and reduce the proliferation of Th1 cells which are active in enhancing cell-mediated immune response. OmalizuMAb binds to IgE receptors and is useful for treatment of allergic asthma. DaclizuMAb binds to IL-2 receptors on the surface of activated T cells preventing acute graft rejection of the transplanted kidney. This antibody has also shown promise against T cell lymphomas.

### 11.7.4 Monoclonal Antibodies and Infectious Diseases

A humanized MAb against respiratory syncytial virus has been approved for treatment of premature infants or infants suffering from bronchopulmonary dysplasia. The MAb called PalivizuMAb has shown great promise in such cases. Similarly, many other MAbs are under trial for combating viral infections. MAbs are also very useful tools for dissecting viral antigenic epitopes which is essential for developing efficacious vaccines for viral infections.

MAbs have also found applications in elucidating host–parasite relationships. In order to understand the relationship between the host and parasites, e.g., protozoa and helminthes in different stages of their life cycle, panels of antibodies have been produced against stage specific antigens. Antigens from some of the parasites have been fractionated using MAbs and the antigens providing protective immunity have been identified. These antigens could be used for vaccination against the parasite.

In case of bacterial pathogens, MAbs have been extremely useful tools for unambiguous diagnosis. Bacterial antigenic epitopes are being characterized with the objective of finding antigens with better immunogenic potential. Serotype specific MAbs have been developed for detection of various leptospira, causing infections in humans and animals.

### 11.7.5 Miscellaneous Uses of MAbs

MAbs can be effectively used for purification of antigens from a complex mixture. MAbs attached to solid particles can be used to prepare a series of columns. The complex mixture is then serially passed through different columns and from each column a single antigen can be obtained. Thus a panel of MAbs can be used for fractionation of antigens from a cell.

MAbs are useful reagent for mapping epitopes on complex antigen. They are also being used for isolating different cells of immune system. $CD4^+$ and $CD8^+$ MAbs are used for separating T helper and T cytotoxic cells. Further with appropriate MAbs TH1 and TH2 cells can also be fractionated.

Panel of MAbs used for generating protein microarrays are highly useful tools for protein characterization. MAbs are used for blood group

analysis. Using chromosome Y specific MAbs, embryos can be sexed. This is being widely used in dairy industry for producing only female calves.

For passive immunization humanized MAbs are used as prophylactic agents. These can also be used for neutralizing pathogens in acute cases.

Monoclonal antibodies are also used in the detection of adulteration in meat. Poultry (chicken and turkey) tissue is a major source of protein and less expensive than red meat, which is consumed and imported throughout the world. Increasing use of mechanically separated poultry meat have a potential for the adulteration of red meat by poultry products. Martin (1989) produced and characterized MAbs against species-specific sarcoplasmic protein of chicken. Three MAbs are capable of distinguishing between muscle extracts of the most frequently, marketed avian (chicken and turkey) and mammalian (beef, pork, horse and lamb) species of meat animals. One of these antibodies has the added advantage of distinguishing between chicken and turkey extracts by ELISA. MAb technology will improve diagnosis and serotyping/pathotyping procedures in poultry disease management.

## 11.8 Conclusion

Monoclonal antibodies are antibodies having a single specificity and are continuously secreted by "immortalized" hybridoma cell, which is a biologically constructed hybrid between an antibody-producing, mortal, lymphoid cell, and a malignant, or "immortal", myeloma cell. MAb are more pure than the polyclonal antibodies and because of their specificity, are used in biomedical research, in diagnosis of diseases, and in treatment of infections and cancer. These have also been used for animal disease diagnosis of poultry diseases, infectious bronchitis, and avian influenza. Commercialization of MAb has led to their large-scale production in fermentors.

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