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Monoclonal Antibodies: Their Production and Applications—A Review

S K GUPTA, T BAGCHI, U DESHMUKH, R GUPTA*, H BAGAVANT and M SHAMIM

Gamete Antigen Lab, National Institute of Immunology, JNU Campus, Shahid Jeet Singh Marg, New Delhi 110 067

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Introduction

Earlier observations on the Chinese custom (fifteenth century) of snuffing dried powder of smallpox 'pocks' to "immunize" against smallpox suggested that the body possessed a defensive system. The experiment by Edward Jenner in 1798 of imparting protection against smallpox by immunization with avirulent cowpox vaccine confirmed the existance of such a defence mechanism. The continued research in this field further pointed to non-specific (e.g., phagocytosis) and specific mechanisms (e.g., immune response). The immune response is further classified into cellular and humoral responses. The humoral immune response is manifested in the form of antibody production by B cells involving a complex network of interaction with other cells of immune network. The scope of this review does not permit us to go into the details of the mechanisms involved in the production of antibodies in response to a given antigen.

An activated B lymphocyte under appropriate conditions of antigen presenting cell and T lymphocyte help will produce homogeneous population of antibody molecules of a defined specificity. However, the duration of the secretion of antibody by plasma cells is short, owing to the short-life span of plasma cell. Employing hybridoma technology, it is possible to immortalize the B lymphocyte and thereby the production of an antibody.

Production of Monoclonal Antibodies

Hybrid cell clones are generated by fusion of two different cells, one capable of multiplying indefinitely in tissue culture medium and the other capable of making a defined antibody. One of the partner cell for fusion, the myeloma cell, is from a cell line which has a drug marker and is HGPRT deficient. The myeloma cells used for fusion are from cell lines selected for resistance by growing in the presence of toxic purine analogs, e.g. 8-azaguanine. Table 1 list some of the myeloma/plasmacytoma cell lines used in the development of hybrid cells. Earlier fusion with P3-X63-Ag8 myeloma, secretor of kappa light chain and γ_1 heavy chain had the disadvantage that the hybrid cells secreted immunoglobulins made up of heavy and/or light chains contributed from both parent cells. Such antibodies will have lower avidity as well as lower titre. Thus for generating hybrid cell clones myeloma cells such as SP2/O-Ag 1.4 or X63-Ag8653 unable to either synthesize or secrete immunoglobulin light or heavy chains are preferred. These do not grow in a medium containing aminopterin as it inhibits dihydrofolate reductase and thereby blocks the *denovo* pathway of DNA synthesis. Myeloma cells can not use salvage pathway as they lack the key

*Fellow of the Talwar Research Foundation.
Correspondence to: Dr S K Gupta, Principal Scientific Officer, Gamete Antigen Lab, National Institute of Immunology, JNU Campus, Shahid Jeet Singh Marg, New Delhi 110 067.

enzyme HGPRT. The other partner e.g. antibody synthesizing B lymphocyte or plasma cell is HGPRT positive. Its own life is limited and it does not *per se* multiply. However, the hybrid cell will replicate as it contains the genes of the myeloma cell (to multiply *in vitro*) and HGPRT gene from antibody forming cell (AFC). They can survive in the medium containing hypoxanthine, aminopterin and thymidine (HAT) by virtue of genetic compensation of HGPRT gene by the other partner. Neither of them alone will multiply in this medium.

In order to obtain stable hybrid cell clones BALB/c mice are preferred for immunization because all the mouse myeloma cell lines are derived from the BALB/c mouse. Moreover, hybrid cell clones obtained with BALB/c lymphocytes will grow as tumour in this strain when injected intraperitoneally, thus generating high titred ascites fluid. Unlike the conventional methods of antibody production, the antigen of interest need not be pure for immunization purposes as selection of specific hybrid cell clones at the later stage will avoid the necessity for purification of antigen. Fusion of immune cells with mouse myeloma is brought about by polyethlyene glycol (PEG). The details of methodology to generate monoclonal antibodies is describe elsewhere (Gupta 1991).

Table 1 *Different Myeloma/Plasmacytoma cell lines used in generation of hybrid cells*

Cell Line	Chromosome No	Derived From	Ig chain secreted
P3-X63-Ag8	65	MOPC-21, BALB/c	K, IgG1
P3/NS1/1-Ag 4-1	65	X63-Ag8	K*
X63-Ag8 6.5.3	58	X63-Ag8	None
SP2/0-Ag 14	72	X63-Ag8 × BALB/c hybridoma	None
210.RCY 3.Ag 1.2.3	39	Lou rat	K
SKO 007	?	Human	Myeloma Protein
GM 1500	?	Human	IgG2, K
KR-4	46(40-50)	Human	IgG, K

*Only synthesizes; does not secrete

The problem due to over growth of non-specific hybrids is usually avoided by cloning which involves growing a single hybrid cell to a group of cells i.e. clone and thus the antibody secreted by them is termed as monoclonal antibody (MCA). Positive stable hybrids can be stored by freezing them in liquid nitrogen and can be revived, whenever required thus providing an unlimited and continued source of antibody.

Hybrid cells can be propagated in tissue culture or as ascites in the peritoneal cavity of the mouse. Ascites thus tapped has titres of the order of 1 to 10 million (2-20 mg antibody per ml of ascites) which are seldom possible by conventional immunization approaches. With the advances in technology of growing hybrid cells at mass scale (microbeads, hollow fiber system, entraping hybrid cells in calcium alginate beads and growing the same in air lift fermentors of capacity ranging from 5 to 1000 litres), it is feasible to obtain economically MCA at the industrial level. Though the technology was introduced in 1975 by Kohler and Milstein, hybridoma products have stood up to the rigorous analysis of its scientific merits and commerce.

To avoid risk of generating anti-mouse antibodies during *in vivo* therapy in humans, human MCAs are preferred. However, in case of human MCA the possibility of development of anti-idiotypic antibodies still exist. There is a slow progress in the field of the development of human hybridomas mainly because of the lack of good non-secreting human plasmacytoma or lymphoblastoid cell lines. The first human MCA against 2,4-dinitrophenyl hapten was produced by fusing B lymphocytes with HAT-sensitive plasma cell line SKO-007 (Olsson & Kaplan 1980). Due to lack of suitable human plasmacytoma cell lines attempts have also been made to fuse human B lymphocyte with mouse myelomas. However, such interspecies hybrids preferentially segregate human chromosomes. Human B lymphocytes

isolated from peripheral blood, bone-marrow, spleen, tonsil or lymph node have been used for fusion. The number of B cells secreting specific antibody are very few in peripheral blood. Their number can be increased by stimulating these cells *in vitro* with pockweed mitogen (PWM) or antigen or a combination of both. One of the alternate possibility is to use Epstein Barr Virus (EBV) isolated from B95-8 marmoset cell line to transform human B lymphocytes thus making them capable of growing as established cell lines and secreting human antibody (Steintz et al. 1977). However, such EBV-transformed cell lines secrete low amount of antibody and are unstable. To avoid these problems attempts have been made to fuse EBV transformed human B cell lines secreting specific antibody with either mouse myeloma (Kozbor et al. 1982a) or human myeloma (Kozbor et al. 1982b) which have led to development of stable hybrid cell lines secreting human antibody.

Relative Merits/Demerits of Monoclonal vs Polyclonal Antibodies

Sera obtained from conventional immunized animals, besides having antibody repertoire against the test antigen, also contain bulk of non-specific immunoglobulins. Affinity chromatography and other techniques theoretically can separate the relevant from the non-relevant antibodies. The elution of high affinity antibodies from such columns is, however, not easy. Moreover, large amounts of the immune serum will be required for preparation of globulins. These difficulties can largely be resolved by hybridoma technology.

The advantages of MCA obtained by hybridoma technology as compared to conventional antisera are summarized in table 2. However, there are some disadvantages of MCAs, such as that the biological function may be limited to the heavy chain class. Most of the MCAs failed to precipitate the antigen in gels while performing either simple immunodiffusion or cross-immuno-electrophoresis. By virture of the fact that MCAs react with single determinant and thus having a single affinity and specificity these properties can be influenced more by change in pH, temperature etc. Using hybridoma technology several murine and human monoclonal antibodies have been generated against a variety of antigens. Table 3 summarizes a partial list against which MCA are available in different laboratories. It is difficult to cover all the applied aspects of hybridoma technology, however, some of its applications are described in brief.

Immunodiagnostic Applications

The ability of an antibody to recognize a specific molecular determinant in a mixture, without the necessity of prior extraction, forms the basis of

Table 2 *Properties of monoclonal and polyclonal antibodies*

Property	Polyclonal Antibody	Monoclonal Antibody
Useful antibody content	Low	High
Composition	Heterogeneous	Homogeneous
Specificity	Multiple; variable from animal to animal	Defined and consistent
Cross-reaction with other antigens	Partial with antigens bearing common antigenic determinants	Usually absent but complete, if antibody binds to a common determinant
Class and Subclass of antibody	Typical mixture of many classes and subclasses	Only one, may be any
Supply	Limited	Abundant

immuno-diagnostic tests. One can detect and quantify a given antigen, be it a virus, bacteria, parasite, a marker of cancer, by the use of antibodies uniquely reacting with it. One of such applications of MCA is in an enzyme linked immunosorbant assays (ELISAs).

Immunodiagnosis of Pregnancy

Oneset of pregnancy is characterized by the synthesis and secretion of human chorionic gonadotropin (hCG), a glycoprotein hormone as early as 170hr after fertilization (Fishel et al. 1984). As an interesting prototype, our laboratory developed a sandwich ELISA for hCG employing two different MCA. The product of one of the hybrid cell clone, P_3W_{80}, is highly specific for hCG (Gupta et al. 1982) whereas the other P_22_3, recognizes α-hCG, hCG and pituitary gonadotrophins i.e. hLH & hFSH (Gupta et al. 1985a). Polystyrene microtitration plate was coated with monoclonal anti-β hCG antibody (P_3W_{80}). Monoclonal anti-α-hCG antibody (P_22_3) was labelled with the enzyme, horseradish peroxidase. The detection limit of the assay was 1ng (10mIU) hCG/ml (Gupta et al. 1985b).

A variety of solid supports such as microtitration plates, polystyrene balls, magnetic polyacrylamide agarose beads (Magnogel) and nitrocellulose discs pasted at one end of plastic strip (dip-stick) have been investigated for their relative merits (Gupta et al. 1985c, Gupta 1986, Gupta & Talwar 1986). Dip-stick and polystyrene balls serves as good solid supports when few samples have to be analysed at the field level. However, microtitration plate is the solid support of choice when many samples have to be analysed simultaneously. An interesting prototype pregnancy detection kit using dip-sticks as solid support have been developed at National Institute of Immunology and has been taken for

Table 3 *Available (Mouse/Human) monoclonal antibodies of clinical interest*

Normal	Blood group antigens, Lymphocyte subpopulation, major histocompatibility complex, Sperm, Oocyte, Neurons & Trophoblast antigens.
Pathogens	
(a) Bacteria	*Streptococcus, E. coli, Pneumococcus, S. typhi, S. paratyphi*-A and -B, Salmonela, *N. meningitdis, N. gonorrhoeal, M. leprae, M. tuberculosis* etc.
(b) Virus	Herpes Simplex Virus I, Hepatitis-B, Influenza virus-A, Foot-and-Mouth diseases virus, polio virus, rota virus, measles, rinderpest, rabies, murine leukemia, bovine leukemia, human immnune deficiency virus, and epstein-barr virus.
(c) Parasites	Plasmodium falciparum.
(d) Mycoplasma	Toxoplasma gondii.
Hormones	Human chorionic gonadotropin, leutinizing hormone, gonadotropin release hormone, progesterone, estradiol α 17 β, vasopressin, growth hormone, gastrin, insulin, adrenocarticotrophic etc.
Tumour Associated Antigens	Leukemia, ovarian carcinoma, colon carcinoma, germ cell tumours and terato-carcinoma, glioblastoma, hodgkin disease, lung cancer, mammary cancer, melanoma, osteoesarcoma, prostatic cancer, neuroblastoma, carcinoembryonic antigen, α-fetoprotein, acid phosphatase, etc.
Others	β2-microglobulin , transferrin receptors, tetanus toxoid, H-Y antigen acetylcholine receptor, complement components, interferons, interleukins, immunoglobulins, estrogen and progesterone receptors, etc.

commercial production by a leading Indian Pharmaceutical Company. The kit can diagnose pregnancy correctly within 3-4 days of the missed period. The assay is easy to perform and results can be obtained in 20 min.

Immunodiagnosis of Viral, Bacterial and Parasitic Infections

Enzyme immunoassays have now been developed for a large number of bacterial, viral and parasitic infections (for review see Talwar 1983). These assays are discriminatory and specific as MCA have been empolyed. As an example typhoid fever which is diagnosed by employing Widal's agglutination test, a commonly used diagnostic test for serum antibodies, gives positive result only one week after the onset of the disease and lacks specificity. Employing monoclonal antibodies it has now been possible to develop enzyme immunoassays, which enable detection of *S. typhi*, *S. paratyphi* -A and -B antigens with. high sensitivity and specificity in cultures of clinical blood samples (Qadri et al. 1990). As the test is designed to detect antigen rather than antibodies, it gives reliable results on the first or second day of pyrexia. Kapoor et al. (1990) developed a MCA (P6) which could distinguish the virulent form of *M. tuberculosis* ($H_{37}Rv$) from the avirulent strains of *M. tuberculosis* ($R_{37}Ra$) and *M. bovis* (BCG). It was also devoid of reactivity with 25 other mycobacteria and 38 bacteria as tested in ELISA. At the National Institute of Immunology this antibody is currently being explored for possible use in the development of an immunodiagnostic assay for tuberculosis.

MCSsareincresasinglybeingusedinWesternbolt to determine the apparent molecular weight of the antigen being recognized by antibody. Due to its single specificity in many situations MCAs give better results as compared to polyclonal antisera to identify an antigen from a complex mixture. For example P6 MCA against *M. tuberculosis* recognize only 45 and 96kDa bands from the extract prepared from *M. tuberculosis* ($M_{37}Rv$) and failed to react against *M. tuberculosis* ($H_{37}Ra$), *M. leprae* and *M. bovis* (BCG).

Children suffering from repetitive throat infection of Group-A *Streptococcus* can develop as post-infective sequelae rheumatic fever and acute glomerulonephritis. A conservative estimation suggests that 2-3 million children suffer every year from rheumatic fever in India alone. To avoid Group-A *Streptococci* post-infective sequelae, it is necessary to diagnose the children infected with *Streptococcus-A* for which a non-culture rapid test will be desirable. For this purpose in our laboratory, we have developed several MCAs against *Streptococcus-A*. The monoclonal anti-*Streptococcus-A* antibody which does not cross-react with *Streptococcus-B, -C, -G* and *Staphylococcus aureus* is being employed to develope a latex agglutination test. Currently we are testing the senstivity and specificity of this assay using clinical samples.

Blood Group Typing

Before blood transfusion, blood group typing is essential. For this purpose, polyclonal antibodies obtained from human volunteers having high levels of "naturally" occurring antibodies to the A and B blood group antigens or from deliberately immunized subjects, constituted the main source. However, in view of the ethical problems associated with production of antibodies in humans and difficulties of their production in animals using conventional immunization protocols, MCA offers an attractive proposition. Monoclonal anti-A and -B have been produced in several laboratories and proved to be superior in many respects to conventional antisera (Munro et al. 1982). Recently at the National Institute of Immunology monoclonal antibodies against blood group antigens A and B have also been developed (unpublished data). For rhesus typing of human red cells the murine monoclonal antibodies are not suitable due to lack of specificity. The technology of Epstein Bar Virus (EBV) transformed human B cell from deliberately immunized human volunteers or Rh − ve mother sensitized by Rh + ve fetus have been attempted by several groups (Boylston et al.

1980). Some of these EBV transformed cell lines have been stablized, maintained high levels of antibody production and found to be suitable for red cell typing (Thompson et al. 1986).

Immunodiagnosis of Tumours

Various groups have generated MCA against variety of tumours (table 3) which have helped in defining and characterization of the tumour associated antigen(s), their correct 'typing' and diagnosis (Levy et al. 1978, Kennett et al. 1980, Greaves et al. 1982). MCA have also been used to monitor the levels of products such as α-fetoprotein, carcinoembryonic antigen and hCG secreted by variety of tumours. Our own study has showed the utility of continuous monitoring of hCG levels in cases of choriocarcinomas to study the response to chemotherapy and as a forewarning for the recurrence of metastasis (Gupta et al. 1985d).

Panel of MCAs against cell surface antigen have been used for immunological classification of lymphocytic and granulocytic leukemia (Foon et al. 1982). It is often difficult to distinguish undifferentiated forms of acute myelogenous leukemia (AML) and acute lymphocytic leukemia for which optimal therapies are quite different. Use of MCA may establish the correct diagnosis and thereby an early appropriate treatment.

In certain cases of neoplastic meningitis, results of cerebral spinal fluid (CSF) cytology are equivocal and repeated examinations often prove to be nondiagnostic. MCA based immunocytological tests have greatly increased the accuracy with which malignant cells in CSF can be identified and characterized (Coakham et al. 1984). With early and correct diagnosis many patients can be spared extensive investigations or inappropriate treatment.

Monoclonal anti-tumour antibodies have great promise for radio-immunodetection of tumours and tumour-metastases. Radio-immunodetection involves intravenous administration of radiolabelled (usually ^{131}I-labelled) antibody recognising a tumour associated antigen followed by detection of the gamma emission (localized at the site of primary tumour and or metastases) by external scintigraphy. Using this approach metastatic cancers in patients with colorectal cancer, breast cancer and carcinoma of pancreas have been correctly diagnosed (Smedley et al. 1983). Radioimmunodetection has proved to be useful in those cases where conventional imaging methods failed to correctly diagnose the presence of tumour.

Immunodiagnosis of Plant Diseases

MCA have also proved to be useful in the diagnosis of plant diseases caused by viruses such as *pranus necrotic ringspot, apple mosaic, tobacco strea* and *alfalfa mosaic viruses* (Jordan 1984). This has helped in diagnosis of disease at a stage when it cannot be detected visibly and helped in the supply of seed stocks free of virus infection.

Identification of Important Viral, Bacterial and Parasitic Antigens

The use of MCAs have helped in the identification of antigenic determinants on viruses, bacteria and parasite membranes which are involved in infectivity and thus help in the development of vaccines to impart protective immunity. Analysis of antigenic determinants of viruses has been done for measles virus (Sheshberdaran & Norrby 1985), hepatitis-B virus (Cote et al. 1982), rabies virus (Lafon et al. 1983), murine leukemia virus (Nowinski et al. 1979) and a host of other enveloped viruses. These are examples of enveloped viruses where the exposed glycoproteins are most often associated with infectivity. Similarly, in the case of non-enveloped viruses where infectivity is associated with the nucleoprotein, analyses with MCAs has revealed epitopes which are associated with infectivity of the virion particle. Such examples include foot and mouth disease virus (McCullough et al. 1987), poliovirus (Ferguson et al. 1981) and rhinovirus (Sherry et al. 1986). These studies have been able to establish not only important epitopes, but also whether these epitopes are conformation sequence dependent. Such information eventually helps in

selecting specific sequences of amino acids which can be used as synthetic peptide vaccines.

Another area where MCAs have been used in the field of virology, is epidemiology. It has been possible to ascertain the antigenic changes that have taken place in the variants of a particular virus. Although a host of virsuses have been analysed for antigenic variation and epidemiology, the influenza virus has been studied in great detail. Based on these studies it is now known that in the case of influenza virus, the hemagglutinin (H) protein has the largest number of variations while the neuraminidase (N) protein has a few. These variations were apparently in a region which had a bearing on viral infectivity and protective immune response (Laver 1982). In the case of measles virus, the H glycoprotein showed highest degree of antigenic variations as compared to Fusion (F) glycoprotein which was most stable in different variants (Sheshberdaran & Norrby 1985). Variations in the nucleoproteins and matrix protein which were difficult to isolate by using polyclonal antibodies has been observed in influenza virus virions by employing MCA (Laver 1982).

Analysis of Antigenic Determinants

Many classification schemes of micro-organisms like viruses are based on their antigenic analysis. For this purpose MCAs are good source of specific reagents capable of defining unambiguously, the antigenic variants of a given type of virus isolated from various geographical locations. Since MCAs could be made in large amounts, these could potentially serve as ideal reference typing reagents for parasites or viruses from different parts of the world (Gerhard et al. 1978). Thus it is possible to 'fingerprint' almost every isolate of virus. By "antigenic fingerprinting" with MCA, it has been possible to separate wild-type from the vaccine strain of rabies virus (Wiktor & Koprowski 1978) and poliovirus (Ferguson et al. 1982). Such an identification has helped in the development of reliable immunodiagnostic tests as well as in the field of vaccine development to monitor immune response against the protective epitope. In case of foot-and-mouth disease virus, MCA helped in identification of four sites involved in the protective immune response (McCullough et al. 1986).

MCA have also helped in the analysis of antigenic determinants of streptococci, Mycobacteria (*M. leprae, M. tuberculosis*, Neisseria, Plasmodium Species, *Streptococce* and Trypanosomes (for reviews see McMichael & Fabre 1982). This has helped in elucidation of life cycle of parasites and designing of new 'subunit' vaccines. An exquisite example is the discovery of the major sporozoite antigen of the *Plasmodium Know lesi*, he monkey malarial parasite. Zawala et al (1983) prepared a panel of MCAs against the parasite antigen. In competition assays each competed fully with the other. In sandwich assay the MCAs could simultaneously bind to the antigen. These experiments led to the conclusion that the parasite has a single dominant epitope. Furthermore such epitope would be repetitive in the antigen. It was found to be composed of 12 repeats of a 12 amino acid long unit.

Immunotherapy

Management of Bacterial and Viral Infection

Monoclonal antibodies due to their homogenous nature and the fact that they can be made available in pure form hold promise for passive immunotherapy. Murine MCA have been found to protect chimpanzees against challenge with *hepatitis-B* virus and rodents against *Haemophilus influenzae, E. coli*, Pseudomonas and Streptococcal infection (James et al. 1984). In the field of virology, it has been observed that some of the MCA which did not neutralise viral infectivity of host cells *in vitro* were found to be protective *in vivo*. The protection *in vivo* is mainly brought about by efficiently opsonizing the pathogen and there by enhancing its phagocytosis. Rector et al. (1982) did demonstrate that *in vitro* non-neutralizing antibodies could protect *in vivo* against herpes simplex virus type I infection through enhancement of macrophage killing of virus-infected cells. Anti-tetanus toxoid

antibodies produced by EBV transformed human B cells have shown to be highly protective in mice challenged with tetanus toxin (Boyd et al. 1984). Sheep passively immunized with MCA against bluetongue virus were conferred protection (Letchworth & Appleton 1983).

Cancer

Chemotherapy constitutes a major therapeutic approach for the treatment of cancer. However, the major drawback of anticancer drugs is that besides killing neoplastic cells, they also have detrimental effect on normal cells, especially the rapidly proliferating cells of gastrointestinal tract and bone marrow. Because of their specificity, MCA against an antigen present on the surface of cancer cell will exert a site specific cytotoxic effect in the presence of complement. Cytotoxicity can also be exercised by other mechanisms such as antibody dependent cell mediated cytotoxicity, phagocytosis of antibody coated cells by reticuloendothelial system and direct antiproliferative effect.

Monoclonal Antibodies in Cancer Therapy

MCA have been used in patients suffering from leukemias, lymphomas, melanomas, colorectal cancers etc. with variable success (Sears et al. 1982, Levy et al. 1983). These clinical trials have delineated a series of limiting factors such as; (a) Heterogeneity of tumour cells: all malignant cells may not carry the relevent antigen (b) Circulating free antigen: the presence of circulating free antigens can effectively block MCA from binding to the target cells (c) Antigenic modulation: antigenic modulation occurs as a consequence of binding of MCA to the cancer cell surface as demonstrated in case of leukemia cells (Ritz et al. 1981). By employing monovalent MCA, this problem to some extent can be circumvented (d) Immune response to xenogenic protein: in almost all cases, the MCAs employed were of mouse origin. Their repeated administration led to the formation of anti-mouse antibodies. An exception was the regime employed by Koprowski, where only one out of seven patients got sensitized (Koprowski 1983). Further studies should clarify whether antibodies employed at this dose are tolerizing. This, if true, would obviate the necessity of human hybridomas for repeated therapeutic use of the hitherto available mouse monoclonals.

For passive therapy hybrid antibody having two different specificities (i.e. two paratopes), one reacting with target cell and the other with effector cells of the immune system, thereby focussing immune attack on the targeted infection holds greater potential. The possibility of generating such hybrid antibodies having two distinct specificities has been demonstrated (Milstein & Cuello 1983).

MCA can also directly interfere in the function of tumour cells. To elaborate this point, transferrin receptors are expressed at higher density on a variety of tumour cells as compared to non-cancerous cells. Trowbridge (1983) showed that antibodies against transferrin receptor can interfere with the growth of T cell leukemia *in vivo*. However, the only disadvantage is that transferrin receptors are also expressed on haemopoetic cells (due to rapid mitotic rate of division) and thus may also affect their functions. Some of the tumours also secrete factors such as α-fetoprotein, CAE and hCG. MCA against these factors may also have a therapeutic value.

Monoclonal Antibodies as a Vehicle for Delivery of Drugs

An antibody can be used for homing of an attached drug (magic bullets) or toxin (immunotoxin) at cancer cells. By this approach, higher concentration of the drug can be built up locally, minimizing systemic toxicity. Indeed a far better survival rate is observed in experimental mice given Daunamycin loaded on MCA raised against mouse lymphomas as compared to those receiving the drug alone (Baldwin et al. 1981). Similarly other cytotoxic drugs such as vindesine, methotrexate and adriamycin have been used (Moller 1982). MCA covalently bound to a toxin or toxin-subunit known as immuno-toxin have been observed to be effective in therapy of some

cancers (Vitetta et al. 1983). MCA-ricin A chain conjugates have been most commonly used. Ricin, a plant toxin, consists of a toxic polypeptide (A-chain) attached to a cell binding polypeptide (B-chain) by a disulfide bond. Whole ricin binds to cell membrane glycoprotein containing galactose via a combining site on the B-chain. This binding interaction facilitates entry of ricin A-chain into the cytoplasm of the cell. Inside the cytoplasm, the A-chain inhibits protein synthesis by enzymatically inactivating the elongation factor-2 binding portion of 60S ribosomal subunit. To overcome the problem of nonspecific binding of B-chain to the cell surface, only A-chain of ricin is conjugated with MCA which are specific for a particular cell surface antigen. By using molecular biology approach, it is now possible to express in suitable vectors a single construct having gene corresponding to antigen binding site of antibody and determinants of toxin responsible for killing the cell.The proteins known as "immunotoxins" are highly potent in killing the cancer cells *in vitro* (Chaudhary et al. 1990). However, the utility of immunotoxins for *in vivo* therapy in cancer patients is awaited.

Use in Immunocontraception

The MCAs have been developed against a variety of hormones and antigens of the reproductive tract. The feasibility of regulating fertility by the use of such antibodies has been demonstrated by various investigators (for review see Talwar 1980). Mouse monoclonals against GnRH are capable of blocking cyclicity and ovulation in rodents and progression of estrus in female dogs (Talwar et al. 1985). Pregnancy was terminated in mice and baboons on administration of anti-GnRH MCA in early phase of pregnancy (Gupta et al. 1985e, Das et al. 1985). A single injection of anti-progesterone MCA was also abortificient in mice (Gupta & Rao 1987). The MCAs against zona pellucida (a translucent layer surrounding the mammalian egg) prevent the binding of the sperm to the egg as well as protect the lysis of zona by proteolytic enzymes *in vitro* (Bamezai et al. 1988). The *in vivo* use of antibodies would demand the conduct of safety studies to exclude side effects. An apparent contra-indicatiion of mouse MCA for repeated use may be the production of antibodies against the mouse immonoglobins.

The effect of passive immunization by MCA is of a short duration limited to the period that antibodies in adequate amounts are in circulation. On the other hand active immunization employing hormone or reproductive tract antigens will be an interesting proposition. The MCAs have helped in elucidation of the functionally relevant epitopes on polypeptide hormones and gamete (Spermatozoa and oocyte) having immunocontraceptive potentials. To illustrate this point two examples are given. By employing different MCAs against hCG it has been possible to delineate the epitopes involved in the association of its α and β subunits and the hormone receptor interaction (Bidart et al. 1987, Troalent et al. 1988). From the information gained from such an investigation it was possible to construct a synthetic vaccine comprising residues 46 to 55 of α-hCG and 106 to 116 of β-hCG, which generated a specific immune response against hCG (Bidart et al. 1990). The antibodies thus generated inhibited the binding of hCG to its receptor. Thus, a synthetic immunogen can mimic a conformational specific epitope and can be useful for vaccine development.

A rat MCA produced against zona pellucida antigen-3 (ZP3) when administered into female mice produced long term reversible contraception by preventing sperm penetration of the zona pellucida (East et al. 1985). This MCA was used to screen a cDNA library prepared from mouse ovaries. A 1.0 kD cDNA containing sequences encoding the epitope recognized by this MCA was identified (Ringuette et al. 1986). The 1.0 kD cDNA was cut into random fragments of 200 to 1000 bp, cloned into the λ gt 11 expression vectors and screened again by this MCA. By screening, 8 positive clones were identified. The nucleic acid sequences of the insert in the eight clones led to the identification of 7 amino acid peptide common in all the clones thus representing the epitope recognized by the antibody. Immunization of

female mice with a synthetic peptide containing this B cell epitope linked to a carrier protein resulted in long-lasting contraception (Miller et al. 1989).

Understanding of Regulation of Immune Response and B & T Cell Ontogeny

Tremendous progress has been made in understanding various aspects of immunology ranging from functions of different subsets of lymphocytes, ontogeny of lymphocytes, involvement of major histocompatibility complex (MHC) molecules and other cell surface antigens in self/non-self discrimination.

The presence and respective functions of different subsets of leukocytes such as B cells, T helper, T suppressor, T cytotoxic and delayed type hypersensitivity T lymphocytes, macrophages and granulocytes have been identified by using allo-antisera and classical rosetting techniques. The MCA confirmed these observations and have helped in understanding the pathways involved in their development, maturation and finally their separation (Moller 1983 a, b). The study of site specific differentiation of a particular leukocyte and the factors influencing such a differentiation is now possible by the use of MCA. MCA have also helped in understanding the structure and function of molecules of the MHC (Betts & Mckenzie 1982). The MCA have also helped in the classification of leukocytes into different subpopulations. As an example macrophages express 4 antigens MAC-1, -2, -3 and -4 (Springer 1982). MAC-1 is a common precursor of monocyte and granulocytes. However, peritoneal macrophages display an increase in the expression of MAC-1 as compared to macrophages obtained from spleen and bone marrow and as well as blood monocytes and granulocytes. MAC-2, -3 and -4 appears to be expressed only on the monocytic line of differentiation, some time after the divergence from the granulocytic line as these are absent on bone marrow cells. They are present in less than 10% of spleen cells and 2% of resident peritoneal macrophages. However, these antigens are expressed to a different extent on a given population of macrophages as more than 90% of thioglycollate induced macrophages carry MCA-2, -3 and -4 in contrast to only 10 to 20% of peptone stimulated macrophages.

In addition to the antigen specific T cell receptor, many accessory molecules regulate T cell activation and impart sensitivity and plasticity to the immune response. The identity and significance of these molecules which include CD2, CD4, CD8 and LFA-1, were initially dedcued by the development of MCAs which inhibit their functions(Macdonald et al.1982, Greenstein et al. 1985, Yang et al. 1986, Peterson & Seed 1987). The CD4 and CD8 molecules are two glycoproteins that are expressed on T cells early in thymic ontogeny. With the help of MCAs it has been possible to differentiate thymocytes into four groups on the expression of these molecules. They are CD4−, CD8− (double negative), CD4+, CD8+ (double positive), CD4+, CD8− and CD4−, CD8+ (single positives) thymocytes. In the periphery, however, they are expressed on mutually exclusive subsets of T cells such as T helper cell CD4+ or cytotoxic T lymphocyte CD8+ (CTL).

Monoclonal antibodies have been used in depleting lymphocytes *in vivo* so as to assess the effect of the depleted subpopulation. Using this approach it has been possible to establish the phenotype of T cells that are responsible for rejecting skin allografts (Cobbold et al. 1986), and other transplanted tissues (Mottram et al. 1987). Resolution of the cellular basis of Graft Versus Host Diseases (GVHD) was possible only after the advent of MCAs. Although it is clear that both CD4 and CD8 phenotypes are involved in this phenomenon, the factors that govern the selective use of either type is yet unclear. The most effective use has, however, been in avoiding GVHD in allogeneic bone marrow therapy (used in anti-leukemic therapy). Using CAMPATH-1M, an IgM MCA, it was possible to lyse human T cells from bone marrow donors thereby reducing the risk of GVHD (Hale et al. 1988).

Investigation of Receptor Ligand Interaction

The use of MCAs in cell biology has been very successful in the study of receptor structure and function. This has included affinity purification of receptor (Momoi & Lennon 1982), biochemical characterization (Gullick & Lindstrom 1983) function (Carpenter et al. 1979) location (Gatter et al. 1983) and identification of receptor-ligand internalization and recycling pathways (Brown et al. 1983).

Besides giving an insight into the structure of various receptors, MCAs have been used in determining their intracellular localization as well as quantitative distribution in various tissues. This has been possible by using MCA in histochemical methods whereby intracellular localization of receptor sites could be established (Pertschuk et al. 1980). Such approaches have helped in the analyses of structure, distribution and function of biochemical techniques are not feasible. The analyses of structure, distribution and function of steroid hormone (estrogen, progesterone etc.) receptors have been studied by using MCA. Use of MCA demonstrated the antigenic differences in the estrogen receptor of mammalian as compared to avian species (Radanyi et al. 1983).

Purification of Therapeutic Agents

As MCAs are homogenous in terms of both affinity and specificity they will prove to be of great value in purification procedures to make available drugs, hormones, allergens and immuno-modulating agents (such as interferon), in highly pure form for laboratory and clinical use. A single step procedure using MCA enabling several hundred fold purification of interferon has been used (Secher & Burke 1980). However, interferons can now be prepared by recombinant technology thus limiting the usefulness of MCA-based affinity purified interferons. Nevertheless, MCA-based affinity chromatography is playing a significant role to purify recombinant DNA engineered products from the secreted products of host expression system such as bacteria, yeast or mammalian cells.

Polyclonal antibodies have antibody population of differing affinities, thus the elution of bound antigen is gradual and in order to have complete elution one requires strong buffers such as 0.2M glycine-HCl (*p*H 2) or 0.5M acetic acid. One can choose MCA of desirable single affinity so that bound antigen can be eluted by mild buffers as shown for the purification of α-fetoprotein (Stenman et al. 1981). Thus MCA can be chosen to give the most desirable result, selectivity (specificity) and ease of purification (affinity).

Generation of Antiidiotypic Antibodies

Idiotope is a single determinant on the variable region of an antibody that can ellicit an immunological response. A collection of such idiotopes on a single antibody is called the idiotype of that antibody. Antibodies generated against the idiotypes are called as antiidiotypic antibodies. Antiidiotypic antibodies have been classified by Jerne (1974) into two types, an Ab2α & Ab2 . Of these Ab2α generally recognizes determinants on Ab1 that are distinct from the antigen binding site of Ab1. The other class Ab2β, also termed as the internal image, recognise the determinants in the antigen binding site of Ab1 and can mimic the original antigen. With the advent of MCAs the field of antiidiotypes has been revolutionized. Monoclonal antiidiotypes or polyclonal antiidotypes raised against MCAs have found applications in diverse fields. In all applications the molecular mimicry of the ligand by the antiidiotypes forms the basis of their use. These can be employed deliberately to stimulate or suppress a given immune arc. Kennedy and Dressman (1984) have successfully employed antiidiotype antibodies to enhance antibody response to hepatitis antigen. A patient with poorly differentiated lymphocytic lymphoma was treated by using antiidiotype MCA (Miller et al. 1982). Reagan et al. (1983) have employed antiidiotypic antibodies to generate immune response which could neutralize rabies virus. Ertl and Finberg (1984) were able to protect mice using an antiidotype MCA against sendai virus-specific Thpy̆mpocyteclone. The antiidiotypic antibodies,

however, mimic an immune response akin to antigen only in a narrow concentration range which is of the order of 10-100ng. Higher concentration do not have any effect or even may suppress the immune response.

Antiidiotypes have been widely used to study receptor-ligand interactions. First antibody (Ab1) is made against the ligand then the antiidiotypes against Ab1 are raised. Some of the Ab2β antibodies can recognize the physiological receptor. The Ab2β can act either as agonists or antagonists. A large number of antiidiotypic antibodies recognizing receptors have been prepared (Strosberg 1989).

Antiidiotypes have also been used to understand the mechanisms underlying the autoimmune disorders like myasthenia gravis (Miriam & Sara 1989). In one study antiidiotypic reagents have been used in the immune suppression of anti-DNA antibody production (Tikeshi 1989).

Understanding the Functions of Catalytic Antibodies

Another area where MCA have begin to have an impact, is in the development of catalytic antibodies. Catalytic antibodies, as the name suggests, are antibodies that have the ability to carry out specific catalytic reactions. These reactions may include ester or amide bond hydrolyses, redox reactions and amide bond formation, besides others. Applications of such antibodies could be wide ranging in the field of medicine, biology and chemistry. They could eventually help in the development of new biomolecules or pharmaceuticals, or use as therapeutic agents to selectively destroy biological targets through enzymatic actions. MCA that have been developed for carrying out specific enzymic reactions include those for hydrolysis of esters (Pollack et al. 1986), amide bond hydrolysis (Janda et al. 1988) and amide synthesis (Benkovic et al. 1988).

Concluding Comments

The MCAs have distinct advantages over the conventional antibodies. They are homogenous in composition and can be obtained in nearly pure form. These can be produced economically in large amount as ascites or by employing air-lift fermentors. The improvement in the design of fermentors and development of making available alternative low cost media will further cut down the cost of MCA production.

Since demonstrating the possibility of generating MCA in 1975, over the years these have found increasing use in both basic as well as applied research. In basic sciences MCA have helped in better understanding of the regulation of immune response, delineation of epitopes relevant for the structure and function of hormones and determinants involved in the infertility and pathogenicity of virus, bacteria & parasites. These studies helped in designing synthetic vaccines. The MCAs substantiated the Jerne's Network therory for regulation of immune response and ushered a new era in the field of vaccinology by employing antiidiotypic antibodies specially in those situations where highly purified antigen was limited.

One of the important impact of MCAs is in the diagnostics. It is apparent that many companies are selling MCAs meant for diagnostic purposes. Moreover, the number of commercially available MCA based diagnostic kits for monitoring levels of hormones, blood group typing, diagnosis and classification of cancers and various diseases caused by virus, bacteria and parasites are increasing day by day.

The *in vivo* therapeutic potentials of MCAs in humans has remained uncertain inspite of the fact that the same MCA have proved to be very effective in experimental animal model sytem. However, with the development of suitable cell lines for producing human hybrids or generating hybrid MCA (with two distinct paratopes) it will find increasing use in prophylactic and therapeutic applications. It has been possible to 'humanize' defined mouse MCAs by genetic engineering. The first generation of humanized mouse MCA were the 'chimeric' MCAs, consisting of mouse variable regions linked to human constant domains

(Boulianne et al. 1984). Another alternative is to only introduce hypervariable regions from mouse/rat MCA into human heavy and light chain genes. Riechmann et al. (1988) have inserted hypervariable sites of CAMPATH-1 rat MCA (reacts with human lymphoid and monocytic cells) into a human IgG1 antibody. The engineered immunoglobulin had similar reactivity as that of parental rat CAMPATH-I MCA. Alternately from spleen genomic DNA library it has been possible to express and secrate only variable domains of heavy chain of immunoglobulin capable of binding with lysozyme in *Escherichia coli* by using molecular biology approach (Ward et al. 1989). Affinity and specificity of antibody can be modified further by directing mutations into specific bases in the hypervariable segments. In studies of antibody directed against hen egg-white lysozyme, when two charged amino acids (glutamic acid and lysine) in hypervariable regions were replaced by noncharged residues (serine and glutamine respectively), the antibody affinity increased by 8-9 fold. Further more, cross-reactivity with closely related antigens decreased (Roberts et al. 1987).

MCA have been the greatest success story in biology over the last 15 years and will continue to play an important role in future.

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