15 *Monoclonal Antibodies in Medicine—Today and Tomorrow*

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Abstract: Ever since Köhler and Milstein in 1975 discovered the technique of production of monoclonal antibodies (MoAb) *in vitro*, it has become all pervasive in every field of medicine and other scientific areas. Be it diagnostic, prognostic or therapeutic areas, MoAbs continue to play a central role and evolve. As immunology is the basis of our survival, MoAbs help us in diagnosing, prognosticating and managing certain malignancies, inflammatory conditions, autoimmune diseases and infectious diseases. Leukemias and lymphomas can not be diagnosed accurately without immunophenotyping. Development of new generation flow cytometer machines has helped in widening the field for diagnostics as well as measuring minimal residual disease (MRD) in hematological malignancies. In the therapeutic area, rituximab, a MoAb against CD20, has become an integral part of managing both low grade as well as high grade B cell non-Hodgkin's lymphomas. Similar MoAbs against various CD antigens are being developed to treat other lymphomas and leukemias. In a number of solid tumors like breast cancer, advanced colon cancers and head and neck cancers, when combined with cytotxic chemotherapy, patients benefit significantly with agents like herceptin, bevacizumab, cetuximab, etc. In the field of autoimmune diseases like rheumatoid arthritis, bronchial asthma, multiple sclerosis, therapeutic MoAbs are being increasingly used. There appears to be a great potential of such agents in infectious diseases too; however, it is a relatively new area and much needs to be understood.

Being biological agents, the MoAbs have their own profile of side effects like hypersensitivity, development of neutralizing antibodies, immunosuppressions leading to enhanced possibilities of opportunistic infections, etc. Of equal importance is exorbitant cost of these agents. Even in developed countries, the cost of them has become a burning issue. Therefore, judicious decisions should be taken in each case.

MONOCLONAL ANTIBODIES IN MEDICINE

Most biologists prefer to credit Paul-Ehrlich for propounding the theory of immunotherapy. In the last decade of nineteenth century, he thought of developing "magic bullet" against most diseases, including cancer. He wrote, "By injecting one animal with the cells of another, we can produce substances in the serum of the first, which have a specific damaging or destructive influence on these cells. This possibility has, within a short time, extended the theoretical doctrines of immunity in various directions.[1]" Although his prediction remains unfulfilled, great strides have been noted in the field of immunotherapy of various diseases. For a long time, the major impeding factors have been difficulty in preparing antisera with desired specificity relatively low titer of these reagents, and inability to produce and administer the large quantities of specific antisera necessary for clinical use.[2] In this article we will not discuss about vaccinations against various infectious diseases which by itself is a separate topic.

We need to mention a number of scientists in the field whose contributions have remained significant till date. They are E Metchnikoff, Emil von Behring, K Landsteiner, M Heidelberger, E Kabat, N Jerne, FM Burnet and others. Their works led to understanding of myeloma immunoglobulins, normal immunoglobulins and related proteins.

The modern era of antibodies, monoclonal antibodies (MAb) to be precise, began with the hybridoma technology developed by Köhler and Milstein in 1975.[3] This revolutionary technique showed that antibody-producing cells of virtually any desired specificity could be fused with a myeloma cell line, which leads to unlimited production of monoclonal antibodies carrying that specificity. This opened up unlimited vistas in the fields of immunology, cellular and molecular biology, developmental biology and biochemistry; in fact, there is no branch in biology that has remained untouched by monoclonal antibodies.[4] These now play crucial roles in diagnosis, disease monitoring, identifying prognostic markers and therapy.[5-7] Till date, close to twenty Nobel prizes have been awarded in the field of immunology and allied branches. Such unrestricted and massive growth in the field could be possible due to unselfishness of Köhler and Milstein who did not patent the technology.

HYBRIDOMA TECHNOLOGY

As the name itself suggests MAb means raising of protein (antibody) against a single clone of antigen. By using hybridoma technology, commercial grade MAbs are developed in the laboratories against any given antigenic molecule. Hybridoma in this case means fusion of a myeloma cell (immortal) and an antibody producing cell. A normal antibody producing cell only expresses one antibody resulting in the well-known phenomenon of allelic exclusion.[3] From numerous animal studies, it has become clear that the role of antibody isotope and complement is crucial. IgG2a appear to act on target cells through antibody-dependent cell-mediated cytotoxicity.[8] Currently used MAbs are usually of IgG2a and IgG3 types.

APPLICATION OF MONOCLONAL ANTIBODIES IN MEDICINE

As mentioned earlier, all branches of medical science have been touched by the hybridoma technology, be it in diagnosis, disease monitoring, prognostication or therapy.[5-7]

Diagnosis

The real strength of MAbs currently lies in detecting various disorders.[5,6] With its specificity against a certain surface or cytoplasmic or nuclear protein, it is now relatively easier to localize the tissue of origin. However, a deeper knowledge of such antigens being expressed in certain physiologically linked or unlinked tissues is crucial to detect the false positivity or cross reactivity.[5,9] For example, CD 10 could be expressed in renal tissues or certain connective tissues. For an accurate diagnosis the composite picture of morphology and immunophenotype could never be overemphasized. Currently, MAbs are extensively used to diagnose malignancies of all types, autoimmune diseases and selective infectious diseases.[5] In the diagnosis and classification of leukemia and lymphoma, MAbs of cluster differentiation (CD) antigens have contributed very significantly.[6] No leukemia or lymphoma should now be treated with inadequate information of its immunophenotypic characteristics. Diagnosing infectious diseases based on immunology alone has its own serious shortcomings. However, it could be an important adjunct to other investigations.

MONITORING OF DISEASE ACTIVITY

In many chronic diseases, the treatment could be prolonged and it becomes imperative to quantify response. Conventional morphology or biochemistry may not be sensitive enough to detect the residual disease. This minimal residual disease (MRD) could be detected, usually in a

semi-quantitative method using flowcytometry.[10] Usually, multicolor MAbs specific to the studied tissues are applied.

MAbs IN CLINICAL THERAPEUTICS

Although MAbs have been showing unlimited promise in vitro and animal models from the view point of therapeutic applications, their use in the human diseases had to (still do) overcome many practical hurdles.[2,11-16] MAbs raised in animals had been minimally or insignificantly active in human diseases. One of the most important issues is development of neutralizing antibodies. Hence, technology had to be developed to manufacture humanized/chimeric MAbs. This has been possible to a certain extent. Currently, MAbs can be manufactured, 1) alone; 2) tagged with cytotoxic agents; and 3) labeled with radioisotopes.

MAbs IN TREATMENT OF MALIGNANCIES

As soon as it was realized that many malignancies could be identified with their immuno-phenotypes, corresponding often to their normal or physiological counterparts, attempts began to target the functional proteins of a given tumor.[2] Antibodies were raised in animals, hence, had faced serious hurdles in clinical activity in human tumors. Over a period of time this could be overcome with production of chimeric (e.g., mouse-human antibodies) MAbs.[17] By mid 1990s clinical trials were initiated with the first chimeric MAb, Mabthera/Rituxan/Rituximab (anti-CD 20) in relapsed follicular NHLs.[18,19] Approximately half of the patients showed favorable response and toxicity profile. The dose of 375 mg/sq. m BSA was an arbitrary dose but, has become a standard dose. Subsequently, many trials were completed with the MAb alone or in combination with chemotherapy. Currently, a combination of anti CD 20 + CHOP chemotherapy has become a standard of care for B-cell lymphomas of both low-grade and high grade biology. The benefit of adding rituximab to the standard chemotherapy regimen of CHOP has been demonstrated in clinical trials even after 5-yr follow-up.[20-22] This benefit was more significant in patients with tumors overexpressing BCL-2 protein.[23] The exact mechanism of rituximab has not been explained, but the possible ones are antibody-dependent cell-mediated cytotoxicity (ADCC), complement-mediated lysis, and induction of apoptosis.[19]

More recently, radio-isotope tagged anti- CD 20 (RIT) molecules have been developed and shown to be effective even in resistant/relapsed B-cell lymphomas. In recent times anti-CD 20 molecule has shown versatility with its effectiveness in managing refractory immune- thrombo-cytopenia, chronic GVHD and certain autoimmune disorders. Attempts are on to develop MAbs against other functional B-cell markers like CD 22. Likewise, MAbs against T-cell NHLs are being developed. Integration of these biologicals into a chemotherapy regimen are expected to improve outcome. Anti-CD 52 molecule (alemtuzumab/Campath H) has shown affectivity in chronic lymphocytic leukemia (CLL)[24] and prevention of acute GVHD in hematopoietic stem cell transplantation setting.

Another MAb developed against the CD33, expressed in myeloid precursor cells and acute myeloid leukemia (AML) has shown effectiveness and is approved for elderly relapsed AML.[25] Clinical trials are on to see its therapeutic impact on newly diagnosed young patients. It is a chemotherapy tagged (calicheamycin) MAb. Newer MAbs that may spare the normal hematopoietic counterparts are being developed.

As the incidence of solid tumors is more common than the hematological malignancies, the opportunities for clinical use of MAbs are higher. Therefore, a number of clinically effective MAbs have been developed.[7] Herceptin (against Her 2 antigen) against breast cancer, gefitinib and erlotinib (against epidermal growth factor receptor—EHFR) in non-small cell lung cancer, bevacizumab (against angiogenesis) and cetuximab (EGFR blocker) in colon cancer, have now become integral part of therapy in metastatic diseases. Alone or in combination with

chemotherapy, they have improved responses and durability of such responses. The studies are being extended into some other epithelial tumors of stomach, pancreas, ovary, etc.

AUTOIMMUNE AND/OR INFLAMMATORY DISEASES

There are number of disorders like Rheumatoid arthritis, Bronchial asthma, Crohn's disease, Multiple sclerosis, Idiopathic thrombocytopenic purpura, Chronic GVHD following allogeneic BMT, etc. are extremely difficult to manage when they become refractory to conventional therapy. These disorders appear to occur as a result of development of autoreactive T-cells or other autoreactive mechanism. Development of MAbs against these T-cells or other antigenic structures including B-cells and their use in clinics are beginning to show promise.[26-30] Currently, these are considered experimental approaches.

MAbs IN INFECTIOUS DISEASES

Considering the enormous antigenic differences between humans and microbes there is a great potential in developing MAbs against various microbial infections. However, the field is nascent at present. Currently, MAbs are being used for diagnostic procedures mycobacterial and viral infections.

ADMINISTRATION OF MAbs

Currently available MAbs are administered intravenously. As these are proteins, and are capable of causing hypersensitivity, adequate premedications with antihistaminics, antipyretics and even corticosteroids should be used prior to MAb administration. The rule of the thumb is to start a very slow infusion and gradually build up to a more rapid one.

SHORTCOMINGS AND COMPLICATIONS OF THERAPEUTIC MAbs

MAb therapies are still a long way from application in most of the diseases due to technical shortcomings and still poorly understood biology of the normal and mutated antigens. Other important issues are costs, development of resistance, etc. Although novel therapies are now a reality in oncology, MAbs like rituximab, trastuzumab, cetuximab and bevacizumab, etc are efficacious alone or in combination with chemotherapy, they are exorbitantly priced.[31,32] This has led to inequalities in delivery of optimum care, particularly in developing countries.

Certain factors limit the therapeutic efficacy of MAbs—toxicity, serum blocking factors, antigenic modulations, immune response to xenogenic proteins, specificity, and perhaps most significantly, inefficiency of natural immune effector mechanism.

Anti-TNF alpha antibody has been found to be effective in certain autoimmune diseases, particularly of the GI tract. However, in recent times several concerns regarding its safety have been raised.[33] These include mycobacterial and opportunistic infections, cytopenias, lymphomas, demyelinating disease, drug-induced lupus, CHF and hepatotoxicity.

Based on some unique features of these biologic agents, a new classification of their adverse side effects has been proposed; 5 distinct types have been recognized, viz., clinical reactions because of high cytokine levels (type alpha), hypersensitivity because of immune reaction against the biologic agent (beta), immune or cytokine imbalance syndrome (gamma), symptoms because of cross-reactivity (delta) and symptoms not directly affecting the immune system (epsilon).[34]

FUTURE OF MONOCLONAL ANTIBODIES

It is hazardous to speculate events, as serendipity always throws up the unexpected good or bad news. Nevertheless, one needs to have a road map with readiness to accept detours. One thing is clear that there will be no Ehrlich's magic bullet given the vast heterogeneity of various proteins

in tissues. But, there is no doubt that monoclonal antibodies will stay with us for centuries to come.[2,7,35] This, I say, because immune system is crucial to our survival and evolution. As we continue to evolve (howsoever slowly) immune system will continue to play crucial roles in it. This will involve not only the humans but also our environment consisting of microbes and other constituents of the ecosystem. We will develop newer tools to diagnose pathogens, monitor their activity in disease and health, newer and more powerful antibodies to fight malignancy, infectious disease and autoimmune diseases.

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