MURINE AND HUMANIZED MONOCLONAL ANTIBODIES AND THEIR THERAPEUTIC APPLICATIONS. *Review Article*

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Abstract

Milstein and Kohler first described monoclonal antibodies in 1975 and they were honored with a Nobel Prize for discovering monoclonal antibodies in 1980.mAb are molecules that identify foreign bodies and accord to targets that are specific in nature called antigens. A single line of cells called B lymphocytes produce mABs so they are known as monoclonal antibodies. Originally monoclonal antibodies were produced using mice, so known as mouse monoclonal antibodies. Monoclonal antibodies that have therapeutic properties have been defined and their generic names end in –nab and –mab as suffixes; origin of monoclonal antibody is defined as a prefix as for human origin u- and for mouse origin 0- ; example adalimumab and muromonab respectively. The prefix that is used finally denotes the disease, the therapeutic purpose of monoclonal antibody like lim- for immune modulation, tu- for cancer, vir-for viral disease. On the basis of origin, monoclonal antibodies are classified in to four types. Two of these are of a single origin as human or murine antibodies, whereas remaining two are from both origins like some part from human and some from murine. Monoclonal antibodies having Fc portion of human and Fab portion of murine known as chimeric monoclonal antibodies. Humanized monoclonal antibody consists of human IgG and complementary determinant region known as CDR region from murine origin. Humanized antibodies are designated with zu- and chimeric are designated with xi-.Over the last few years' progression in the development of monoclonal antibodies have matured rapidly. Over forty years ago the technique widely used for the generation of monoclonal antibodies was Hybridoma technology. In this technology myeloma cell lines are fused with primary B cells that produce antibodies. Through a strenuous process of sub cloning hybrids that produce antibodies can be developed and isolated for the binding of antigens.Monoclonal antibodies are derived from two main sources that is murine and human source, they are classified also on the basis of their origins mainly, and hybridoma and phage display techniques are chiefly used for their isolation and characterization. Monoclonal antibodies have many therapeutic uses as well such as in cardiology, gastroenterology, rheumatology, oncology, hematology. New and effective ways should be used to produce new antibodies that are efficient and host specific.

Key words: Monoclonal, Antibodies, lymphocytes, Cancer and Derived

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INTRODUCTION

Milstein and Kohler first described monoclonal antibodies in 1975 and they were honored with a Nobel Prize for discovering monoclonal antibodies in 1980.mAb are molecules that identify foreign bodies and accord to targets that are specific in nature called antigens. A single line of cells called B lymphocytes produce mABs so they are known as monoclonal antibodies. Originally monoclonal antibodies were produced using mice, so known as mouse monoclonal antibodies [1]. These monoclonal antibodies were initially used for diagnostic and experimental purposes but later on monoclonal antibodies were made using both human and mice sources known as humanize mouse monoclonal antibodies, these had therapeutic purposes for humans as well. Current developments have made the possibility of full developing the human mono clonal antibodies. mAB can be of chimeric, humanized of murine nature. mAB are produced by a single line of B lymphocytes and work by acting on a same epitope. FDA has approved approximately thirty antibodies that are used for therapeutic purposes like heart disease, cancer and various other transplantation. Inflammatory autoimmune diseases like rheumatoid

arthritis are also being treated with monoclonal antibodies[2].

Nomenclature and classification of monoclonal antibodies

Monoclonal antibodies that have therapeutic properties have been defined and their generic names end in –nab and –mab as suffixes; origin of monoclonal antibody is defined as a prefix as for human origin u- and for mouse origin 0-; example adalimumab and muromonab respectively. The prefix that is used finally denotes the disease ,the therapeutic purpose of monoclonal antibody like lim- for immune modulation, tu- for cancer, vir-for viral disease [3].

On the basis of origin, monoclonal antibodies are classified in to four types. Two of these are of a single origin as human or murine antibodies, whereas remaining two are from both origins like some part from human and some from murine. Monoclonal antibodies having Fc portion of human and Fab portion of murine known as chimeric monoclonal antibodies. Humanized monoclonal antibody consists of human IgG and complementary determinant region known as CDR region from murine origin. Humanized antibodies are designated with zu- and chimeric are designated with xi- [4].

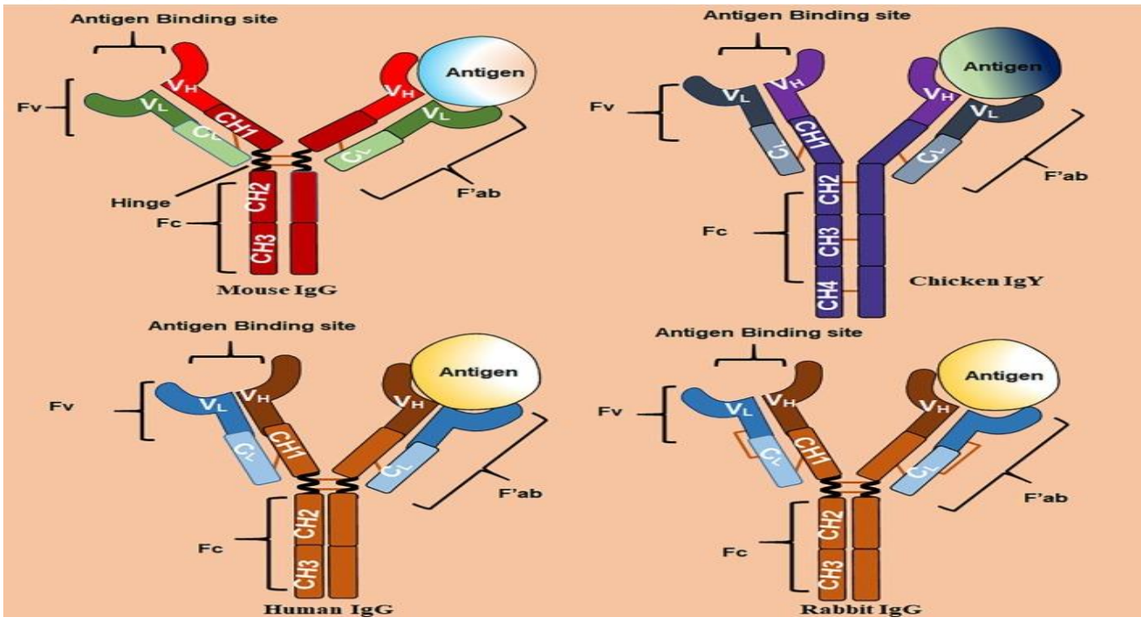

Figure 1 : Structure representation of mouse, chicken , human and rabbit monoclonal antibodies[5]

Absolute genetic sequence of mouse is acquired by murine antibodies, seventy percent human sequence is possessed by chimeric monoclonal antibodies, ninety five percent human sequence is possessed by humanized monoclonal antibodies, and an absolute quantity of human sequence is possessed by human monoclonal antibodies [6].

ISOLATION OF MONOCLONAL ANTIBODIES

Over the last few years' progression in the development of monoclonal antibodies have matured rapidly. Over forty years ago the technique widely used for the generation of monoclonal antibodies was Hybridoma technology. In this technology myeloma cell lines are fused with primary B cells that produce antibodies. Through a strenuous process of sub cloning hybrids that produce antibodies can be developed and isolated for the binding of antigens. Advantage of this method is that once the antibodies are isolated the Hybirdoma culture of B cells that are used for the production of desired antibody directly and can be cryo preserved for the unspecified prospective usage. Conversely as input B cells are not selected typically for the positivity of the antigen; extensive screening is required for antigen specific hybridomas in this method though current progression in robotic screening has made the process easier somewhat. Furthermore, the efficiency of initial cell fusion can be very low regardless of current improvements in approaches [7].

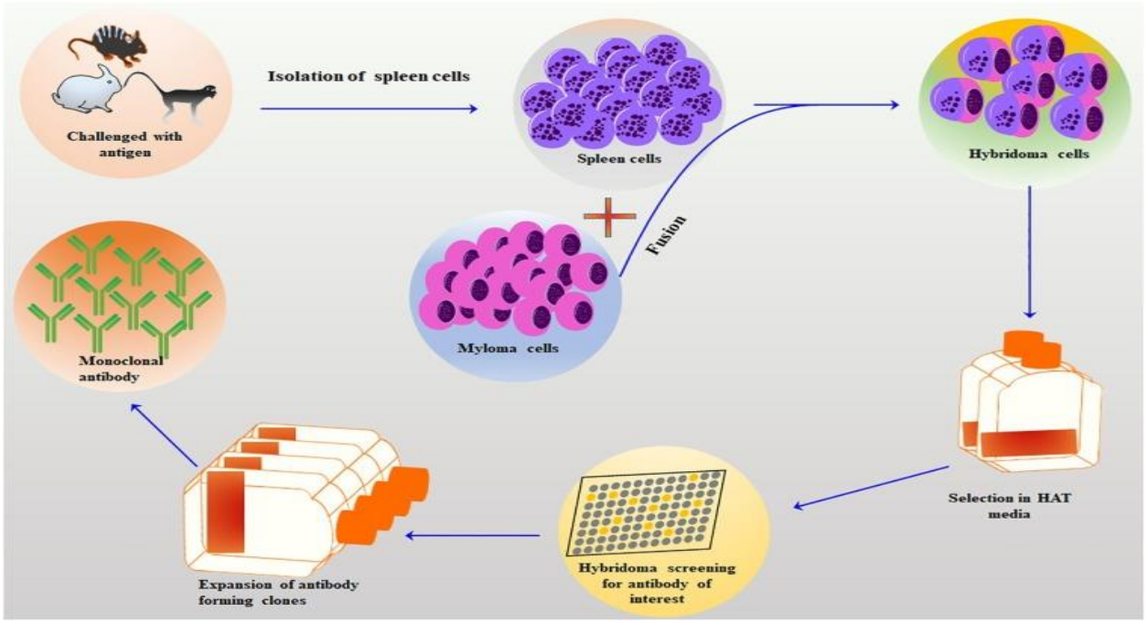

Figure2: Hybirdoma technique used for the production of monoclonal antibodies ([5])

Phage display is another method for the generation of monoclonal antibodies, in this process there is a random pairing of LC and HC sequences for the creation of a library for the potential combination of antibodies. There could be disparagation of libraries iteratively against of interest for the selection of antigen binding LC + HC combinations. But there is loss of native pairing of LC and HC from the B cell. Furthermore, since the consequential antibodies are artificial, there is amplified possibility of separating self-reactive mAbs. Such techniques were beneficial for creating components, but had restricted effectiveness to progress our consideration of humoral immunity *in vivo*. Moreover, such procedures are particularly toil rigorous and ineffective. Consequently, while beneficial in producing prospective therapeutics and reagents, this technique is not superlative for labors to comprehend humoral immunity compared to pathogens or vaccines[8].

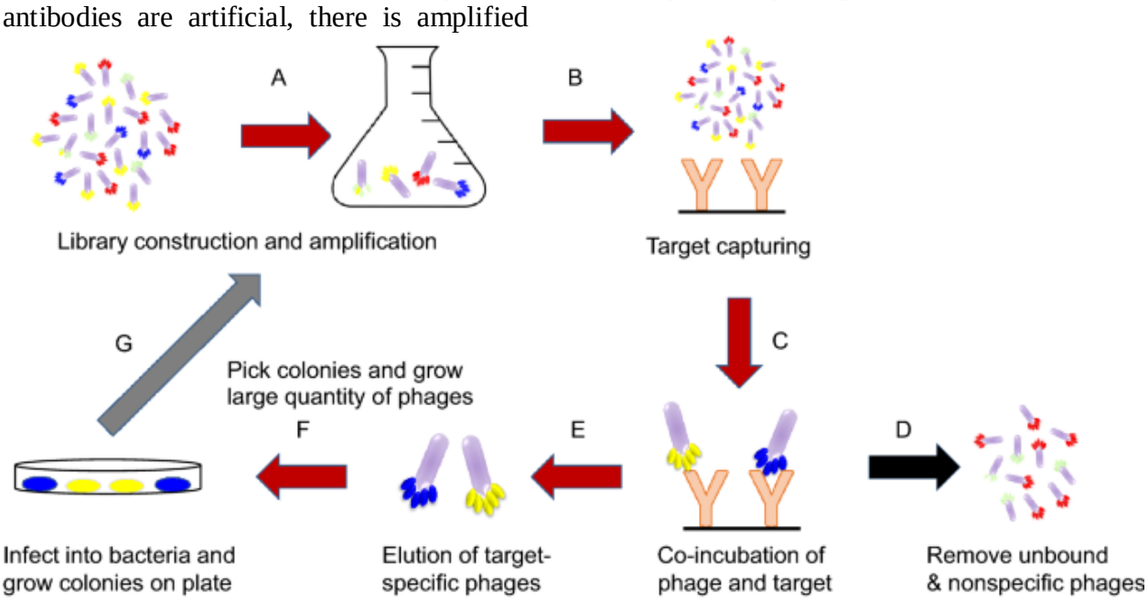

Figure 3: Phage display technique [9]

The advancement of approaches to separate and liberate natively coordinated heavy and light chain pairs from single antigen-positive B cells promptly progressed the research area [10]. One significant characteristic feature of this approach is that the primary stage chooses for antigen-positive B cells by FACS (fluorescence-activated cell sorting). Then, the sequences of heavy- and light-chain are liberated by RT-PCR. In some approaches, the organized B cells are developed in culture and separated for binding proceeding to the liberation, though approaches with murine B cells are not soundly defined. In former approaches, the B cells are arranged openly into sequences of heavy- and light-chain and lysis buffer, and are augmented and sequenced without secondary selection. In such circumstances, the secondary selection takes place

succeeding the manifestation and fabrication of recombinant mAbs. Significantly, single B cell segregation by FACS is changeable through species, and has been defined for rhesus macaques,humans, mice and rabbits [11].

Applications of monoclonal antibodies

Rheumatology In rheumatology two targets of antibodies are used ,particularly in rheumatoid arthritis, (IL-6) interleukin -6 and (TNF- α) tumor necrosis factor alpha, in 1980s it was discovered that tumor necrosis factor alpha plays a vital role in pathogenesis of rheumatoid arthritis [12]. The key mediator causing joint disruption that is inflammation induced is tumor necrosis alpha. Monoclonal antibodies that bind to tumor necrosis alpha cause a decrease in the immune response that is induced by TNF .These immune responses include production of cytokines, production of matrix metalloproteinase, activity of neutrophils, functions of dendritic cells and differentiation of osteoclasts [13, 14].

Infection Currently, the only anti-infective monoclonal antibody in the market is palivizumab. It is a humanized monoclonal antibody that is produced by DNA recombinant DNA technology and is IgG-sourced antibody; it is 5 % murine and 95% human. In the respirational syncytial virus it binds to the antigenic epitope region [15]. It is used to inhibit RSV-virussourced lower respiratory tract infections in newborns; it can also be used for children who have either premature birth histories or bronchopulmonary dysplasia [16].

Oncology /Hematology Preference is given to monoclonal antibodies because of their less nonspecific binding affinity and the target specificity.Monoclonal antibodies show their tumoricidal activity in three specific ways, antibody dependent cellular cytotoxicity, receptor related signalization and complement dependent cytotoxicity. These receptors can be stimulated by monoclonal antibodies by activation of cell surface receptors [17]. Different quality of signaling is observed in different cases. Such as when apoptosis is induced by CD-20 monoclonal antibody, ligand binding is blocked by specific epidermal growth factor receptor. Monoclonal antibodies are of two types, unconjugated and conjugated monoclonal antibodies. Conjugated monoclonal antibodies are conjugated with structures like radioisotope's, chemotherapeutic agents and enzymes. These antibodies target areas that are affected with cancer cells. Unconjugated monoclonal antibodies are responsible for blockage of cancer cell specific antigens that cause the death of the tumor cell by the activation of ADCC and CDC [18].

Transplantation In clinical terms the word transplantation means transfer of islets of Langerhans and transfer of organs. Knowledge is required regarding the immunology of transplantation so host verses graft reaction or response can be overawed and regeneration can also be blocked. Moreover, not only host versus graft reaction is important but the response of graft versus host should also be taken in to account respectively for the transplantation of cells like hematopoietic stem cells. Peripheral blood cells and bone marrow contain some mature T cells that are prepared for transplantation therefore there is a great risk of graft versus host response after the transplantation. A murine originated monoclonal antibody murmonab-CD3 is parentally used for the treatment of acute allograft rejections in patients who have undergoneheart, liver or kidney transplants. In the membrane of T lymphocytes muromonab recognizes the CD3 antigens and reacts with them [19].

Cardiology Few studies include monoclonal antibodies in cardiovascular medicine, like to visualize the necrosis of cardiac muscle with monoclonal antibodies that are myosin specific and intoxication of reverse digitalis with specific antibodies of digioxin.Four main monoclonal antibodies are used in clinical cardiology like 7E3,OKT3, Digibind and Myoscint. These antibodies have great potential in clinical cardiology for treatment [20].

Gastroenterology Over the last decade ,to treat autoimmune diseases, the gold standard is anti-tumor necrosis factor –α treatment, the outcome of clinical studies proved that adalimumab, certolizumab pegoal and infliximab have beneficial therapeutic effects against ulcerative colitis and Crohn's disease [9, 15].

Conclusion

Monoclonal antibodies are derived from two main sources that is murine and human source, they are classified also on the basis of their origins mainly, and hybridoma and phage display techniques are chiefly used for their isolation and characterization. Mono clonal antibodies have many therapeutic uses as well such as in cardiology, gastroenterology, rheumatology, oncology, hematology. New and effective ways should be used to produce new antibodies that are efficient and host specific.

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