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A BRIEF REVIEW ON MONOCLONAL ANTIBODIES

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ABSTRACT

Introduction: Clubfoot is a birth defect where one or both feet are rotated inwards and downwards. The affected foot, calf, and leg may be smaller than the other. Most cases are not associated with other problems. Without treatment, people walk on the sides of their feet which cause issues with walking. The treatment of clubfoot has developed over time and can generally be divided into many approaches like: Kite method, Ponseti method, French Method and other surgical method. **Objective:** In this study our main goal is to evaluate the efficiency of Ponseti Technique for the Treatment of Congenital Club foot. **Method:** This study was a cross sectional studywas done at tertiary medical college and hospital Bangladesh and the sample was 100 patients under Ponseti clubfoot treatment over a period of one years from 1st October 2017 to 1st October 2018. **Results:** During the study, most of the clubfoot patient age range is 1month-5month (57%) and lowest patient age range is 3-year 6 month-4-year age patient (.50%).casting treatment by the Ponseti method in the present study indicated that the results were good in (75%) cases, medium in (25%) cases, and poor in (5%) cases.clubfoot reoccurred only in (3%) cases. Moreover, noncompliance with the treatment was seen in (5%). In addition, the results revealed that the treatment of clubfoot by Ponseti method was successful in (92%) cases. **Conclusion:** From our result we can conclude that, Ponseti method is very much useful and effective treatment for clubfoot patients.

KEYWORDS: Congenital Club foot, Ponseti Technique.

INTRODUCTION[1]

In the year 1975, Kohler and Milstein provided the proof of clonal selection theory by the fusion of normal cells and the constantly dividing myeloma cells. This discovery was so spectacular that they were awarded with the prestigious Nobel Prize in the year 1984.There were few immunological reactions due to the murine nature of the older antibodies.in year 1988 Greg Winter pioneered and mastered the technique of humanizing these antibodies. This is first FDA-approved therapeutic monoclonal antibody was a murine IgG2a CD3 exclusive transplant rejection drug, OKT3 (also called muromonab), in 1986. The drug found use in the solid organ transplant recipients who happen steroid resistant. Hundreds of therapies are undergoing clinical trials. Most are concerned with immunological and oncological targets. Monoclonal antibodies(MAbs) are an integral part of targeted therapy approach for various diseases which result in decrease in adverse effects and increase in efficacy.

In the briefly technique of production of these monoclonal antibodies, the mechanism of action in therapy and clinically important monoclonal antibodies will be discussed. MONOCLONAL ANTIBODIES (MAbs) are antibodies that are identical because they are produced by one type of immune cell; all are clones of a single parent cell. Given any substance, it is possible to create monoclonal antibodies that specifically bind to that substance; they can then serve to detect or purify that substance. This has become an important tool in biochemistry, molecular biology and medicine.

Advantages of using Monoclonal Antibodies[2,3]

- Hybridoma serves as an immortal source of monoclonal antibody.
- Same quality of the antibody is maintained amongst the different production batches.
- Highly reproducible and scalable, unlimited production source.
- Speed and sensitivity and specificity of assays.
- Can produce antibodies when needed.
- No need to worry about maintaining the animals.
- Antigen or immunogenic need not be pure.
- Selection helps to identify the right clones against the specific antigen

Disadvantages of using Monoclonal Antibodies

- Time consuming project - anywhere between 6 months -9 months.
- Very expensive and needs considerable effort to produce them.

- Small peptide and fragment antigens may not be good antigens-monoclonal antibody may not recognize the original antigen.
- Hybridoma culture may be subject to contamination.
- System is only well developed for mouse and rat and not for other animals.
- More than 99% of the cells do not survive during the fusion process – reducing the range of useful antibodies that can be produced against an antigen.

TYPE OF MONOCLONAL ANTIBODIES

Progress in antibody engineering has yielded various types of mAbs for application in life science and biomedicine. These types of antibodies may have similar principles, but different targets and applications. In addition, the choice of one method over another may be guided by several factors, including purpose of application, availability and effectiveness.

Murine Monoclonal Antibodies[4-11]

The use of murine antibodies produced by hybridoma technology in human therapy (clinical medicine) is limited, attributable to differences between the human and rodent immune systems. This usually results in treatment failure, with the exception of some particular circumstances. Murine antibodies have mild effects of stimulation of cytotoxicity. Thus, their continuous administration often results in allergic reactions and anaphylactic shock, a result of the production of human anti-mouse antibodies (HAMA) that invariably attack the administered murine mAb and in turn stimulate allergic response. Anti-CD3 mAb of murine origin (OKT-3) was the first therapeutic mAb that was approved for clinical use in human medicine. However, the mAb failed in treatment of transplantation rejection, primarily because it causes severe human anti-mouse antibody (HAMA) response in patients. In order to minimize immunogenic effects of murine mAbs in human therapy, murine immunogenic components are removed with increased efficiency through various approaches. Since murine mAbs contain foreign protein molecules, majority of the early reagents for clinical use stimulated unwanted immune responses in human patients Recently, molecular biology advances have brought about *in vitro* gene manipulation and subsequent expression of these manipulated sequences in mammalian, bacterial or fungal cell culture protocols. This has thus ensured a better option for re-engineering murine mAb to partly substitute the rodent antibody fragment with a corresponding human antibody sequence. The total immunogenicity of the mAb is therefore reduced without affecting the recognition ability of the original antibody. Antibodies resulting from humanization are becoming more relevant in the treatment of inflammatory diseases and cancer, with several antibody products readily available in the market, and others undergoing clinical trials.

Chimeric Monoclonal Antibodies[12,13]

Chimeric antibodies are special types of therapeutic antibodies made by the combination of genetic ingredients from humans and nonhumans (mice). They are produced through manipulation of human constant regions and mouse variable regions. These antibodies are made up of about 65% human genetic component in order to minimize the risk of unwanted reactions to foreign antibodies. Interestingly, the Food and Drug administration has approved some drugs that are based on chimeric antibodies for use in human therapy and research. The nomenclature for naming chimeric mAbs ends with the suffix "ximab" e.g Infliximab, Rituximab, Abciximab.

Humanized Monoclonal Antibodies[14,15,20]

Human mAbs (HMA) have been considered natural drugs due to their safety for in vivo activities. Modifications in the field of mAb technologies has made human mAbs to be widely applied in the therapy of various diseases, as well as in the development of novel immunodiagnostics. A number of about 20 mAb drugs, including humanized mice mAbs, have been accepted as therapeutic reagents during the past few decades. Other mAbs at different stages of clinical trial, and controlled by different research institution, and/or in collaboration with pharmaceutical companies. Use of human mAb technologies are not only limited to strategic research, but are also of great values in health economics. In humanized antibodies, the hyper variable regions are grafted onto human variable domain framework. The antibody molecule is nearly 95% human origin. They are sometimes weaker than the parent murine monoclonal antibodies in terms of binding with antigens. To increase the antibody-antigen binding affinity, techniques such as chain-shuffling randomization can be employed to introduce some transformations into the complementarity determining region (CDR). Examples of FDA-approved humanized antibodies include daclizumab, omalizumab, and alemtuzumab.

Fully Human Monoclonal Antibodies[7,16-20]

Human mAb production by the conventional hybridoma techniques is relatively difficult because of the stress involved in maintaining immortalised cell lines and human hybridomas. It is also not feasible for in vivo immunization of humans with many different antigens compared to the use of animal models. However, methods for the production of human mAbs are made possible through the expression of antibody fragments or single cell variable fragment (Fab or ScFv) in bacteria. Similarly, antibody fragments can be displayed on filamentous bacteriophages for screening of antibody libraries. Generation of fully human mAbs serves as an alternative to reengineer murine mAbs with a source of low immunogenic therapeutic antibodies. Most of these drugs were generated using either transgenic mice or phage display platforms. However, there is still no clear distinction between them. The phage display technique is a wellestablished and the most widely used method for

the development of new human antibodies. Alternatively, using transgenic mice containing human immunoglobulins may be a strategy for the production of human mAbs. Hybridomas that produce human antibodies can be generated due to a human antibody response resulting from the immunization of transgenic mice. In 2003, Humira®, the first fully human mAb drug was lunched for the treatment of rheumatoid arthritis. Adalimumab® and Panitimumab® are among the marketed fully human therapeutic mAbs, while several others are in various stages of human clinical testing. Two basic platforms have demonstrated to yield active and welltolerated therapeutics for the clinical use of fully human mAbs. These include transgenic mice and phage display platforms.

Production of Monoclonal Antibodies[21,22]

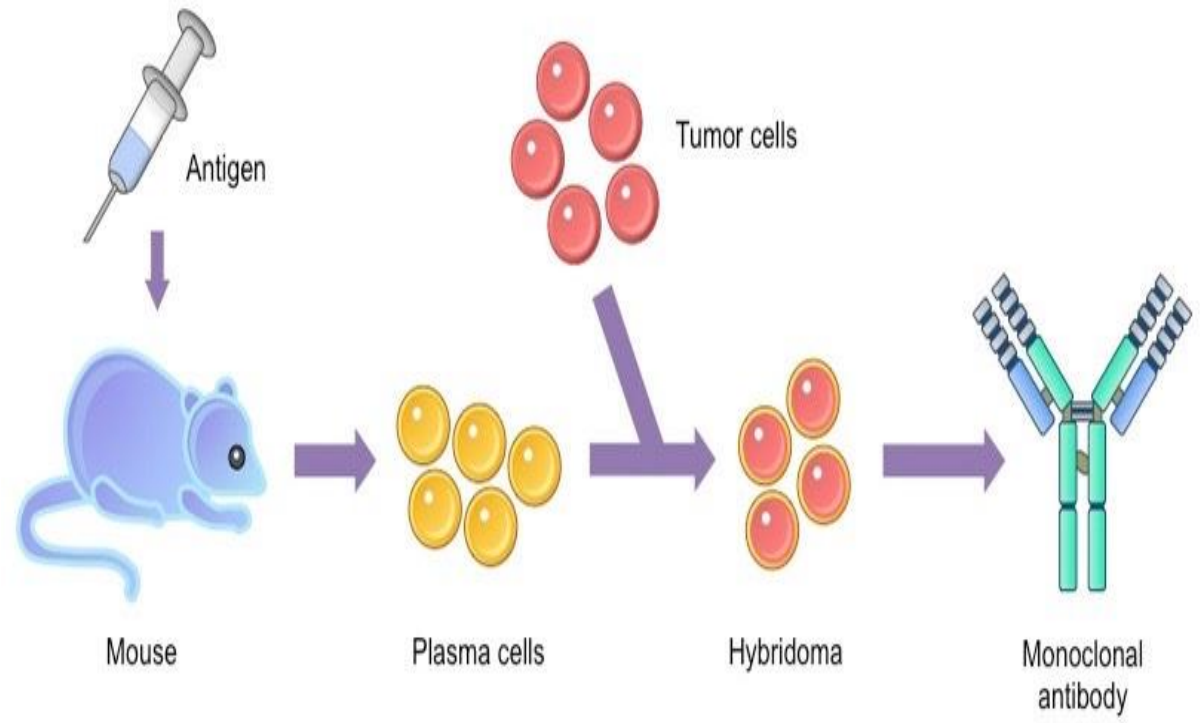

Fig. -1- production of monoclonal antibodies.

Immunization

Most myeloma lines used in cell fusion originate from BALB/c mice. These mice can be immunized with exogenous proteins (50–100 _g/ml), with cells (107 cells) or with peptides conjugated with carrier proteins, such as the keyhole limpet hemocyanin (KLH). The purpose of immunization is to sensitize the B lymphocytes (in the spleen) against the antigen for which monoclonal antibodies required.

Fusion

Myeloma cells are generally of the SP2Ag14/0 line; they are cultivated in RPMI 1640 medium containing 10% fetal calf serum until semiconfluence and then collected from the culture flasks by centrifugation. The mixture contains 2-3x107 myeloma cells for each 108 spleen cells. These cells are allowed to sediment and are then washed twice with a serum medium and centrifuged. The cell mixture is then resuspended in 1 ml of a 10% DMSO and 50% PEG solution. This solution is added slowly to the cells over a period of 2.5 minutes. The first 60 seconds are at room temperature, after which the temperature is increased to 37°C (for the final 90 seconds). The volume of the cell suspension is then slowly increased to a total of 50 ml with culture medium or physiological saline solution. After 5 minutes the cells are allowed to sediment and washed twice by centrifugation. The final sediment is resuspended in HAT medium containing 20% fetal bovine serum. In the standard procedure, the cells are now plated at a density of 105 per well in 96-well plates containing a feeder layer of macrophages, although in some protocols, subsequent selection is simplified by plating 24 wells with a density of 106 cells per well. The feeder layer is prepared by seeding the wells with macrophages collected from the peritoneal cavity of normal mice some 48 hours prior to the fusion procedure.

Selection of hybrid cells

The fused cells are grown in HAT (Hypoxanthine, Aminopterin and Thymidine) medium. The selection of hybrid cells formed between only B lymphocyte and the myeloma cell is achieved by growing the fused cells in the HAT medium.

Cloning of hybrid cells

The hybrid cells growing in HAT medium are then screened for their ability to produce and secrete the

desired antibody. This is done be enzyme linked immunosorbent assay (ELISA). Then cloning under limiting dilution is done to ensure the monoclonality of the hybrid cells. Limiting dilution is a dilution technique by which hybrid cells are distributed at a concentration of 1 cell per well. Within 2 weeks, the 'one' cell in the well form a group of cells and secrete monoclonal antibodies against single epitope of the antigen used in the immunization.

Large scale production of monoclonal antibodies

For large scale production, hybridomas can be grown either in tissue culture, where they secrete up to 100 ug / ml (usually 10 – 50 ug / ml) or in vivo as tumors in the peritoneal cavity of BALB/c mice (hybridoma) where they produce up to 40 mg / ml (usually 2 – 20 mg / ml).

ROLE OF MONOCLONAL ANTIBODIES IN CANCER THERAPY

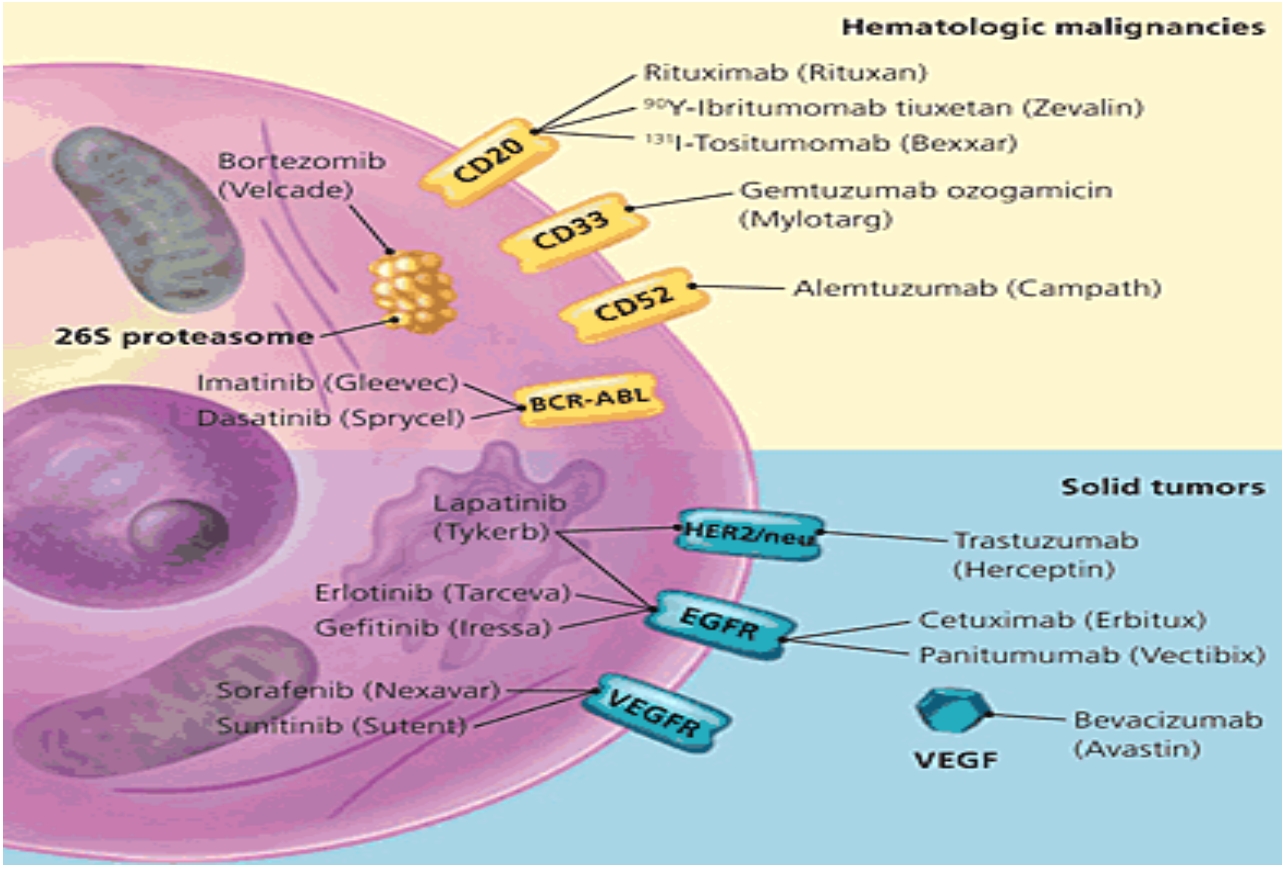

Fig – 2- Targeted therapy: monoclonal antibodies and small molecules in chemotherapy of various cancers.

APPLICATION OF MONOCLONAL ANTIBODIES

1) Diagnostic Application

- Detect protein of interest either by blotting or immunofluorescence
- Cardiovascular diseases
- Deep vein thrombosis
- Location of 1*C and 2*C metastatic tumors
- Immunosuppressive therapy
- Pregnancy testing kits

2) Therapeutic applications

- Radioisotope immunoconjugates
- Toxic and drug immunoconjugates
- Immunoliposome based kits
- In cancer

CONCLUSION

Although the first mAbs approved as human therapeutic agent were generally reported to beintolerable as therapeutics, advances in hybridoma technology have resulted in mAbs that are currently more effective and safer. Many of these new generation mAbs have been approved for human therapy. Multiple engineering efforts have led to the evolution of modified therapeutic antibodies in the hope of improving their efficacy and safety as antibodybased drugs. These efforts include

antibody chimerization, humanization and the development of fully human antibodies. The ability to engineer variable regions that encode multiple specificities into a single molecular entity has been an advantage for optimizing antigenbinding capabilities. There is no doubt that the future of mAbs will sway the treatment of infectious diseases, cancers and other conditions like Alzheimer and Parkinsonism. Whether therapeutic mAbs will be commercially more abundant and affordable in the near future, is in part, a matter of rapid advances in biotechnology and biomolecular sciences as well as the outcomes of extensive clinical trials.

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