Title: MONOCLONAL ANTIBIDY TO RABIES VIRUS AND ITS ROLE IN IMMUNOPROPHYLAXIS

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Keywords

Abstract

Rabies is one of the dreadful Zoonotic diseases and hyperimmune immunoglobulin preparation against rabies virus is one of the vital curative tool in WHO category III exposure.

Review Article

Monoclonal Antibody to Rabies Virus and its role in Immunoprophylaxis

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INTRODUCTION

Rabies is one of the dreadful Zoonotic diseases and hyperimmune immunoglobulin preparation against Rabies virus is one of the vital curative tool in WHO category III exposure.

Annual Rabies death in India is estimated to be 20,000. The burden of Rabies in India has to be reassessed. Developed countries are free from Rabies but bat related Rabies is endemic in pockets of Western Europe and sporadic cases are reported. 95% mortality due to Rabies occurs in Asia and Africa, of these major contribution is from India and its neighbouring countries. USA and Canada are free from Canine Rabies. In these countries cycle of transmission is maintained in wild animals such as Bats, Foxes, etc. and infection is transmitted to domestic animals and general population by wild animals. Statistics reflect that 16000 – 39000 people receive post exposure prophylaxis in these countries.

There was constant effort by the scientific community since 1890 to develop passive immunoprophylaxis against Rabies as depicted below:

1890-1950 - Efficacy of antirabies serum in animal and human evaluated.

1975 - Kohler and Milstein developed an indigenous method for large scale production of monoclonal antibodies against any desired antigen and they were awarded Nobel Prize in 1984 for their pioneer work. A single clone of plasma cells/lymphocytes undergo selective proliferation Eg. Multiple Myeloma and produce antibodies. Such antibodies produced by a single clone and react with a single antigenic determinant are called Monoclonal antibodies.

Kohler and Milstein developed hybridoma technology. As per this technique first the mice is immunised with desired antigen. Then lymphocytes are obtained from spleen cells of immunised mice. A single clone suspension of lymphocytes is made and is added to the culture of mouse myeloms cells. These mouse myeloma cells are devoid of enzyme hypoxanthine phosphoribosyl transferase (HPRT) and they do not produce immunoglobulins. In culture cells are exposed to polyethylene glycol which facilitates the fusion of lymphocytes with myeloma cells resulting in the formation of somatic cell hybrids which are known as hybridomas. Fused cells are placed in basal culture medium i.e. H.A.T. medium containing hypoxanthine, aminopterin and thymidine. In HAT medium only hybrid cells survive. Hybrid cells possess both the properties i.e. multiply continuously and produce antibodies. These hybridomas are cloned by limited dilution method and recloning is performed to assure monoclonicity. Clones producing antibodies against desired antigen are selected for continuous cultivation and production of monoclonal antibodies. Hybridomas are injected into mice intraperitoneally and monoclonal antibodies can be obtained by harvesting ascitic fluid. Hybridomas can be frozen for prolonged storage. Human hybridomas have also been developed. Large quantities of desired antibody can also be prepared by Recombinant technology.

1980-2012 - Crucell Massachusetts Biologic Laboratories and others have put continuous effort in the commercial production of Rabies Specific Monoclonal Antibodies.

Several studies were conducted to gauge the efficacy of combination of active and passive immunoprophylaxis. It is concluded from the studies that combination is most effective in

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inducing neutralizing antibodies against Rabies. After vaccination Antibodies against glycoprotein and nucleoprotein are produced. Glycoprotein antibodies neutralise Rabies virus and Nucleoprotein antigen in conjunction with CD4+ T Lymphocytes assist in B cell immunoglobulin class swithching and immunoglobulin production. There is no method to assess cellular immune response against Rabies virus. Humoral immunity is important for protection against Rabies infection. Glycoprotein antibodies are neutralising antibodies and thus after vaccination titres of RVNA (Rabies virus neutralising antibodies) are crucial. There are two methods by which RVNA can be assayed, these are Rapid fluorescent focus inhibition test (RFFIT) and Fluorescent antibody virus neutralisation test (FAVN). Both these tests are recommended by WHO for the assessment of humoral immune status after Rabies vaccination.

RABIES PASSIVE PROPHYLAXIS

Active immunisation needs time to induce production of antibodies, thus along with Rabies Immunoglobulin (R.I.G.) in treatment of post exposure prophylaxis, it prevents viral spread. Neutralisation of virus is effective when R.I.G.is injected directly into wounds, whereas R.I.G. given only intramuscularly at a distant site increases the risk of the virus reaching Central Nervous System.It is estimated that it takes one week for the vaccine to induce the antibody production. During this period R.I.G. is vital in providing protection against virus. If the patient has received preexposure propholyxis, R.I.G. is not recommended. In postexposure, combination of active immunisation with TCV+ infiltration of the wounds with R.I.G. is 100% effective. When R.I.G. and vaccine is given after the onset of symptoms, the outcome is controversial i.e. few studies suggest early death and few suggest prognosis is unaffected. The inference can be drawn that the combination is not beneficial after the onset of symptoms, because Immuno-globulins cannot cross blood brain barrier. In H.I.V. infected patients with CD4 count less than 200, when exposed to Rabies, treating these patients is a challenge. These patients do not respond to vaccination. Local infiltration of the wound with immunoglobulin is the only remedy and it can be life saving.

R.I.G. AND ITS DRAWBACKS (Polyclonal)

Human Rabies Immunoglobulin (HRIG) is of human origin, there is inherent risk of transmitting infections. Other drawbacks are limited supply, high cost in addition to batch to batch variation.

Similarly Equine Rabies Immunoglobulin (ERIG) has several drawbacks namely ethical issues leading to discontinuation of production by some manufacturers, reduced half life of F(ab)2 fragments, high cost, anaphylaxis and serum sickness.

Because of aforesaid reasons i.e. safety, cost and supply, the viable alternative is Monoclonal Antibodies .

Use of Monoclonal Antibodies in the treatment of Rabies:

ADVANTAGES

1. Monoclonal antibodies are effective against various strains of Rabies virus
2. Monoclonal antibodies specifically bind to the Rabies virus glycoprotein thus inhibit adhesion of virus to receptors which is important in the initial stage of pathogenesis, also prevents adhesion of virus to plasma membrane and endosomal membranes. Fc fragment of the antibody also contributes indirectly by activating complement in neutralising virulence,
3. R.I.G. also contain non neutralising antibodies where as Monoclonal antibodies specifically reacts with glycoprotein, thus enhancing the specificity and efficacy when compared to RIG.
4. Volume of the dose is less compared to RIG, thus causing minimal local reactions and improved tolerance.
5. Experimentally in laboratory animals it has been proved that monoclonal antibodies give more than adequate protection.

DISADVANTAGES

Animals are the reservoir of Rabies infection. They harbour various strains of Rabies virus and virus is prone for mutation Thus therapeutic monoclonal antibodies should contain Rabies specific cocktail of non competitive monoclonal antibodies which will react specifically against various virulence factors (antigens).

Several Rabies specific monoclonal antibodies are undergoing clinical trials. The results are encouraging and suggest monoclonal antibodies as potential candidates for use as an alternative to HRIG and ERIG. Mouse monoclonal and human monoclonal antibodies have been shown to protect rodents from lethal Rabies virus challenge.

CONCLUSION

HRIG is costly and not easily available . In country like India, majority of patients are poor and cannot afford. Commercial production of Rabies specific human or murine monoclonal antibody will help in replacing non affordable polyclonal products. Large scale production will; reduce the cost, will be easily available and thus decreasing the mortality and morbidity drastically.

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