Traditional monoclonal antibodies and recombinant antibodies

Introduction:

Antibody is a special molecule that present in our bodies to fight against infections and stimulate immune response. Typical antibody is a “ Y” shaped molecule consists of two H (heavy) and two (light) chains. Two antigen-binding fragments (Fabs) are linked with a constant region (Fc)(Brekke and Sandlie 2003). After the discovery of murine monoclonal antibodies produced by hybridoma cells developed by Kohlor and Milstein(Kohler and Milstein 1975), the role of monoclonal antibodies in therapeutics and clinical diagnostics are increasingly important in the last three decades (Laffly and Sodoyer 2005). The term monoclonal antibody is defined as an antibody molecule which is monospecific and derived from a single B cell clone. Results in using fully murine monoclonal antibodies in therapeutics are not ideal and problems aroused in triggering unwanted human immune responses. These problems force the generation of recombinant antibodies in the replacement of traditional monoclonal antibodies. Tailor-made recombinant antibodies fragments increase flexibility both in immunotherapy and immunodiagnostics. The application of minimal form of functional antibodies single-chain antibodies (scFvs) are the most popular form of recombinant antibodies fragments as diagnostic agents (Hagemeyer et al. 2009). In this assignment, the comparison between traditional monoclonal antibodies and recombinant antibodies as therapeutics agents and diagnostics tools will be discussed. From the example of tragedy TGN 1412, the potential risk of using recombinant antibodies in therapeutic agents should not be ignored. Finally, future perspective of recombinant antibodies

in gene therapy and using polyclonal antibodies as novel immunotherapeutic strategy will be discussed.

Theoretically, probably any kind of monoclonal antibodies can be produced with the aid of hybridoma technique. The continuous culture of hybridoma cells creates an inexhaustible supply of monoclonal antibodies in the laboratories by cell culture or rodent(Nelson, Reynolds et al. 2000). Its highly specificity, stability and homogeneity are ideal for diagnostics and in therapeutic purposes. After the introduction of the first FDA approved drugs OKT3 launched into the market in 1986, the results of using fully murine monoclonal antibodies in human was not promising (Chatenoud, Baudrihaye et al. 1986; Chatenoud, Jonker et al. 1986). This is because murine originated monoclonal antibodies triggered several immunogenic responses in human body. One of the problems arise is human anti-mouse antibodies (HAMA) or anti-globulin antibodies (HAGA) response (DeNardo, Bradt et al. 2003; Presta 2006) generated against the administrated murine antibodies. Studies showed that around 30-75% of patients with solid tumors and relapsed B-cell malignancies developed HAMA response after exposure to murine antibodies (Smith, Nelson et al. 2004; Majidi, Barar et al. 2009). The activation of HAMA response is mainly due to the host antibodies generated against the idiotopes of the administered murine antibodies. Moreover, rapid clearance of murine Abs shortens its serum half-life and relatively ineffective to trigger cytotoxic effect (e. g. ADCC and CDC) compared to human antibodies hindered murine Abs as therapeutics agents (Presta 2006).

Based on the above unresolved problems, with the aid of genetic engineering, murine monoclonal antibodies are modified to become less

immunogenic and enhance potency in therapeutics and diagnostics. Three different types of recombinant antibodies are generated: chimeric, humanized and human antibody. In chimerization, the murine variable region is fused with the human constant region forming chimeric antibodies(Presta 2006). This modification imitates the human immune system. Although chimeric antibodies is less immunogenic but may trigger human anti-chimeric antibody responses (HACA)(Baert, Noman et al. 2003). Further maturation technique is humanization (hyperchimeric). In this technique, only the complementarity determining regions (CDRs) from the murine antibody was grafted into a human constant and variable regions (Smith, Nelson et al. 2004). By resurfacing, reshaping and hyperchimerisation of hyperchimeric antibody, the antibody binding affinity improved. Although the above methods minimize immunogenicity, but immune response result of xenografting may occur. Finally, human antibodies can be generated by transgenic mice and in vitro combinatorial libraries (Brekke and Loset 2003; Brekke and Sandlie 2003; Presta 2006). Antibodies which generated under this method are expected to be identical to human antibodies with clinical significant without any side effects. One of the examples in combinatorial library approach for the selection of antibodies is by phage display technology in which antibody variables domain are expressed as fusion protein as coated on the surface of the bacteriophages. Under combinatorial library approaches and transgenic mice, the chance of getting fully human antibodies are higher when compared to hybridoma and chimeric antibody technologies. In addition, single-chain variable fragment (scFv) and Fab fragment can be isolated (Brekke and Loset 2003; Brekke and Sandlie 2003).

Therapeutics application

Fc portion in an intact antibody trigger effector function which is undesirable for therapeutic applications. Therefore, for a desirable antibodies design for cytokine inactivation or receptor blockage, the main considerations of antibody design are: size, tissue penetration, distribution, half-life, effector function, affinity, stability and immunogenicity. scFv and Fab fragments are preferred as choice of preference when compared to traditional antibodies because of smallest in size, high binding affinity, specificity, good tissue penetration and reducing immunogenicity due to HAMA response. scFv and Fab antibodies have a shorter half-life than whole antibodies and this drawback can be overcome by PEGylation. In addition, the attachment of PEGylation of murine monoclonal antibody reduces HAMA response of the host after administration(Laffly and Sodoyer 2005).

Applications of monoclonal antibodies are vastly employed in therapeutic agents (e. g. treatment of cancer) and in clinical diagnostic (e. g. histopathological diagnosis).

1. Humanized mAbs (transgenic mice) (resurfacing, reshaping and hyperchimerisation, etc)
2. Phage display technology (Fabs and Fvs)

In recent years MAbs have become very important commercial reagents, and currently contribute to over 30% of biopharmaceuticals in development and production. To date, 10 different MAbs have achieved FDA approval, with

others in phase III trials. 4

Applications

1. Antibody conjugates (Majidi, Barar et al. 2009)
2. Unconjugated mAbs (Majidi, Barar et al. 2009)
3. rAbs for cancer therapy
4. immunohistology
5. genetic immunotherapy(Pelegrin, Gros et al. 2004)
6. scFv for diagnostics tools (size, immunosensor, inhibition of imnflammation and complement system) (Hagemeyer, von Zur Muhlen et al. 2009)

Problems

1. polyclonal vs monoclonal therapeutics (Haurum 2006)
2. TGN1412 incident (Self and Thompson 2006)

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