BISPECIFIC ANTIBODIES: PRODUCTION AND USE

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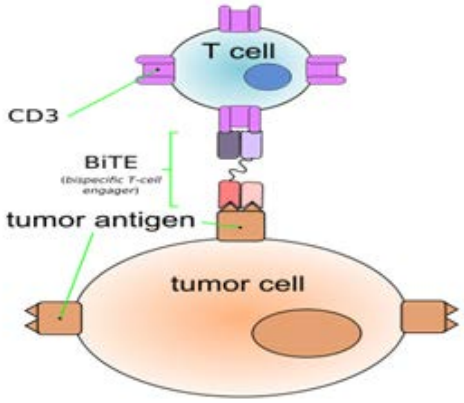
INTRODUCTION

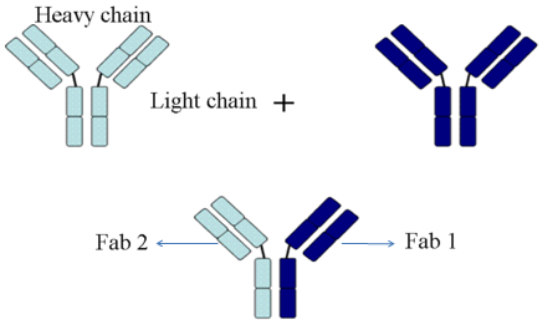
Bispecific antibodies are specific type of monoclonal antibodies, which can bind to two different types of antigens. These monoclonal antibodies do not occur naturally but can be produced by genetic engineering or by cell fusion. However, one exceptional case of natural bispecific antibody was recently obtained from allergic patients receiving therapeutic injections with 2 different allergens during specific immunotherapy[1]. The main advantage of these types of antibodies is that they can recognize more than one protein on the surface of different cells. Bispecific antibodies are mostly designed to recruit cytotoxic effector cells (cytotoxic T-lymphocytes or NK cells) of the immune system effectively against pathogenic target cells thus improving effector cell cytotoxicity[2]. Bispecific T-cell engagers (BiTEs) is one of the typical example of bispecific monoclonal antibody which direct a host's T cells cytotoxic activity against diseased cells like cancer cell (figure 1).



(Figure 1) Bi-specific T-cell engagers (BiTEs) is atypical example of bispecific monoclonal antibody.
Photo taken from wikipedia

Bispecific Antibodies structure:

Bispecific antibodies also represent as a "Y" shaped molecule, and has two different arms, each arm can bind two different types of molecules. Each Y-shaped unit contains 4 polypeptides, 2 identical copies of a polypeptide known as the heavy chain and 2 identical copies of a polypeptide called the light chain. Bispecific antibody, as its name indicates, usually consists of 2 distinct Fab (antigen binding fragment) arms, by which it is capable of simultaneously binding two different antigens, while normal antibody has two identical Fab arms. The third fragment, which forms the base of the Y, is called Fc fragment. This special characteristic of BsAbs confers bispecific antibody huge potential for a wide range of clinical applications as targeting agents for immunodiagnostic and therapeutic. It was specified that the antibody with bispecificity belonged to the IgG4 subclass. In bispecific antibodies one Fab arms by swap with a heavy chain and attached light chain (half-molecule) with another heavy-light chain pair from another molecule bispecific antibodies[1,3] (figure2).



(Figure 2) Bispecific antibody(BsAb) with 2 different Fab regions from 2 different antibodies

Production of Bispecific antibodies:

Bispecific antibodies are mainly produced by two methods: 1st by fusion of two hybridoma cell lines producing and 2nd chemically links two antibody molecules together.

1- Fusion of Hybridoma cells:

As a result of allelic exclusion, plasma cells produce only one set of light and heavy chain of Immunoglobulin (antibody) (figure3).
Specific Mabs producing Hybridoma cells are modified in such a way that they are sensitive to hypoxanthine-aminopterin and thymidine (HAT) containing medium using the drug 8-azaguanine and resistance to neomycin (neo). As a results of this hybridoma cell line (neo /s HAT) is only capable of growing in neomycin but not in HAT-containing medium. The second hybridoma cell line for producing Bispecific antibody will be HAT resistant / neomycin sensitive, is then selected on neo / HAT selection. After fusion of the two these two different hybridoma, the resulting quadroma cell line can be selected by culturing and cloning in HAT- and neomycin-containing medium[4]. Producing Bispecific antibodies by hybridoma is easier and resulting Bispecific antibody is naturally assembled. The drawback of this technology is that Bispecific antibodies produced by somatic fusion of two different hybridoma cell lines expressing monoclonal antibod-

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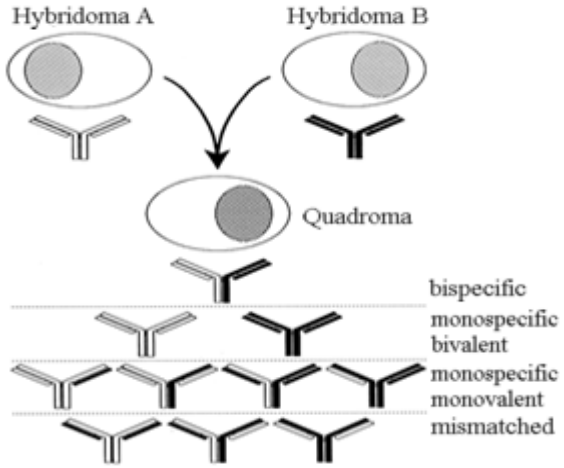
ies with the desired specificities of the bispecific antibody random pairing of two different immunoglobulin heavy and light chains within the resulting hybrid–hybridoma (or quadroma) cell line, up to ten different immunogloblin species are generated of which only one is the functional Bispecific antibody. Another disadvantage of this technology is in stability of the resulting cell lines, low yields, difficulty in purification of the Bispecific antibodies.

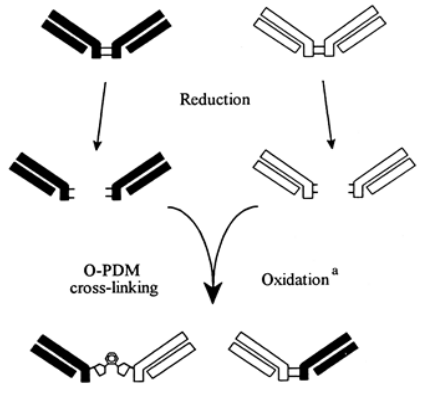


(Figure 3) Bispecific antibodies production by Hybridoma technology: Representation of 10 different bispecific antibodies secreted by a hydrid hybridoma cell as a result of different random association of light and heavy chain from parental hybridoma

2- Chemical method of producing Bispecific Antibodies:

This technique was established by Nisonoff & Rivers in 1961. This technique does not require any cell fusion as the name indicates. The advantage of this technique is that, it is more quick to produce Bispecific antibody and antibody molecule is easy to purify compare to hybridoma technique[5].

Chemical combination can be achieved in two different ways: 1st is direct coupling of the whole antibody molecules or their derivatives, and 2nd is dissociation and reassociation of heterologous immunoglobulin (figure 4).



(Figure 4) Chemical method of producing Bi specific Antibodies

2nd method of producing Bispecific antibodies by chemical method initially requires chemical manipulation to detach immunoglobulins into half molecules and to keep the antigen binding sites intact, then in second step reform the disulphide bonds linking the heavy chains without allowing any interfering side reactions such as the formation of intrachain or mismatch disulphide bonds.

In this straightforward method coupling of two parental antibodies was performed by heterobifunctional cross linker. As a result of this bispecific antibodies production method, there is a significant molecular heterogeneity because reaction of the cross linker with the parental antibodies. To obtain more homogeneous preparations of Bispecific antibodies two different Fab fragments have been chemically cross linked at their hinge cysteine residues in a site directed manner[6].

Various challenging methods have been applied to make immune protective Bispecific antibodies.

A novel Bispecific antibody has been produced by fusion of DNA encoding a single chain antibody (ScFv) after C terminus (CH3-ScFv) or after the hinge (Hinge-ScFv) with antibody of different specificity. Transfection method has been applied to express this fusion protein. Transfected cells secrete a homogenous population of the recombinant antibody with 2 different specificity, one with N terminus (antidextran) and other with C terminus (antidansyl). The CH3-ScFv antibody, maintains the constant region of human IgG3, has some of the associated effector functions such as long half-life and Fc receptor binding[7].

Use of Bispecific Antibodies:

Bispecific antibodies have been used for various medical purposes till now and these are found to be very effective tool for medical purpose specially in human diseases.

1- Molecular Advances in Pre-targeting Radioimunotherapy with Bispecific Antibodies[8]:

For the targeted delivery of radionuclides to treat tumour, Antibodies were used for the first time before 20 years and promising results were achieved using this radiolabled antibodies especially on hematopoietic malignancies. Pretargeted delivery of radiolabeled antibodies has seen to be very effective where this pre-targeted delivery significantly increase the radioactive uptake in tumour cells compare to normal cells and thus enhances the usefulness of both detection and therapy of tumour and cancer. Separations of the radionuclide from the tumor targeting antibody permits slow and effective process of specific antibody localization into tumour cells. Peptide macromolecules are used in pretargetting. These macromolecules are capable of binding with a high affinity to a radioactive agent of low molecular weight and selectively target the tumour antigen. As a treatment of tumour/cancer these mac-

romolecules are administered first into the patient, and the radioactive agent ("the effector") is then given at a later stage, when the concentration of the macromolecule in the tumour is greater than in other tissues. Also the variation in radioactivity between tumour and normal tissue is easily reachable. So this is two steps procedure where the success is based on the clearance of macromolecule sufficiently from the blood and normal tissues, otherwise radioactive effector molecule will remain wherever the macromolecule is allocated. In this study they have produced two bispecificdia bodies (BS1.5 and BS1.5H) for pretargeted delivery of radiolabeledbivalent haptens to tumors expressing CEA. BS1.5 (Mr 54,000) consists of two heterologous polypeptidechains associated noncovalently to form one binding site for CEA from the variable domains of hMN14 (a humanizedanti-CEA antibody from Immunomedics, Inc., Morris Plains,NJ) and one binding site for HSG from the variable domains of 679 (a murine monoclonal antibody specific for HSG).

2- Recombinant Bispecific antibodies for cancer therapy:

Bispecific antibodies have been used for variety of biological applications with potential use in cancer therapy. Most commonly used applications are retargeting of effector molecules (produrg-converting enzymes, radio-isotopes, complement components, effector cells (CTLs. NK cells) and adenoviral vectors. Various approaches have been used to treat cancer using bi-specific antibodies. One study showed that intracellular expressed Bispecific antibodies were used to induce a functional knock out of two cell surface receptors. In another study a bispecific tandem ScFv with cell-penetrating abilities was applied to restore p53 wild type function by intracellular binding to p53. Most application for cancer therapy involves the retargeting of effector cells of the immune system to tumour cells. Wide range of work has been done on the retargeting of cytotoxic T lymphocytes (CTLs) through binding to the T cell co-receptor.

In a recent study, Bispecific antibodies were used as a molecule for immunotherapy against pathogen that infect the heart and it was utilized to cross linking between bacteria and polymorphonuclear (PMN), as these PMN neutrophillic leukocytes are the first line of immune resistance in the body against intramammary infections[(9)].

3- Use of Bispecific antibodies in HIV1[(10)]:

During the HIV-1 infection, HIV virus infects monocytes and T lymphocytes. Viral gp120 binds to CD4 cell surface protein. It has been found that some specific antibodies bind to CD4. This also led to think of making recombinant CD4 to block CD4 binding site on HIV-1 gp120. Monocytes express 3 types of Fc receptor for these antibodies. Bispecific antibodies have been used to target HIV-1 to either Type I, II or III FcγR on these cells. Bispecific antibodies specific for soluble CD4 and Fc region of IgG, on phagocytic cells have been made which potentially link to HIV-1 to the Fc receptor for IgG on phagocyte cells. The disadvantage of this antibody that, antibodies have difficulty in affinity for different strains of HIV-1. An alternative approach involves the use of anti-FcγRIcontaining Bispecific antibodie to target HIV-1 and HIV-1 infected cells for removal by FcγRI-expressing myeloid effector cells. Using these types of antibodies could be useful in target specific removal of HIV infection and to boost immune system of the infected HIV individual.

4- Bispecific antibodies in Hodgkin's Disease[(11)]:

This research explained that refractory Hodgkin's disease was treated with an anti-CD16/CD30 bispecific antibody during the Phase I/II trials. These Bispecific antibodies (HRS-3/A9) were generated against the Fc (gamma)-receptor III (CD16 antigen) and the Hodgkin's-associated CD30 antigen. Promising results were seen when median counts of NK cells and of all lymphocyte subsets were considerably decreased in the patients before therapy. These Bispecific antibodies administered everyday starting with 1mg/m2. As expected there was no clear-cut dose-side effect or dose-response correlation. These promising results obviously prove the effectiveness of necessity of these antibodies and further clinical trials would also require getting it for daily therapeutic role.

5- Bispecific antibodies for malaria[(12)]:

Attempts have been made to treat malaria using Bispecific antibodies. Antibodies have been synthesized against plasmodium antigen and could be used for therapeutic purposes. These antibodies designed in such a way that these could be act like a drug and kill parasites directly or same strategy could be used for vaccine development using these antibodies. Vaccination with these antibodies could protect individual from infection.

Based on the recent research, it has been seen that Bispecific antibodies are very good and advance tool in the therapeutics use. It would be an effective tool in near future to treat various type of tumour or cancer and other challenging diseases to man. Bispecific antibodies are also used in treating HIV infection. There are various other challenging diseases which cause a huge number of death tolls every year, including MTB infection, hepatitis infection, human pappiloma (HPV) virus infection, herpes simplex virus (HSV). Using the recombinant DNA technology Bispeicific antibodies could be used in all these disease and successful clinical trials could be achieved and this way we can lower down the death toll per year due to these diseases. Attempted has already made successfully for High risk HPV infection. To treat these malignant diseases, monoclonal antibodies needs to be routinely modified to get the

better response against these diseases. Extensive work is still going on in the construction, evaluation, and in vivo and in vitro testing of BsAb that target tumor-associated antigens and other disease pathogen and effector cells.

It is not hidden that, lots of poor countries are suffering from various threatening diseases and there is poor disease control management. There is a serious need of production and development of Bispecific antibody based treatment in these poor countries. Cheap antibody based therapy would be a very good alternative to treat these disease where poor people could easily approach these therapy.

All in all Bispecific antibodies production and their use is an important process in novel clinical therapy as it will develop new tools in immune disease diagnosis.

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