Heterohybridoma for the production of non murine monoclonal antibodies

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Abstract

Hybridoma technology described by kohler and Milstein produce only mouse immunoglobulins. Such immunoglobulins have limited use due to its negative side effects such as the recipient's immune response. The production of a non murine monoclonal antibody to combat the problems of murine monoclonal antibody is again difficult due to the lack of a suitable myeloma cell line. Heterohybridoma formed by the fusion of lymphocyte of one species with the myeloma cell of a different species is the solution, which can be used for the production of non murine monoclonal antibodies.

Keywords: heterohybridoma, non-murine, lymphocytes, myeloma cell, monoclonal antibodies

Introduction

Heterohybridoma also called as xenohybridoma is formed by the fusion of lymphocyte of one species with the myeloma cell of a different species. Usually the myeloma cell line is of BALB/C mouse origin. In heterohybridomas the source of lymphocyte is a species other than mouse.

Why heterohybridoma is required?

Classical methods of making hybridomas as the described by Kohler and Milstein produce only mouse immunoglobulins. The application of murine monoclonal antibody is widespread in many areas from biological and medical research to clinical diagnostics. Monoclonal antibodies also have potential in human therapeutic applications (Scott and Welt, 1997; Hudson 1999). However their usefulness is limited due to negative side effects including the recipient's immune response.

Clark (2000) has put forth two major problems in the therapeutic use of murine monoclonal antibodies-

1. Although murine antibody has exquisite specificity for therapeutic targets they did not always trigger the appropriate human effector systems of complement and receptors.
2. Even when murine antibody were identified that did work in vivo the patients immune system normally cut short the therapeutic window as murine antibodies were recognized as foreign by the recipient.

Attempts have been made to produce human monoclonal antibody by the same hybridoma technology but it has been hampered by the lack of a suitable human myeloma cell line (Little et al., 2000). Campbell (1980) has reported that there is no non secreting human myeloma or lymphoblastoid cell line human antibody produced by human-human hybridoma was a mixture diluting the desired antibody. Alternatively human antibody secreting cells can be immortalized by infection with Epstein-Barr virus (EBV). However EBV infected cells are very difficult to clone and usually produce low yields of immunoglobulin (Little et al., 2000). Those successfully transformed cells also tend to show unstable growth (Shay, 1985) and therapeutic use of antibodies derived from EBV infected cells involves safety concerns (Netzer, 1983).

According to Tizard (1998), although it is possible to make intraspecies hybridomas by for example fusing bovine cultured bovine lymphoblastoid cell line, it is easier to make hybridomas by fusing B cells from the species under the study with mouse myeloma cells.

Preparation of heterohybridoma

The success rate in hetrohybridoma production will vary depending on the nature of the immunizing antigen and the immune history of the vaccine recipient. Jessup et al. (2000) has provided a protocol for preparation of human-mouse heterohybridomas against an immunizing antigen. The procedure consists of the following steps:-

1. Sample preparation: Either untransformed or EBV transformed lymphocytes can be used (Thompson et al., 1986). Peripheral blood is collected in heparinized tubes and lymphocytes are harvested by density gradient centrifugation using Ficoll-hypaque.
2. Myeloma cell preparation: The choice of fusion partner is of utmost importance when preparing heterohybridomas. Thompson et al. (1986) found that the mouse myeloma X63-Ag8.653 is a suitable fusion partner with EBV-transformed B cells in the efficient production of human monoclonal antibodies. Myeloma cells from the media usually RPMI1640 is harvested and resuspended in PBS (Phosphate buffered saline).
3. Fusion: Fusion frequencies are known to be greater if the lymphocyte partner has been transformed with EBV (Kozbor et al., 1982) or has been stimulated with pokeweed mitogen (Larrick et al., 1983). Fusion is done by using PEG (Polyethylene glycol). Fusion efficiecy may also be increased by the addition of DMSO (Dimethyl sulfoxide) to the fusion mix. An emerging technique to dramatically increase the efficiency and reproducibility of fusion is electrofusion, where cells are fused by tightly contolled electronic pulses (Foung et al., 1990; Yoshimari et al., 1995; Zimmerman et al., 1990). This technique claims high fusion efficiencies and can be used with fewer starting cells.
4. Tissue culture: The cells are allowed to grow in HAT (Hypoxanthine Aminopterin Thymidine) medium at 37oC with 5% CO2. When medium turns yellow supernatants are tested by ELISA (Enzyme Linked Immuno sorbent Assay) against the antigen used. Positive wells are retested and cloned by limiting dilution or by using the single cell sorting function on an automated cell sorter. The cloning efficiency is less than that of murine hyubridomas by both limiting dilution and automated cell sorting. Heterohybrid cells appear larger and more irregular than murine hybridomas.
5. Scaling up of cultures and purification of antibody: Expand clonal cell populations to a doubled size tissue culture flasks. Once confluence is reached, turn the flasks upright and top up media every 3 days until the flasks are full. Collect the supernatants and purify the antibody by affinity chromatography on a protein-G Superose column.

Problems with heterohybridoma and their solution

1. Mycoplasma infection: Mycoplasma infection can go undetected in murine cell lines with no obvious impact on myeloma proliferation rate or the success of pure murine hybridoma production. Human-mouse heterohybridomas cell lines are less robust and it is imperative that fusion partners are confirmed mycoplasma negative by DNA PCR and or growth in mycoplasma selective media. Myvoplasma growth may be suppressed by standard tissue culture antibiotics so prior to supernatant sampling, cell lines must be grown in antibiotic-free media for three passages and then in continuous culture for 72 hr (Uphoff et al., 1992).
2. Unstable: Unfortunately, these heterohybridomas are unstable and tend to lose the non murine chromosomes as they divide. As a result they may cease immunoglobulin synthesis prematurely. The loss of chromosome is not random. Chromosome 14 and 22 which codes for heavy and lambda chains respectively tend to be retained while chromosome 2 encoding the kappa chain is lost more easily (Croce et al., 1980; Erikson et al., 1981). Improved stability can be achieved by first growing the xenohybridoma cells in the presence of 8-azaguaine to select for aminopterin sensitivity. These xenohybridomas are then used as fusion partners with lymphocytes from immunized animals of the correct species (Tizard, 1998).

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