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Perspective

Monoclonal Antibodies Mechanisms of Action: Hybridoma Technique

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DESCRIPTION

Monoclonal Antibodies (mAbs) are a type of antibodies made by identical B lymphocyte clones in response to a specific antigen. The protein sequence, antigen-binding site area, binding affinity for their targets, and identical downstream functional effects are only a few of the characteristics that monoclonal antibodies possess. These characteristics of mAbs emphasise how their differ from polyclonal antibodies, which have variable activity and identify various antigen epitopes.

When exposed to antigens, differentiated B lymphocytes known as plasma cells create the glycoproteins known as antibodies or immunoglobulin (Ig). The hyper-variable areas of antibodies undergo gene recombination, which results in a diversity of antibody responses to various antigens. Antibodies undergo gene rearrangement during recombination, which enables them to bind a variety of molecules. Antibodies are well-liked molecules with very high efficiency in a variety of therapeutic or diagnostic applications due to their great specificity and diversity. The first generation of monoclonal antibodies created using hybridoma technique was murine mAbs.

Hybridoma technique

A single B lymphocyte clone produces monoclonal antibodies, which bind to the same antigen epitope. Several steps are involved in this method. Mice are first inoculated with particular antigens that have been properly emulsified with an adjuvant. Once enough antibodies have been produced, the animal is usually sacrificed two weeks after the booster dose. Blood is collected to test the adequate level of antibody production using methods like ELISA and flow cytometer. After sacrificing, the spleen is isolated, and tissue digestion using an enzymatic or mechanical process may be used to release B cells. Centrifugation using a density gradient could be used to separate B cells.

The next step is making a fusion between B lymphocytes and myeloma cells (that are immortal like cancer cells). Prior to fusion, myeloma cells should be prepared by culturing with 8 azaguanine, making them sensitive to Hypoxanthine Aminopterin Thymidin (HAT) medium. The fusion process is carried through using Poly Ethylene Glycol (PEG), resulting in cell membrane fusing. Blood is collected to test the adequate level of antibody production using methods like ELISA and flow cytometer. After sacrificing, the spleen is isolated, and tissue digestion using an enzymatic or mechanical method may be used to release B cells. Centrifugation using a density gradient could be used to separate B cells. However, because unfused B cells have a short lifespan, they cannot develop normally. Only hybridomas, or fusing B cell and myeloma cells, may therefore develop in the medium. It should be emphasised that the presence of aminopterin in HAT media also inhibits the "*de novo*" process, another mechanism of nucleic acid synthesis. Therefore, in this selective medium, only the HGPRT-positive cells could be cultivated. Then, the stable clone will be chosen after the antigen-binding capacity of the produced antibodies by various B cell clones has been evaluated using ELISA, antigen microarray assay, Radio Immune Assay (RIA), or immune-dot blot. Liquid nitrogen can be utilized to store the synthesised mAbs and fused hybridomas.

The hybridoma technique has a fairly drawn-out development phase because it takes roughly 6 to 8 months to produce enough mAbs. The antibodies' murine origin, on the other hand, means that upon repeated administration, they may cause the Human Anti Mouse Antibody (HAMA) response in the host, which could hasten mAb clearance and result in unfavourable allergic reactions. The solution to this problem was to create less immunologic chimeric or humanised antibodies by developing antibody engineering techniques. The objective of these designed antibodies was to reduce the HAMA response while maintaining target specificity.

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