• BIOLOGY •

Monoclonal Antibodies

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The antibody is a protein produced by plasma cells to fight pathogens. Though infinitesimal in their sizes, antibodies are able to anchor themselves onto antigens and initiate neutralization and destruction of antigens by altering their chemical structures to disintegrate their complexities. As suggested by the epithet "monoclonal", monoclonal antibodies are produced in large quantities by a plasma cell and used to target a specific antigen.

The process of monoclonal antibody engineering

The process of mAbs (monoclonal antibody) engineering, also known as hybridoma technology, entails the following steps.

Colonizing B plasma cells

Scientists first insert an antigen into an organism, often mice, to induce the production of antibodies against the specific antigen. Witnessing the invasion of a foreigner, the B plasma cell is immediately activated to secretes large quantities of mAbs against the antigen. The activated B plasma cell is then extracted and cultivated artificially to create more of this specific antibody applicable for an unique antigen.

Fusion

Unfortunately, these plasma cells cannot survive on their own and die within three weeks. One of the hallmarks of cancer cells is its ability to grow exponentially while maintaining immortality. The Myeloma cell is a cancerous plasma cell that lives indefinitely, and by fusing myeloma cell with B plasma-cell, the resulting hybridoma is now able to confer the longevity of cancer cells and conjure the ability of plasma cells to create antibodies. During this hybridization process, polyethylene glycol undertakes the role of fusion by disrupting the plasma membrane *(Enzosciences)*.

In results, three hybridomas are created: myeloma cell fused with myeloma cell, B cell fused with B cell and myeloma cell fused with b cell.

HAT medium selection

To select the desired hybridoma, these fused cells undergo the HAT medium selection, a process that utilizes two principal pathways of nucleotides synthesis to incubate the desired hybridoma and eliminate others: the

de novo pathway and the *salvage pathway*. The *de novo* pathway is the synthesis of complex molecules from simpler molecules, such as the biosynthesis of pyrimidines and purine nucleotides from amino acids, carbon dioxide, and other enzymes (*Wikipedia*). *The salvage pathway* is the conversion of existing free nucleotide bases into nucleotides.

The acronym in HAT medium stands for hypoxanthine, aminopterin, and thymidine. Aminopterin blocks the *de novo* pathway. HGPRT is a key enzyme used in the *salvage* pathway, and cells with a non-functional HGPRT gene die in the HAT medium where the other alternative, *de novo* pathway is obstructed by aminopterin. In the three hybridomas above, the fused B-cell and B-cell dies shortly due to their short lifespan outside the host body; fused myeloma cells will die because they are HGPRT negative. Only the hybridoma of B-cell fused with myeloma which has both nucleotide pathways intact survives.

Monoclonal antibodies in research

In cancer immunotherapy research, there are three types of monoclonal antibodies: naked, conjugated, and bispecific.

Naked monoclonal antibodies are most commonly used in cancer therapies. They flow around the body searching for their specific antigen. Once discovered, naked mAbs bind to their specific antigen and call in the body's immune system to initiate a response against the cancerous cell.

Conjugated monoclonal antibodies are attached to a toxin. Patrolling the body, the attached toxin will be used against cancer cell once the antibody finds its particular antigen present on the cancer cell and binds.

Bispecific monoclonal antibodies contain parts from two different antibodies, allowing them to bind to 2 different antigens at the same time. Usually, one antibody binds to the cancer cell and another binds to a cytotoxic immune cell. In this way, the cancer cells and immune cells are congregated to induce the destruction of malignancies.

ADCC

(antibody-dependent cellular cytotoxicity)

ADCC is a process of *cell-mediated immune defense* whereby an immune cell actively lyses a target cell with membrane antigens bounded by specific antibodies. (*Wikipedia*) With antibody markings, the effector cells, which are cells susceptible to signaling and stimulus such as natural killer cells, are recruited to attack the marked cell.

ADCC occurs when the Fc domains of the antibody lock with the Fc receptors present on the surface of immune effector cells (*Steplewski Z, Lubeck MD, Koprowski H Science. 1983 Aug 26; 221(4613):865-7.*). The interaction of activating Fc receptors facilitates the activation of immune effector cells, promoting them to induce phagocytosis and cytolysis. This results in the undertake of antigen processing and presenting via MHC I and II, which are genes called Major histocompatibility complex that code for

surface proteins in assisting the recognition of foreign substances. The process leads to the induction of anti-tumor immunity imposed by tumor-directed cytotoxic cells. (Weiner, L., Dhodapkar, M. and Ferrone, S. (2009))

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