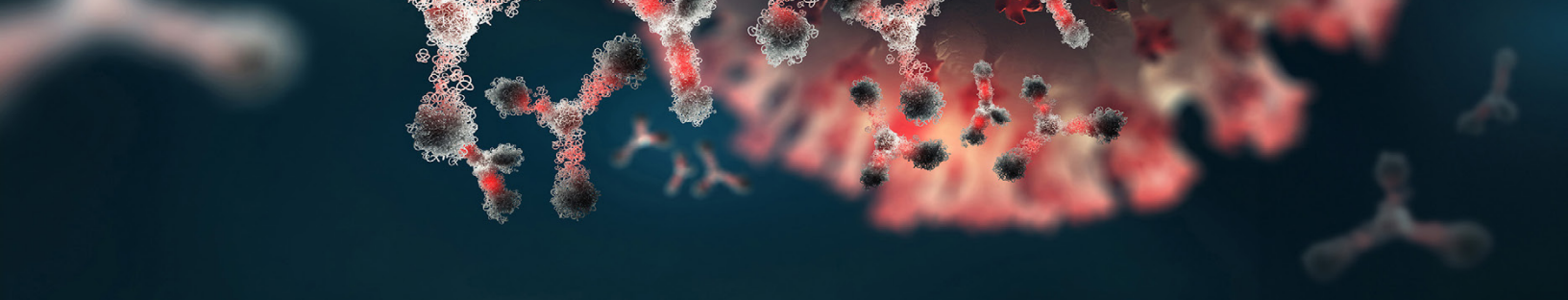


Comparison of Traditional Antibody Generation Methods

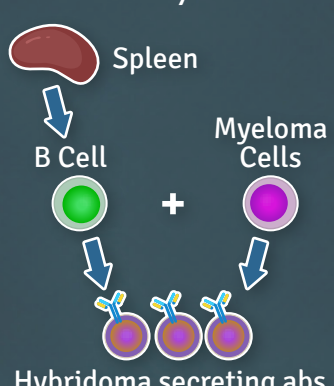
Traditional antibody generation methods, including mouse hybridoma, phage display, transgenic animals, and single B cell sequencing, have been widely used for several decades and have contributed to significant advances in research and medicine. This infographic will delve into these powerful techniques, shedding light on the processes as well as advantages, disadvantages, and applications.

Mouse Hybridoma

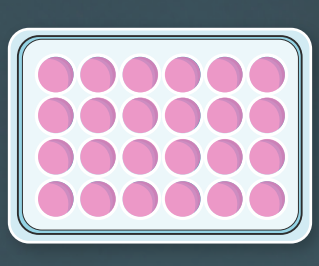
The original antibody generation method, developed in 1975 by Kohler and Milstein, in which immortalized B lymphocytes are used to produce monoclonal antibodies (mAbs).

Mice immunized with antigen

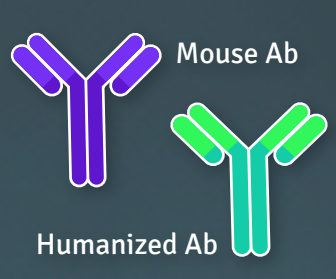
B cells harvested and fused with myeloma cells



Hybridoma clones selected and screened



Chimeric/Humanized versions developed



Advantages

- Technique familiar to many scientists
- An unlimited supply of antibodies
- Highly reproducible and scalable
- Purity of antigen or immunogen is not a prerequisite

Disadvantages

- Time-consuming method, often requiring six to nine months
- Not suitable for producing antibodies against small peptides and fragment antigens
- Some patients treated with these antibodies will develop a rapid human anti-mouse antibody (HAMA) response, which will expedite the clearance of monoclonals, or may cause allergic reactions

Applications

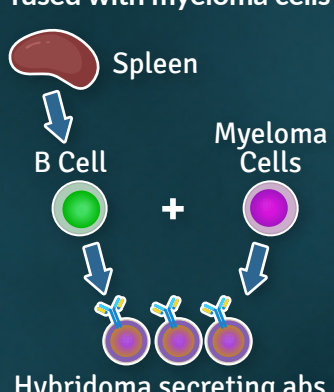
- Many FDA-approved monoclonal antibodies were developed with hybridoma technology including Aducanumab, Risankizumab, and Isatuximab

Transgenic Animals

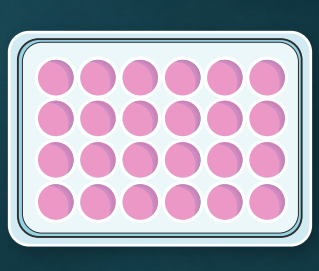
Advancements in gene-editing have made it so that scientists can now introduce human antibody genes into the mouse germline allowing for natural recombination and affinity maturation. Transgenic animals, along with phage display, produced the first human antibodies. The success of this approach accelerated the shift away from chimeric or humanized antibodies.

Mice immunized with antigen

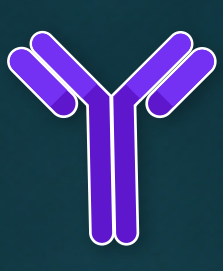
B cells harvested and fused with myeloma cells



Hybridoma clones selected and screened



Humanized versions developed



Advantages

- Unlike the traditional hybridoma method, antibodies do not have to be humanized, can be matured *in vivo*, and an be optimized through clonal selection

Disadvantages

- The mouse genetic background influences antigen processing and B cell regulation, preventing the precise replication of human immune responses

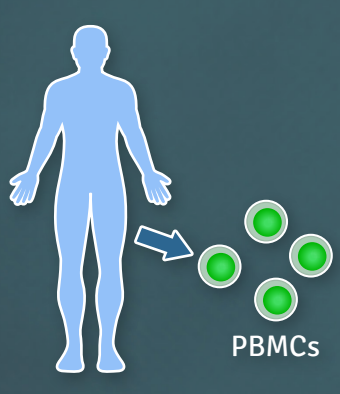
Applications

- The first drug developed by transgenic mice, panitumumab, which blocks EGFR signaling in cancer, was approved by the FDA in 2006

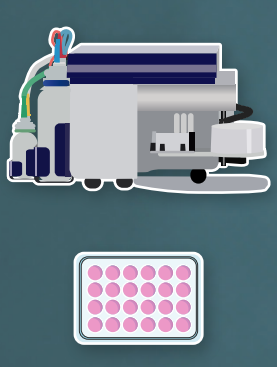
Single B Cell sequencing

An efficient monoclonal generation approach in which V_H and V_L encoding genes are amplified directly from B cells, which may come from any species, including human patients. Unlike phage display, B-cell sorting preserves the native V_H and V_L pairing; these regions are critical for antigen recognition and binding. This pairing helps to develop stable, specific, high affinity antibodies.

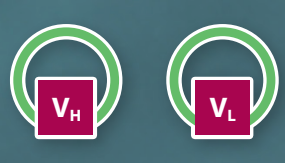
B cells isolated from bone marrow/blood



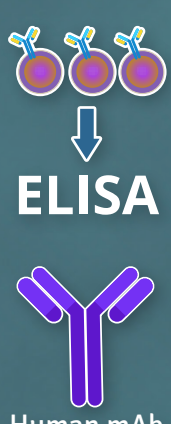
Fluorescence-single antigen specific B cells sorted



V_H & V_L segments cloned from monoclonals



Expression in cells, validation & production



Advantages

- These systems tend to be high-throughput
- Ideal for discovery of rare antibodies to challenging antigen targets
- In addition to maintaining the native pairing of V_H and V_L regions, this method especially allows for the direct screening of fully human antibodies from peripheral blood, which makes this especially useful for antibody-based treatments for infectious diseases

Disadvantages

- Expensive and complex systems, requiring significant expertise

Applications

- The current approved COVID neutralizing antibodies were developed from single B cell cloning

Phage Display

Developed by Nobel laureate Greg Winter, phage display technology involves the fusion of the antibody sequence to a bacteriophage coat protein gene. The antibody-coding DNA sequence is packaged within the phage that expresses the antibody protein on its surface, linking phenotype to genotype. Expansive phage display libraries of antibody fragments are generated; up to 100 billion peptides can be screened as potential high-affinity binding partners for the target antigen.

Advantages

- Known for high efficiency, *in vitro* nature, and rapidity, it can be used for any species, and for naive libraries, animals are not needed
- Used for the discovery, isolation, and modification of antibodies, as well as for antibody maturation
- Huge libraries can be derived from any species including humans and are “naive” or “immunized” depending on whether the model or patient has been immunized or carries a specific disease

Disadvantages

- Diversity and stability of displayed library can be an issue
- Can result in randomly paired V_H and V_L chains, which can lead to the antibody losing binding affinity, or developing self-reactivity

Applications

- Diagnostics and therapeutics for coronavirus diseases
- Immunotherapy
- Regenerative medicine

Antibody generating technology continues to evolve as evidenced by the rapid growth and expansion of monoclonal clinical trials and approved therapeutic antibodies. The development of monoclonal antibodies for disease treatment is becoming more efficient with fewer side effects. When combining newer advancements with artificial intelligence and innovations from other fields such as chimeric antigen receptor T cells (CAR-T) antibody therapies will continue to dominate the drug market while becoming an invaluable resource at the basic science level.

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