Monoclonal Antibodies

Monoclonal Antibodies

- In order to understand **monoclonal** antibodies, we have to think of **polyclonal** first.
- Most antigens offer multiple epitopes and therefore induce proliferation and differentiation of a variety of B-cell clones, each derived from a B-cell that recognises a particular epitope.
- Serum antibodies are heterogenous…a mixture of antobodies for one epitope.

Monoclonal Antibodies

- Such a polyclonal antibody response facilitates the localisation, phagocytosis, and complement mediated lysis of antigen; clear advantages *in vivo*
- Unfortunately, the Ab heterogeneity that increases immune protection *in vivo* often **reduces** the efficacy of an antiserum for various *in vitro* uses.

Monoclonal Antibodies

- For most research, diagnostic and therapeutic purposes, monoclonal antibodies, derived from a single clone and thus specific for a single epitope, are preferable.
- Direct biochemical purification of a monoclonal antibody **from a polyclonal** preparation is not feasible.

Monoclonal Antibodies

- 1975 - Georges Kohler and Cesar Milstein devised a method for preparing monoclonal antibodies…..quickly became one of immunology's key technologies.
- By fusing a normal activated, antibody producing B cell with a myeloma cell (a cancerous plasma cell), they were able to generate a hybrid cell, called a hybridoma, that possessed immortal growth properties of the myeloma cell and secreted the antibody produced by the B-cell.

Monoclonal Antibodies

- Resulting hybridoma clones -
  - Secrete large quantities of monoclonal antibody
  - Can be cultured indefintely
- Nobel prize awarded to Kohler and Milstein

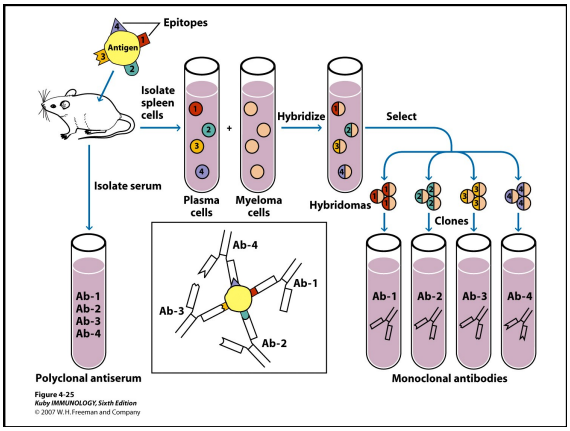

Figure overview

- Polyclonal antiserum produced in response to a complex antigen contains a mixture of monoclonal antibodies, each specific for one of the four epitopes shown (the square).
- In contrast, monoclonal Ab, derived from a single plasma cell, is specific for one epitope on a complex antigen.

Importance of Monoclonal Abs

- Useful as diagnostic, imaging and therapeutic reagents
- Detection - eg. Pregnancy, presence of pathogens, measuring blood levels of various drugs, matching histocompatibility antigens, and detecting antigens shed by certain tumours.

Importance of Monoclonal Abs

- Radiolabelled monoclonal Antibodies can detect and locate tumour antigens, permitting earlier diagnosis of metastatic tumours.

Humanise a monoclonal Ab?

- Recently the availability of monoclonal antibodies and the option to ‘humanise’ them by genetic engineering techniques has given new vigour to this area.
- Early attempts to use mouse monoclonal antibodies to treat humans faced the possibility that there would be a strong reaction against these foreign proteins.
- Human anti-mouse antibody (HAMA) response

Use human?

- Use of hybridoma technology for human mab prep hampered by numerous technical problems, and the generation of hybridomas secreting human antibodies remains a major challenge.
- Use recombinant technology
- Chimeras

Humanise a monoclonal Ab?

- Recent products - made in human systems **or** are genetically engineered to incorporate V regions or CDRs of non human antibodies into C regions and frameworks of human antibodies.
- Chimeric
- Reduce possibility of raising an immune response to them.

Chimeric Antibodies

- One approach to engineering an antibody is to clone recombinant DNA containing the promoter, leader and variable region sequences from a mouse antibody gene and the constant region exons from a human Ab gene.
- Far less immunogenic

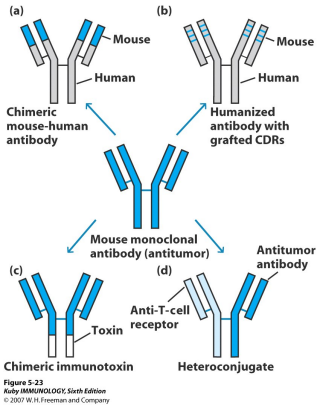

Figure 5-23
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Different ways to generate chimeras

- HAC based - human artifical chromosome including the entire human heavy and light chain loci.
  - Human antibodies and generation of hybridomas possible
- Phage display - bacteriophage
- scFv library - cloned into phages - link scFv gene to gene for phage protein coat - into bacteria - screen - identify V_H and V_L genes for cloning.

mab

- Multi-billion dollar industry.
- Cancer treatment and alleviation of arthritic disorders are two big areas
- Method of making the monoclonal antibodies is critical

Method for making Mabs

- Activated B cells from immunised mice are taken around 3 days after immunisation with the desired antigen and fused (with Polyethylene glycol - PEG) to a mouse plasmacytoma** cell line variant that has lost its rearranged Ig loci and also cannot make the enzyme hypoxanthine-guanine phosphoribosyl transferase (**HGPRT**).
- The fused cells (known as hybridomas) are then selected for their ability to grow in medium in which HGPRT expression is required (called **HAT** medium, composed of Hypoxanthine, Aminopterin & Thymidine)
- **Plasmacytoma - cancerous plasma cells…human cancers of this type are often called multiple myelomas, from the propensity of these cells to grow in the bone marrow.

Method for making Mabs

- The antibiotic aminopterin in HAT medium blocks de novo purine synthesis and therefore makes cell growth dependent on the use of the salvage pathway for nucleoside utilisation, which requires HGPRT.

Method for making Mabs

- Medium is supplemented with Hypoxanthine and thymidine to facilitate the salvage pathway and allow rapid growth of HGPRT-expressing cells in the presence of aminopterin.

Method for making Mabs

- The unfused plasmacytoma cells die - because they cannon maintain purine levels needed for DNA replication.
- The unfused B cells can't grow continuously in culture, but the hybridoma cells have the ability to grow continuously because they have the HGPRT gene from the B cell partner and the continuous growth and plasma cell differentiation state derived from the plasmacytoma parent.

Method for making Mabs

- Hybridomas are screened for production of the desired antibody and then cloned so that a single antibody is produced.
- Fortuitously, activated B cells fuse with myeloma cells much more efficiently that do resting B cells, so hybridomas of the desired specificity are relatively easy to obtain after most immunisations.