Poly clonal Antibodies

The antibodies are produced by B-cells. The B-cells are differentiated in the bone marrow from the lymphoid precursor cells and then stored in the secondary lymphoid organs.

Stimulation of B-cells can occur when foreign bodies enter the body. These foreign bodies will be phagocytized, processed and presented to B-and T-cells by phagocytic cells.Activation of T-cells and B-cells will stimulate B-cells to produce specific antibodies to that foreign body. The antibodies produced by different clones of B-cells. They are polyclonal depending on the number of epitopes present on the antigen (many types of antibodies).

Polyclonal Antibodies:

Are antibodies produced by multi-clones of B-cells. About one billion of B-cells are produced per day. Each B-cell bears 100 million B-cell receptors(BCR). All are specific for one epitope on an antigen.

Some of specific activated B-cells are stored in the secondary lymphoid organs as memory B-cells. The other converted into plasma cells as a final maturation stage containing the antibodies. Each B-cell is specific for single epitope on an antigen.

Polyclonal antibodies arise from many B-cell clone have heterogeneous collection of binding sites on an antigen. Each recognizing different epitope on one antigen.

In vivo: advantage

it has an advantage that they offer multiple ways to attack a pathogen.

*Antigen-antibody interaction is highly specific.

*An antibody can detect one antigen molecule among 100 million antigen molecules.

*It is a great tool of diagnostic immunology to locate and identify antigens. To purify, characterize, quantitate antigens.

In vitro:

It has disadvantages:

1. The desired antibody presents in a low concentration.
2. There is a cross reaction.
3. Reduced specificity and sensitivity.
4. Isolation of a desired antibody is expensive, time consuming, and less efficient.

Production of polyclonal antibodies:

Polyclonal antibodies can be produced experimentally in rabbits and goats. Intravenous injection of antigen three times in two weeks intervals. Then blood is collected and serum isolated. The antibodies are collected and concentrated. The serum contains specific antibodies to that antigen which are polyclonal.

Mono clonal antibodies

In contrast to polyclonal antibodies, monoclonal antibodies are derived from single activated B-cells with single specificity which recognize single specific epitope on an antigen. They are identical antibodies with the same specificity.

The technique was discovered by Koehler and Milstein in 1975.

It is called hybridoma technology.

Production of Monoclonal Antibodies:

Monoclonal antibodies are produced from mouse B-cells.

The first step of production of the monoclonal antibodies is:

1. **Immunization of Mice with the target antigen:**

The immunization of mice with the target antigen. This is done for three weeks with a booster dose every week.Then the spleen of the immunized mice is taken and the lymphocytes are separated by using mechanical and enzymatic disruption then centrifugation by concentration gradient using ficol-isopague solution. The activated lymphocytes are taken

2. **The second Step: Cell fusion:**

   Activated B-cells are fused with myeloma cells.

   -Myeloma cells are cancerous B-cells (plasma cells).

   -Can divide indefinitely in a culture.

   -HGPRT gene is non- functional.

   -Don't produce antibodies.

   The cell fusion is done between activated B-cells with the myeloma cells in the presence of the polyethethylene glycol(PEG).

In this process we will have five types of cells which are:

a. Unfused B-cells.
b. Fused B-cells.
c. Unfused Myeloma cells.
d. Fused Myeloma cells.
e. Hybridoma cells (Hybridomas).

Step 3: **Selection of Hybridomas:**

HAT selection:

H: Hypoxanthine

A: Aminopterine

T: Thymidine

Step 4: Isolation of Hybridomas with single specificity:

The cells will be distributed in multiwall culture plates at very low density, single cell per well.

Step 5: Screening of the products:

The ELISA technique and Radioimmunoassay are used for recognition of the specificity of the monoclonal antibodies produced.

Step 6: Clonning and propagation:

Single cells are cultured in tissue culture plates for production of specific monoclonal antibodies.

Step 7: Characterization and storage of the monoclonal antibodies:

After culturing the produced antibodies are collected and harvested, characterized, and stored in special tube for future distribution and use.