

Antibody and Antigen

Antibody (Immunoglobulin's)

An antibody is an immunoglobulin. It is a Y shaped molecule which is basically a protein that is produced by the B cells of the immune system. Antibodies are produced in response to the exposure to antigens

* Definition:

Glycoprotein in serum and tissue fluid

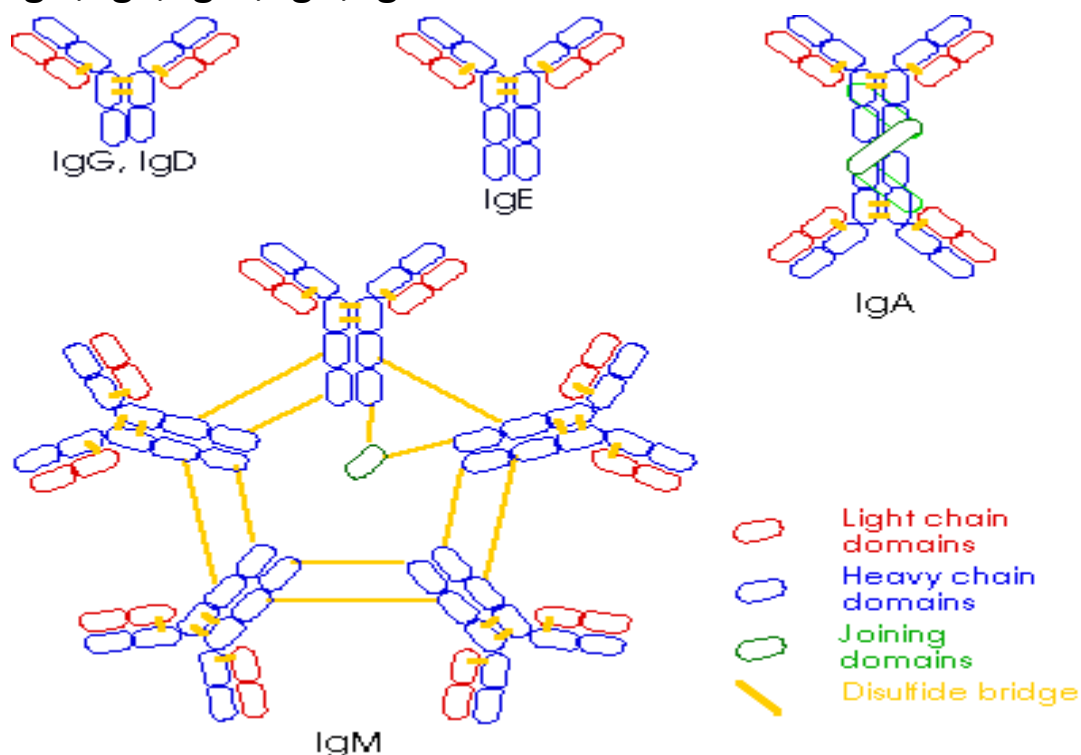
* Produced by:

B-lymphocytes in response to exposure to antigen

* React specifically with antigen

* Five classes of Antibodies:

IgG, IgE, IgM, IgA, IgD



Immunoglobulin's are glycoproteins made up of

a- Two light (L) polypeptide chains

Light chain types are based on differences in the amino acid sequence in the constant region of the light chain. These differences are detected by serological means.

1. Kappa light chains (κ)
2. Lambda light chains (λ)

Antibodies of all isotypes may contain one of two type of L chain: kappa or lambda.

b- Two heavy (H) polypeptide chains- H-chains are distinct for each of the five immunoglobulin's (IgG,IgM,IgE,IgD,IgA).

- The four chains are linked by disulfide bonds
- Terminal portion of L-chain contains part of antigen binding site
- Terminal portion of H-chain participate in antigen binding site
- The other (Carboxyl) terminal portion forms Fc fragment



Antibody Fragments

1-Fab fragment: antigen binding site

2-Fc (crystallizable fragment) effector function

Antibody functions:

1-Opsonization

2-Neutralization of toxin

3-Activation of complement system

Antigen (Immunogenicity)

Foreign molecular enters the body and can react with the immune product's such as antibodies or T. lymphocytes

Antigenicity: The ability of a molecule to be recognized by an antibody or lymphocyte.

Immunogenicity: The ability of a molecule to elicit an immune response.

Haptens :A small molecules that cannot initiate an immune response unless first bound to an immunogenic carrier molecule.(Examples: drugs, hormones with less than 1000 Da are too small to induce immune response Many drugs allergies result from drugs binding to normal proteins and so acting as haptens(Penicillin).)

Epitopes: Antigenic determinants (Part on the antigen that antibodies bonded with like key and lock)

A specific region on the surface of an antigen against which antibodies are formed.

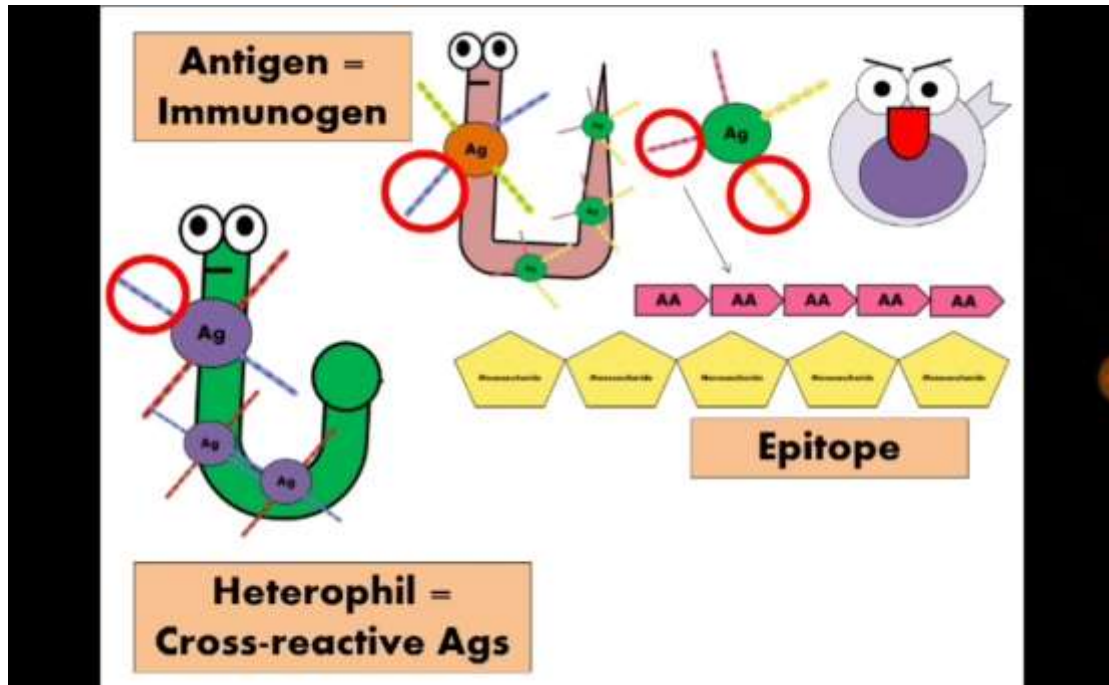
Sometimes two different proteins may share some antigenic determinants. This will lead to what known as cross-reaction: for example

Antiserum against *Proteus vulgaris*

can react with antigenic determinants of

Rickettsia typhi There are at least one antigenic determinant for each 5000 Dalton of Ags and

each antigenic determinant is composed of at least 4-6 aminoacids.



Preservation of Antibodies and Antigens

Preservation of Antibodies

Since antibodies are designed to retain biological activities over periods of many weeks at body temperatures in the 41-42°C range, they are quite thermostable.

Issues facing antibodies preservations are:

- Freezing: All antibodies are labile to one degree or another by freezing conditions, presumably due to the damage incurred during ice crystal formation. Therefore, it is essential that multiple freeze-thaw cycles be avoided at all cost.
- Light: All antibodies (and most proteins) are also labile to light. It is essential, therefore, to keep them in the dark when storing antibodies for long periods of time.
- Contamination: Similarly, all proteins, including antibodies, are labile to degradative enzymes release by bacteria and other microorganisms, especially proteinases. It is essential, therefore, to keep aliquots of antibodies biologically sterile by adding sodium azide (NaN₃) at a final concentration of 0.02-0.05% as an antimicrobial agent to all preparations of antibody in order to discourage the growth of bacteria and fungi. Instead, thimerosal (Merthiolate) at a final concentration of 0.01 % (w/v) may be used.

Methods for antibodies preservations:

1. Aqueous at 4°C:

For short-term (up to 1 year), it is recommended to store at 4°C in the dark. When removing aliquots, it is essential that sterile techniques be followed.

2. 25-50% glycerol or ethylene glycol at -20°C:

For longer-term storage (from 1-5 years), it is recommended diluting the antibodies 1:1 with glycerol, re-filter sterilizing them, and then storing them at -20°C in the dark.

3. frozen at -80°C or in liquid nitrogen:

For very long-term storage (more than 5 years), we recommend storage at -80°C. Although there will be some loss of biological activity accompanying the freezing (typically about 50%), the remaining biological activity will be stable indefinitely.

4. lyophilized:

Freeze-drying (lyophilization) is the method of choice for long term storage stabilization of monoclonal antibodies that are otherwise unstable in liquid. Compared with other methods, lyophilization dries antibodies at a lower temperature, reducing the damage to the products and retaining the molecular integrity. It extends the shelf life of antibodies, reduces the temperature requirement for shipping and preserves their chemical and biological properties. Lyophilized antibodies are stable for 3-5 years without losing activity if stored at -20°C or below.

Preservation of Antigens:

Methods for antigens preservations:

1. refrigerator.
2. formalin 0.2%.
3. phenol 0.5%.
4. or by adding some pigments such as (Rose Bengal, Methylene Blue, and Gentian Violet).
5. Merthiolate (1:10,000).
6. Sodium azide (0.1 %).