Antibodies

Introduction

Antibodies are a class of serum proteins which are induced following contact with antigen. They bind specifically with antigen which induced their formation.

Immunoglobulin (Ig) is a synonym for antibody. Most antibodies are found in the gamma globulin fraction of serum.

Antibody molecules are **structurally heterogeneous** although they are all built up from **units** which share basic **four polypeptide chain structure**.

Basic structure

The basic immunoglobulin unit consists of **two identical heavy chains** and **two light chains**, held together by a chemical link (**disulphide bonds**). Light chains are named by the Greek letters kappa (K) and lambda **(λ),** and the heavy chains by gamma **(γ),** alpha **(α)**, mu **(μ**), delta **(δ),** and epsilon **(ε)**.

The various immunoglobulin **classes** (**IgG, IgA, IgM, IgD, and IgE**) are distinguished by their heavy chains.

Only one type of light chain is found in any individual molecule. These heavy and light chains can be divided into **domains** on the bases of sequence similarity, Figure 11. Light chains have two domains and heavy chains have four or five.

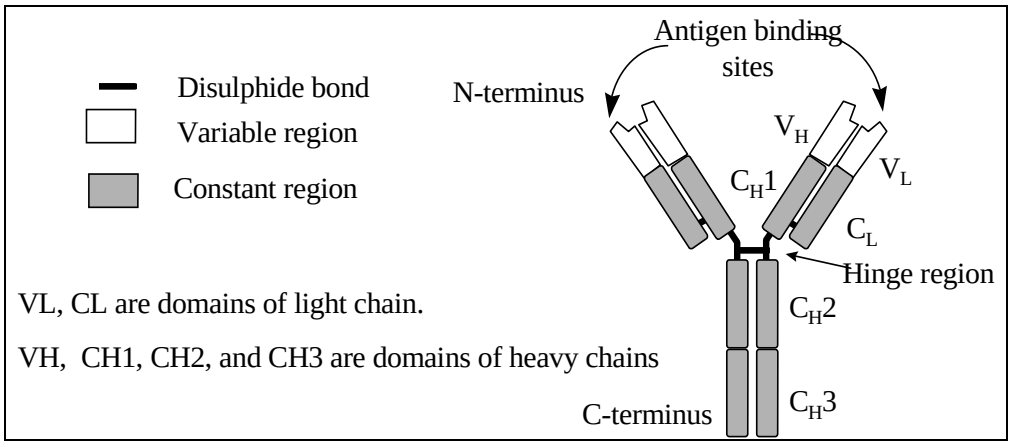

Figure 11: The basic structure of immunoglobulin divided into domains.

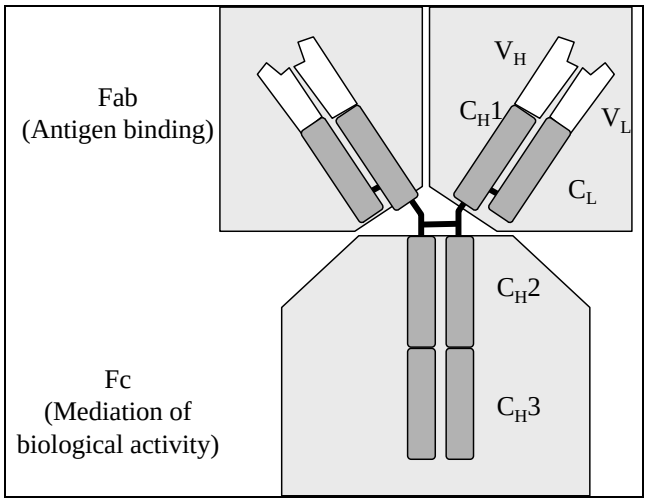

Figure 12: Antibody fragments mediating antigen binding and biological activities.

Papain, a protein-digesting enzyme, splits the antibody molecule into three large pieces. Two of the pieces are identical and contain the antibody binding site. These have been termed the **antibody binding fragment of Fab fragment**. The Fab portions contain what is known as the **'variable' regions** of the molecule in which the amino acid sequence is very different from molecule to molecule. It is this variable region that provides the specific for the binding of one particular antibody to an antigen.

The third fragment plays no part in combining with antigen but has many other important functions; for example, the structure of this site determines whether the antibody will cross the placenta or not. When the molecule is chemically split, this fragment can be obtained in a crystalline form; therefore, it is **termed the Fc fragment**.

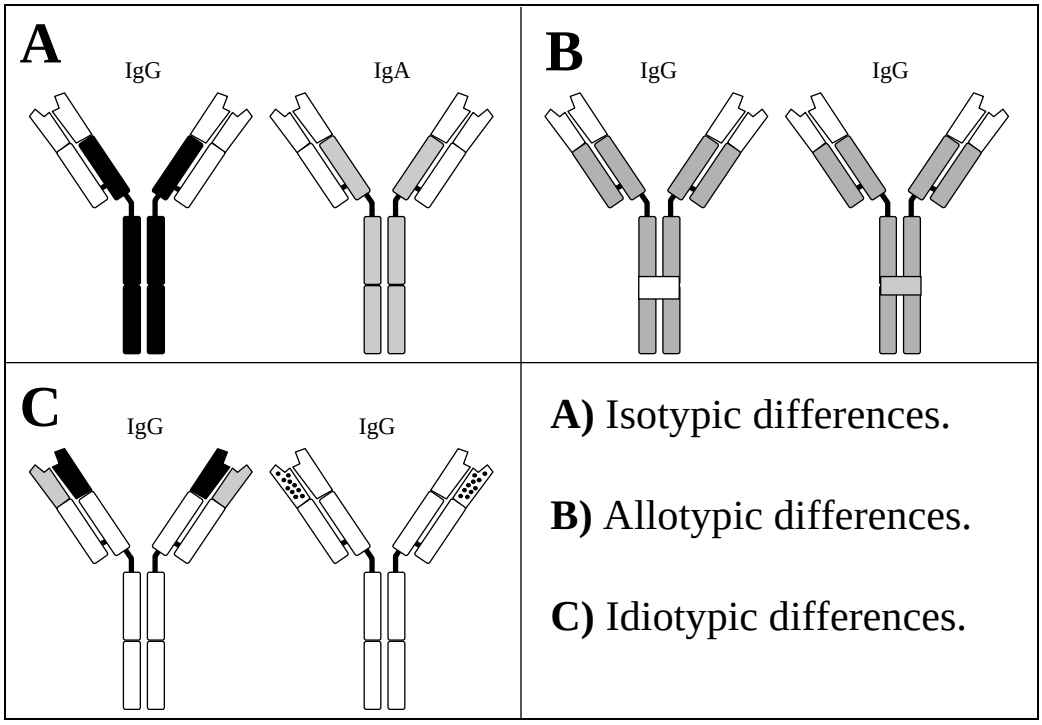

Figure 13: Structural variations of antibody

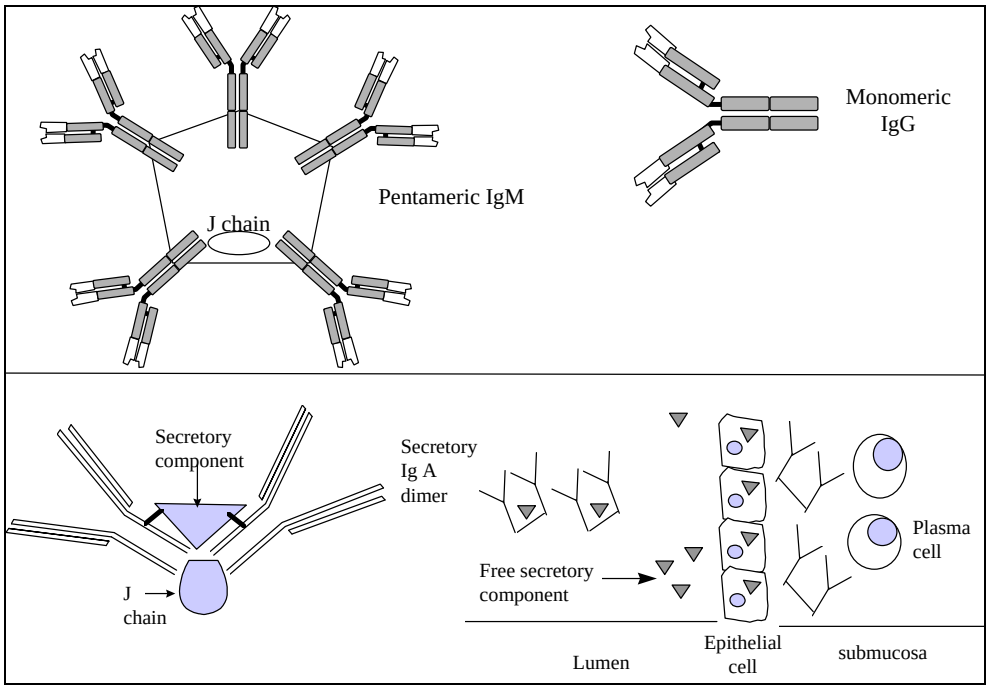

Figure 14: The Structure of IgG as compared to IgM, and secretory IgA

Structural variations

The **genes for antibodies** are present at three gene loci on separate chromosomes. These are κ, λ and heavy chain genes.

Differences between constant region genes are called **isotypes (classes),** these between two alleles of the same constant genes are called **allotypes** and amino acid changes specific to particular variable gene are called **idiotypes**.

The structural variations of antibody is illustrated in Figure 13 and the differences between IgG, IgM, and IgA structures are shown in Figure 14.

Biological functions of IgG, IgA and IgM

The five classes of Ig have distinct chemical structure and a specific biological role. Table 6 summarizes the features of each one of them.

	Isotype				
	IgG	**IgM**	**IgA**	**IgE**	**IgD**
Molecular weight	150,000	900,000	160,000	200,000	180,000
Approximate concentration in serum (mg/ml)	12	1	1.8	0.00002	0-0.04
Percent of total Ig	80	6	13	0.002	0.2
Distribution	Equal: intra and extravascular	Mostly intravascular	intravascular and secretions	On basophils and mast cells present in saliva and nasal secretions	present on lymphocyte surface
Additional protein subunits	-	J	J, S	-	-
Half-life (days)	23	5	5.5	2.0	2.8
Placental passage	++	-	-	-	-
Presence in secretion	-	-	++	-	-
Presence in milk	+	0 to trace	+	-	-
Activation of complement	+	+++	-	-	-
Binding to Fc receptors on macrophages, polymorphonuclear cells, and NK [a] cells	++	-	-	-	-
Relative agglutinating capacity	+	+++	++	-	-
Antiviral activity	+++	+	+++	-	-
Antibacterial activity (gram-negative)	+++	+++ with complement	with lysozyme	-	-
Antitoxin activity	+++	-	-	-	-
“Allergic activity”	-	-	-	++	-

Table 6 : The most Important Features of Immunoglobulin Isotypes.

Agglutination

IgG molecules can cause the agglutination of particulate (insoluble) antigens such as microorganisms. These formed antigen-antibody complexes are easily phagocytosed and destroyed by phagocytic cells, Figure 15.

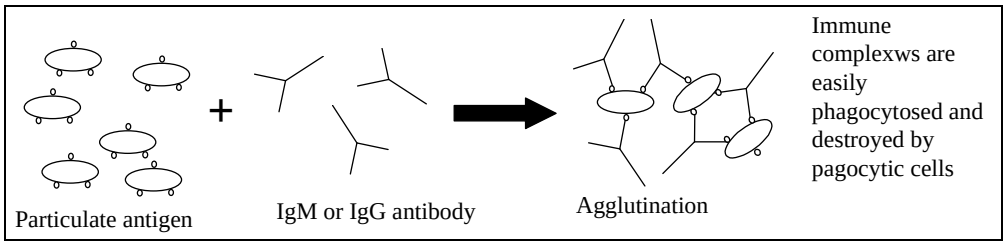

Figure 15: IgG and IgM can eliminate particulate antigen by agglutination.

IgM molecules are efficient agglutinating antibodies. Because of their pentameric form, IgM antibodies can form macro-molecular bridges between epitopes on molecules that may be too distant from each other to be bridged by the smaller IgG antibodies.

Furthermore, because of their pentameric form and multiple valences, the IgM antibodies are particularly well suited to combine with antigens that contain repeated patterns of the same antigenic determinant, as in the case of polysaccharide antigens or cellular antigens, which are multiply expressed on cell surfaces, Figure 15.

The IgM antibodies include the so-called natural **isohemagglutinins** - the naturally occurring antibodies against the red blood cell antigens of the ABO blood groups. These antibodies are presumed to arise as a result of immunization by bacteria in the gastrointestinal and respiratory tracts, which bear determinants similar to the oligosaccharides of the ABO blood groups.

Secretory IgA is also an efficient agglutinating antibody. In contrast serum IgA has no known biological function. Most IgA is present not in the serum, but in secretions such as tears, saliva, colostrum, sweat and mucus, where it serves an important biological function such as being part of the mucosa-associated lymphoid tissue (MALT). The IgA found in secretions (i.e., **secretory IgA**) is always present in dimeric form. Its protective effect is thought to be due to its ability to prevent the invading organism from attaching to and penetrating the epithelial surface.

Passage through the placenta

The IgG is the only class of immunoglobulin that can pass through the placenta, enabling the mother to transfer her immunity to the fetus.

Opsonization

IgG is an opsonizing antibody (from the Greek opsonin, which means to prepare for eating). It reacts with epitopes on microorganisms via its Fab portions; but it is the Fc portion that confers the opsonizing property, since any phagocytic cells, including macrophages and polymorphonuclear phagocytes, bear receptors for the Fc portion of the IgG molecule, Figure 16.

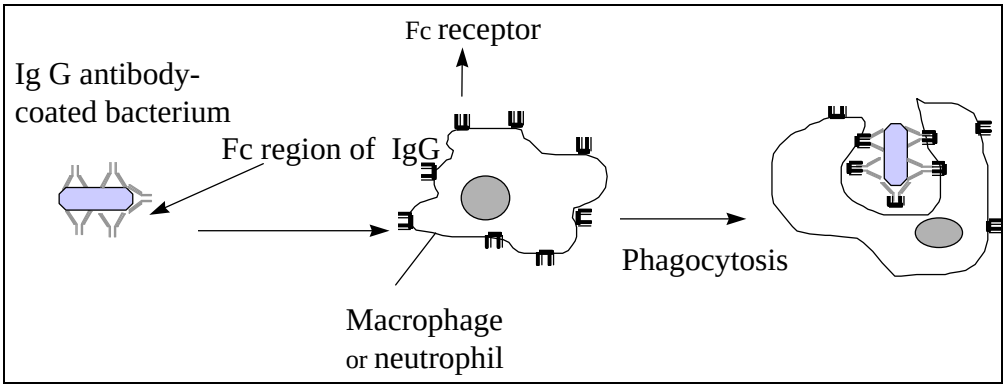

Figure 16: IgG and IgM can opsonize bacteria and facilitate immune adherence and phagocytosis.

These cells adhere to the antibody-coated bacteria by virtue of their receptors for Fc. The net effect is a zipper-like closure of the surface membrane of the phagocytic cells around the organism, leading to the final engulfing and destruction of the microorganism.

Antibody dependent, cell mediated cytotoxicity

The **IgG** molecule plays an important role in antibody-dependent, cell mediated cytotoxicity (ADCC). The Fab portion binds with the target cell, whether it is a microorganism or a tumor cell, and the Fc portion binds with specific receptors, for Fc, that are found on certain large granular lymphocytic cells called **natural killer cells or NK cells**. By this mechanism, the IgG molecule "focuses the killer cells on their target.

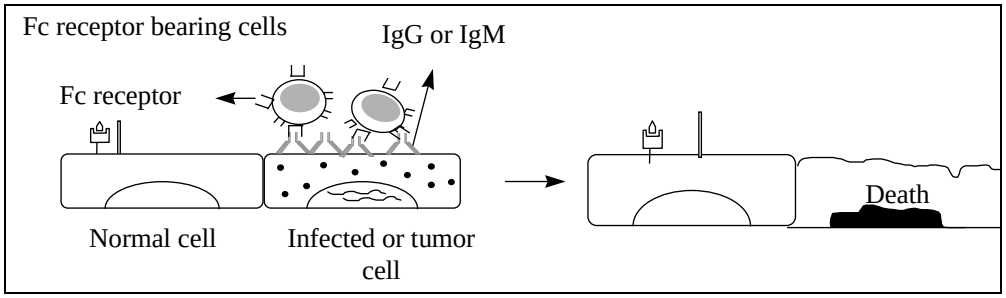

Figure 17: Antibody Dependent Cell mediated Cytotoxicity (ADCC).

Neutralization of toxin

The **IgG** molecule is an excellent antibody for the neutralization of toxins such as tetanus and botulinus, or for the inactivation of, for example, snake and scorpion venom. Because of its ability to neutralize such poisons (mostly by **blocking their active sites**) and because of its long half-life, compared to that of other isotopes, the IgG molecule is the isotype of choice for passive immunization (i.e., the transfer of antibodies) against toxins and venom, Figure 18.

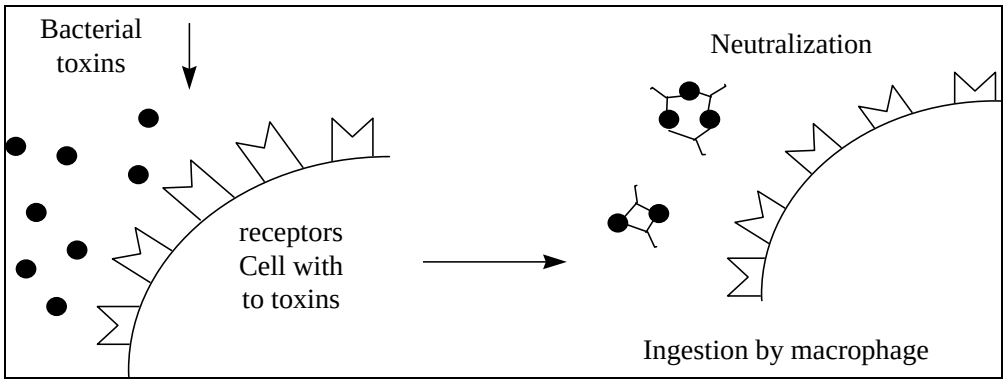

Figure 18: The IgG antibody neutralizes toxins.

Because of its presence in secretions, such as saliva, urine and gastric fluid, **secretary IgA** is of great importance in the primary immunologic defense against local infections in such areas as the respiratory or gastrointestinal tract. For example, in the case of cholera, the pathogenic Vibrio organism attaches to, but never penetrates beyond, the cells that line the gastrointestinal tract, where it secretes an exotoxin responsible for all symptoms. **IgA antibody, which can prevent attachment of the organism to the cells**, provides protection from the pathogen.

Immobilization of bacteria

IgG molecules are efficient in immobilizing various motile bacteria. Reaction of antibodies specific for the **flagella and cilia** of certain microorganisms causes them to clump, thereby arresting their movement and preventing their ability to spread or invade tissue.

Neutralization of viruses

The **IgG** antibody is an efficient virus neutralizing antibody. One mechanism of neutralization is that in which the antibody binds with antigenic determinants on various portions of the virus coat, among which is the region that used by the virus for attachment to the target cell. Inhibition of viral attachment effectively

arrests infection, Fig (4.9). **Secretory IgA** is an efficient antiviral antibody, preventing the viruses from entering host cells.

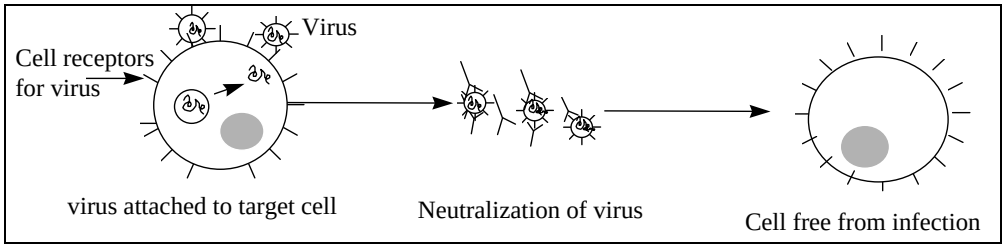

Figure 19: IgG and Ig A can neutralize virus and prevent its attachment to target cells.

Biological functions of IgD and IgE.

IgD is present on the surface of B lymphocytes during certain stages of maturation. It has been suggested to be involved in the **maturation** of these cells.

IgE also termed reagenic antibody, has a half-life in serum of 2 days, the shortest half-life of all classes of immunoglobulins. Another distinction of IgE antibodies is that they are present in serum at the lowest concentrations of all immunoglobulins. These low levels are due in part to a low rate of synthesis and in part, to the unique ability of the Fc portion of IgE to bind with very high affinity to mast cells and basophils.

Both mast cells and basophils have specific receptors for this region, and thus they effectively remove the IgE from the circulation.

IgE, is of paramount importance in **hypersensitivity reactions**. It also appears to be of importance in protection against **parasitic infections**.

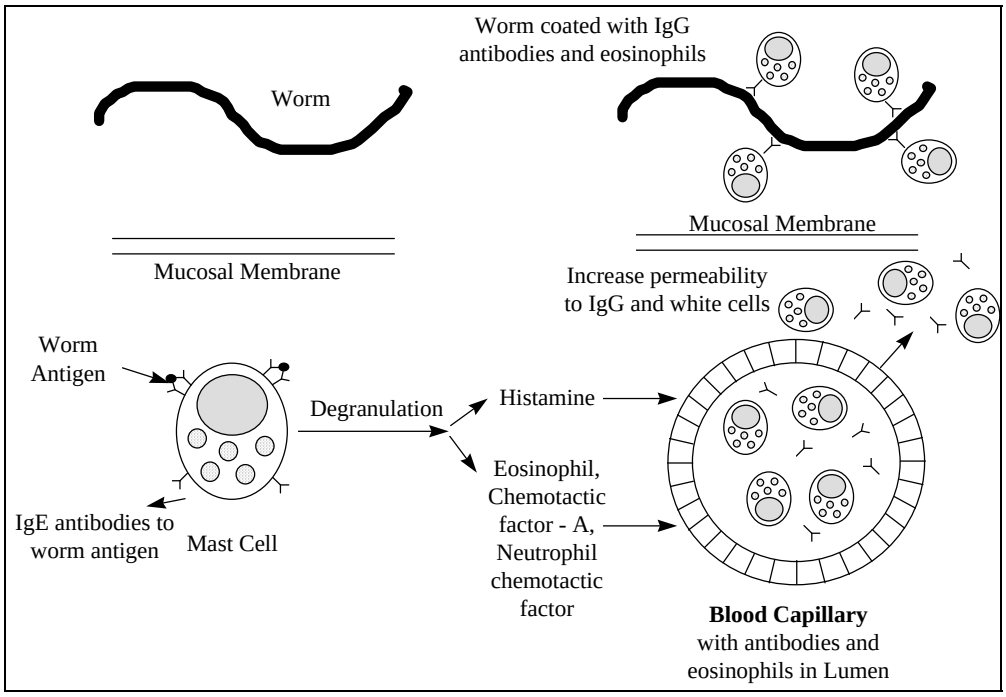

Figure 20: IgE is important in protection against parasitic infection.

Kinetics of antibody response following immunization

Following **first immunization**, the **primary response** consists mainly of the production of IgM antibodies. The second exposure to the same antigen results in **a secondary or anamnestic (memory)** response, which is much quicker than the primary response and in which the response shifts from IgM production to the synthesis of IgG and other isotypes. The secondary response lasts much longer than the primary response, Figure 21.

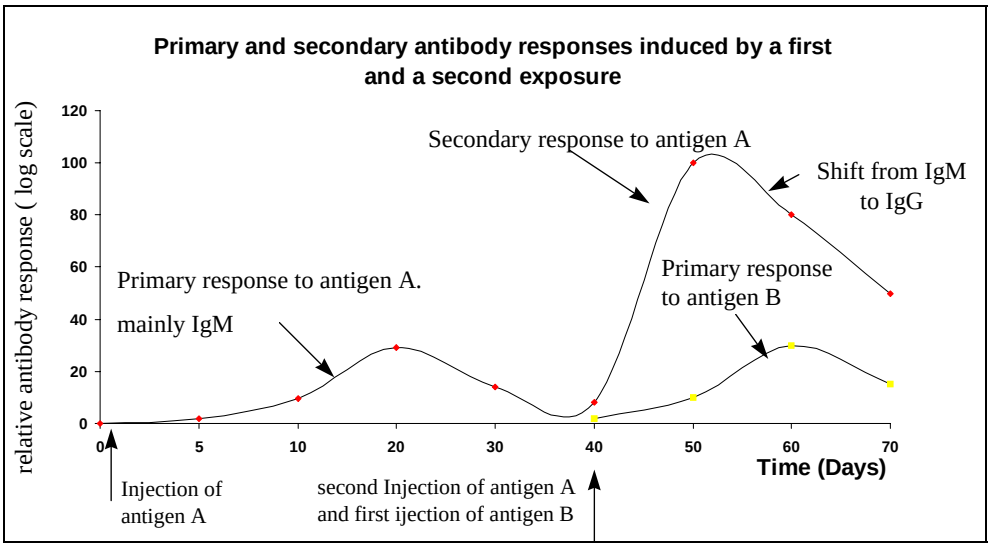

Figure 21: Kinetics of antibody response following immunization.

The secondary immune response is characterized by

- There is a **shift in class response**, with IgG antibodies appearing at higher concentrations and with greater persistence, than IgM. This may also be accompanied by the appearance of IgA and IgE.
- A maturation of the response occurs, such that the average affinity (binding constant) of the antibodies for the antigen increases as the secondary response develops.

The capacity to make secondary or anamnestic (memory) response may persist for a long time (years in humans), and it provides selective advantage for an individual who survives the first contact with an invading pathogen. Establishment of this memory for generating a specific response is of course, the purpose of public health immunization programs.