IMMUNO TECHNOLOGY

LECTURE 03: ANTIGENS, ANTIBODIES

Antigens and antigenicity: Types, structure and requirements for immunogenecity. haptens, adjuvants, cross reactivity. Immunoglobulin: structure, function and biological properties of Ig classes – organization and expression of immunoglobulin genes – Generation of antibody : effector functions of antibodies.

Antigens and antigenicity: Types, structure and requirements for immunogenecity

Substances that can be recognized by the immunoglobulin receptor of B cells, or by the Tcell receptor when complexed with MHC, are called **antigens**.

Immunogenicity Versus Antigenicity

Immunogenicity and antigenicity are related but distinct immunologic properties that sometimes are confused. **Immunogenicity** is the ability to induce a humoral and/or cellmediated immune response:

B cells + antigen ⟶ effector B cells + memory B cells

↓

(plasma cells)

T cells + antigen ⟶ effector T cells + memory T cells

↓

(e.g., CTLs, T_Hs)

Although a substance that induces a specific immune response is usually called an antigen, it is more appropriately called an **immunogen. Antigenicity** is the ability to combine specifically with the final products of the above responses (i.e., antibodies and/or cell-surface receptors). Although all molecules that have the property of immunogenicity also have the property of antigenicity, the reverse is not true. Some small molecules, called *haptens,* are antigenic but incapable, by themselves, of inducing a specific immune response. In other words, they lack immunogenicity.

Immunogenicity is not an intrinsic property of an antigen but rather depends on a number of properties of the particular biological system that the antigen encounters.

The Nature of the Immunogen Contributes to Immunogenicity

Immunogenicity is determined, in part, by four properties of the immunogen: its foreignness, molecular size, chemical composition and complexity, and ability to be processed and presented with an MHC molecule on the surface of an antigen- presenting cell or altered self-cell.

Foreignness

In order to elicit an immune response, a molecule must be recognized as nonself by the biological system. The capacity to recognize nonself is accompanied by tolerance of self, a specific unresponsiveness to self antigens. Much of the ability to tolerate self antigens arises during lymphocyte development, during which immature lymphocytes are exposed to self-components. Antigens that have not been exposed to immature lymphocytes during this critical period may be later recognized as nonself, or foreign, by the immune system. When an antigen is introduced into an organism, the degree of its immunogenicity depends on the degree of its foreignness.

IMMUNO TECHNOLOGY

Generally, the greater the phylogenetic distance between two species, the greater the structural (and therefore the antigenic) disparity between them. For example, the common experimental antigen bovine serum albumin (BSA) is not immunogenic when injected into a cow but is strongly immunogenic when injected into a rabbit. Moreover, BSA would be expected to exhibit greater immunogenicity in a chicken than in a goat, which is more closely related to bovines. There are some exceptions to this rule. Some macromolecules (e.g., collagen and cytochrome *c*) have been highly conserved throughout evolution and therefore display very little immunogenicity across diverse species lines. Conversely, some self-components (e.g., corneal tissue and sperm) are effectively sequestered from the immune system, so that if these tissues are injected even into the animal from which they originated, they will function as immunogens.

Molecular Size

There is a correlation between the size of a macromolecule and its immunogenicity. The most active immunogens tend to have a molecular mass of 100,000 daltons (Da). Generally, substances with a molecular mass less than 5000–10,000 Da are poor immunogens, although a few substances with a molecular mass less than 1000 Da have proven to be immunogenic.

Chemical Composition and Heterogeneity

Size and foreignness are not, by themselves, sufficient to make a molecule immunogenic; other properties are needed as well. For example, synthetic homopolymers (polymerscomposed of a single amino acid or sugar) tend to lack immunogenicity regardless of their size. Studies have shown that copolymers composed of different amino acids or sugars are usually more immunogenic than homopolymers of their constituents. These studies show that chemical complexity contributes to immunogenicity. In this regard it is notable that all four levels of protein organization—primary, secondary, tertiary, and quaternary—contribute to the structural complexity of a protein and hence affect its immunogenicity (Figure 3-1).

Lipids as Antigens

Appropriately presented lipoidal antigens can induce B- and T-cell responses. For the stimulation of B-cell responses, lipids are used as haptens and attached to suitable carrier molecules such as the proteins keyhole limpet hemocyanin (KLH) or bovine serum albumin (BSA).By immunizing with these lipid-protein conjugates it is possible to obtain antibodies that are highly specific for the target lipids. Using this approach, antibodies have been raised against a wide variety of lipid molecules including steroids, complex fatty-acid derivatives, and fat-soluble vitamins such as vitamin E. Such antibodies are of considerable practical importance since many clinical assays for the presence and amounts of medically important lipids are antibody-based. For example, a determination of the levels of a complex group of lipids known as leukotrienes can be useful in evaluating asthma patients. Prednisone, an immunosuppressive steroid, is often given as part of the effort to prevent the rejection of a trans-planted organ.

Susceptibility to antigen processing and presentation

The development of both humoral and cell-mediated immune responses requires interaction of T cells with antigen that has been processed and presented together with MHC molecules. Large, insoluble macromolecules generally are more immunogenic than small, soluble ones because the larger molecules

are more readily phagocytosed and processed. Macromolecules that cannot be degraded and presented with MHC molecules are poor immunogens. This can be illustrated with polymers of D-amino acids, which are stereoisomers of the naturally occurring L-amino acids. Because the degradative enzymes within antigen-presenting cells can degrade only proteins containing L-amino acids, polymers of D-amino acids cannot be processed and thus are poor immunogens.

Genotype of the recipient animal

The genetic constitution **(genotype)** of an immunized animal influences the type of immune response the animal manifests, as well as the degree of the response. For example, Hugh McDevitt showed that two different inbred strains of mice responded very differently to a synthetic polypeptide immunogen. After exposure to the immunogen, one strain produced high levels of serum antibody, whereas the other strain produced low levels. When the two strains were crossed, the F1 generation showed an intermediate response to the immunogen. By backcross analysis, the gene controlling immune responsiveness was mapped to a subregion of the major histocompatibility complex (MHC). Numerous experiments with simple defined immunogens have demonstrated genetic control of immune responsiveness, largely confined to genes within the MHC. These data indicate that MHC gene products, which function to present processed antigen to T cells, play a central role in determining the degree to which an animal responds to an immunogen. The response of an animal to an antigen is also influenced by the genes that encode B-cell and T-cell receptors and by genes that encode various proteins involved in immune regulatory mechanisms. Genetic variability in all of these genes affects the immunogenicity of a given macromolecule in different animals.

Immunogen dosage and route of administration

Each experimental immunogen exhibits a particular dose-response curve, which is determined by measuring the immune response to different doses and different administration routes. An antibody response is measured by determining the level of antibody present in the serum of immunized animals. Evaluating T-cell responses is less simple but may be determined by evaluating the increase in the number of T cells bearing TCRs that recognize the immunogen. Some combination of optimal dosage and route of administration will induce a peak immune response in a given animal.

An insufficient dose will not stimulate an immune response either because it fails to activate enough lymphocytes or because, in some cases, certain ranges of low doses can induce a state of immunologic unresponsiveness, or tolerance. The phenomenon of tolerance is discussed in chapters 10 and 21.

Conversely, an excessively high dose can also induce tolerance. The immune response of mice to the purified pneumococcal capsular polysaccharide illustrates the importance of dose. A 0.5 mg dose of antigen fails to induce an immune response in mice, whereas a thousand-fold lower dose of the same antigen (5 _ 10_4 mg) induces a humoral antibody response. A single dose of most experimental immunogens will not induce a strong response; rather, repeated administration over a period of weeks is usually required. Such repeated administrations, or **boosters,** increase the clonal proliferation of antigen-specific T cells or B cells and thus increase the lymphocyte populations specific for the immunogen.

Experimental immunogens are generally administered parenterally (*para,* around; *enteric,* gut)—that is, by routes other than the digestive tract. The following administration routes are common:

→ Intravenous (iv): into a vein
→ Intradermal (id): into the skin
→ Subcutaneous (sc): beneath the skin
→ Intramuscular (im): into a muscle
→ Intraperitoneal (ip): into the peritoneal cavity

The administration route strongly influences which immune organs and cell populations will be involved in the response. Antigen administered intravenously is carried first to the spleen, whereas antigen administered subcutaneously moves first to local lymph nodes. Differences in the lymphoid cells that populate these organs may be reflected in the subsequent immune response.

Adjuvants

Adjuvants (from Latin *adjuvare,* to help) are substances that, when mixed with an antigen and injected with it, enhance the immunogenicity of that antigen. Adjuvants are often used to boost the immune response when an antigen has low immunogenicity or when only small amounts of an antigen are available. For example, the antibody response of mice to immunization with BSA can be increased fivefold or more if the BSA is administered with an adjuvant. Precisely how adjuvants augment the immune response is not entirely known, but they appear to exert one or more of the following effects:

→ Antigen persistence is prolonged.
→ Co-stimulatory signals are enhanced.
→ Local inflammation is increased.
→ The nonspecific proliferation of lymphocytes is stimulated.

Aluminum potassium sulfate (alum) prolongs the persistence of antigen. When an antigen is mixed with alum, the salt precipitates the antigen. Injection of this alum precipitate results in a slower release of antigen from the injection site, so that the effective time of exposure to the antigen increases from a few days without adjuvant to several weeks with the adjuvant. The alum precipitate also increases the size of the antigen, thus increasing the likelihood of phagocytosis.

Water-in-oil adjuvants also prolong the persistence of antigen. A preparation known as **Freund's incomplete adjuvant** contains antigen in aqueous solution, mineral oil, and an emulsifying agent such as mannide monooleate, which disperses the oil into small droplets surrounding the antigen; the antigen is then released very slowly from the site of injection. This preparation is based on **Freund's complete adjuvant,** the first deliberately formulated highly effective adjuvant, developed by Jules Freund many years ago and containing heat-killed *Mycobacteria* as an additional ingredient. Muramyl dipeptide, a component of the mycobacterial cell wall, activates macrophages, making Freund's complete adjuvant far more potent than the incomplete form. Activated macrophages are more phagocytic than unactivated macrophages and express higher levels of class II MHC molecules and the membrane molecules of the B7 family. The increased expression of class II MHC increases the ability of the antigen-presenting cell to present antigen to TH cells. B7 molecules on the antigen presenting cell bind to CD28, a cell-surface protein on TH cells, triggering co-stimulation, an enhancement of the T-cell immune response. Thus, antigen presentation and the requisite co-stimulatory signal usually are increased in the presence of adjuvant.

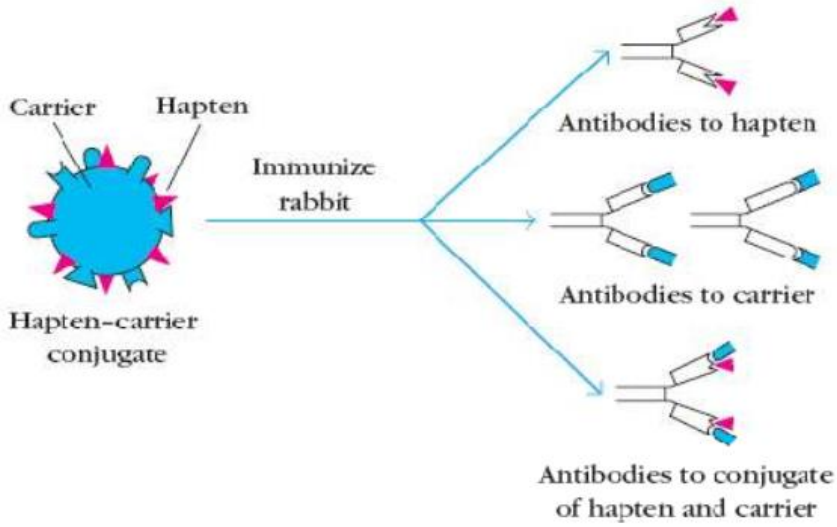
Alum and Freund's adjuvants also stimulate a local, chronic inflammatory response that attracts both phagocytes and lymphocytes. This infiltration of cells at the site of the adjuvant injection often results in formation of a dense, macrophage-rich mass of cells called a **granuloma.** Because the macrophages in a granuloma are activated, this mechanism also enhances the activation of TH cells.

Other adjuvants (e.g., synthetic polyribonucleotides and bacterial lipopolysaccharides) stimulate the nonspecific proliferation of lymphocytes and thus increase the likelihood of antigen-induced clonal selection of lymphocytes.

Haptens and the Study of Antigenicity

The pioneering work of Karl Landsteiner in the 1920s and 1930s created a simple, chemically defined system for studying the binding of an individual antibody to a unique epitope on a complex protein antigen. Landsteiner employed various **haptens,** small organic molecules that are antigenic but not immunogenic. Chemical coupling of a hapten to a large protein, called a **carrier,** yields an immunogenic **hapten-carrier conjugate.**

Animals immunized with such a conjugate produce antibodies specific for
(1) the hapten determinant,
(2) unaltered epitopes on the carrier protein, and
(3) new epitopes formed by combined parts of both the hapten and carrier (see Figure).



By itself, a hapten cannot function as an immunogenic epitope. But when multiple molecules of a single hapten are coupled to a carrier protein (or nonimmunogenic homopolymer), the hapten becomes accessible to the immune system and can function as an immunogen.

The beauty of the hapten-carrier system is that it provides immunologists with a chemically defined determinant that can be subtly modified by chemical means to determine the effect of various chemical structures on immune specificity. In his studies, Landsteiner immunized rabbits with a haptencarrier conjugate and then tested the reactivity of the rabbit's immune sera with that hapten and with closely related haptens coupled to a different carrier protein. He was thus able to measure, specifically, the reaction of the antihapten antibodies in the immune serum and not that of antibodies to

the original carrier epitopes. Landsteiner tested whether an antihapten antibody could bind to other haptens having a slightly different chemical structure. If a reaction occurred, it was called a **cross-reaction.** By observing which hapten modifications prevented or permitted cross-reactions, Landsteiner was able to gain insight into the specificity of antigenantibody interactions.

Many biologically important substances, including drugs, peptide hormones, and steroid hormones, can function as haptens. Conjugates of these haptens with large protein carriers can be used to produce hapten-specific antibodies. These antibodies are useful for measuring the presence of various substances in the body. For instance, the original home pregnancy test kit employed antihapten antibodies to determine whether a woman's urine contained human chorionic gonadotropin (HCG), which is a sign of pregnancy. However, the formation of drug-protein conjugates in the body can produce drug allergies that may be life-threatening.

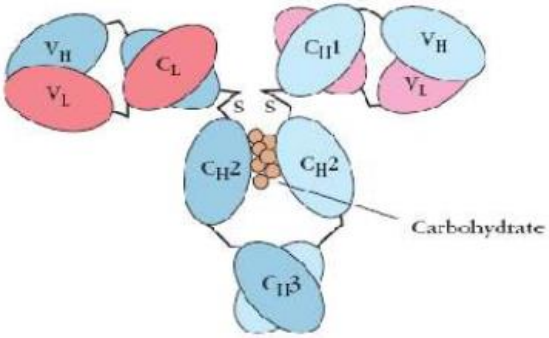
IMMUNO TECHNOLOGY

GENERAL STRUCTURE OF IMMUNOGLOBULINS

Immunoglubulins are immunologically active serum proteins formed in response to an antigen.

Evidence:

Tiselius and E.A.Kabat (1939) found that antibodies were present in serum protein fractions.



Source:

The immunoglobulins are generally found in

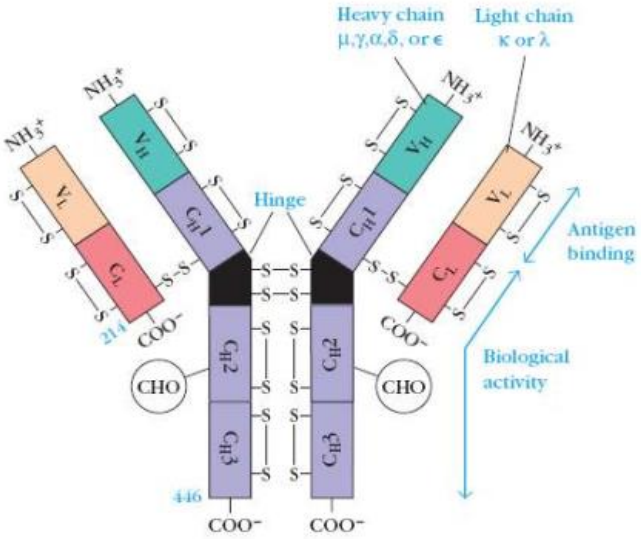
- Serum,
- Body fluids and
- Tissues.

Shape of immunoglobulin molecule:

Immuunoglubulins appear mostly in Y-shape and sometimes as T-shape.

General structure of immunoglobulin molecule:

Rodney porter (1950s) and Gerald Edelman (1960s) proposed the basic structure of the immunoglobulin molecule.



Light chains and heavy chains:

An immunoglobulin molecule is composed of four polypeptide chains.

1. Two identical short chains

These chains are called Light chains or L-chains. The molecular weight of each light chain is 25,000 and

2. Two identical long chains

These chains are called Heavy chains or H-chains. The molecular weight of each heavy chain is 50,000

Amino and carboxyl terminal ends:

One end of each chain is called amino terminal end or N-terminal end. The other end of the chain is called carboxyl terminal end or C-terminal end.

Disulfide linkages:

1. Intra-chain disulfide bonds:

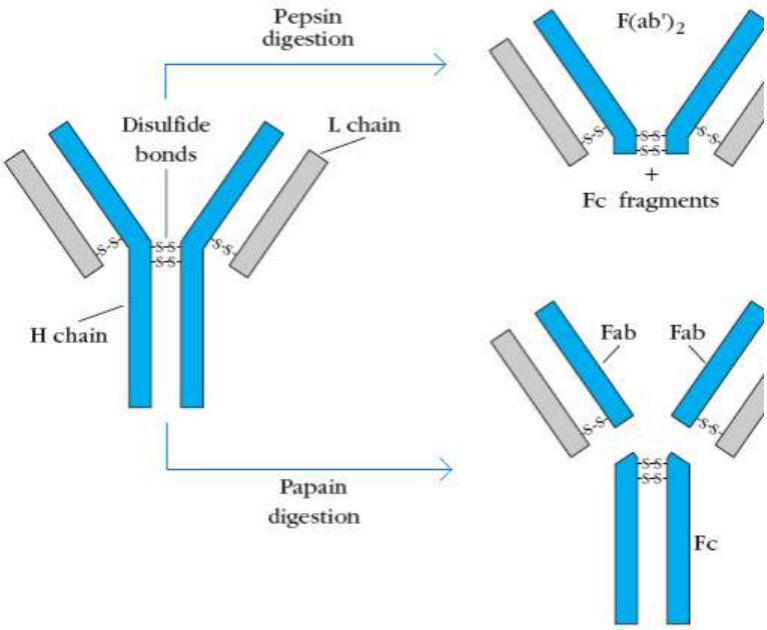
Each L-chain has two Intra-chain disulfide bonds and each H-chain has four intra-chain disulfide bonds. Totally, four exist in two light chains and eight exist in two heavy chains.

2. Inter-chain disulfide bonds:

IMMUNO TECHNOLOGY

Each light chain is linked to a heavy chain by a single inter-chain disulfide bond. The two heavy chains are linked by two inter-chain disulfide bonds. The number of inter-chain disulfide bonds may vary in the hinge region according to the class of the immunoglobulin molecule.

On the whole, a single immunoglobulin molecule possesses twelve intra-chain disulfide bonds and four inter-chain disulfide bonds.



Fragments in immunoglobulin:

Porter had digested the serum fraction containing the immunoglobulin with the papain enzyme. He obtained two identical fragments and non-identical fragment. He named the identical fragments as Fab fragments (**F**ragment **a**ntigen **b**inding) because the fragments had exhibited antigen binding activity.

Regions of immunoglobulin:

The immunoglubulins is composed of two regions namely,

1. A Variable region or V-Region – consists of,
    a. Hypervariable regions or HV regions and
    b. Framework regions or FR regions
2. A Constant region or C-Region – consists of various domains

Variable regions: (V-Region / V-Domains)

The first 100-110 aminoacids of the amino terminal region of a L-chain or a H-chain varies greatly among antibodies of different specificity. These segments of highly variable sequence are called V-Regions or Variable regions or V-domains.

V_L-Regions:

The V-Region present in the light chain is called V_L regions or variable light chain regions.

V_H-Regions:

The V-Region present in the heavy chain is called V_H regions or variable heavy chain regions.

Hypervariable regions or HV regions:

The aminoacid sequences of V_L and V_H regions contain certain highly variable zones called Hypervariable regions or HV regions.

Framework regions or FR regions:

Apart from the HV regions, the remaining regions in the V_L and V_H domains exhibit far-less variation. These stretches of sequence are called Framework regions (FRs).

IMMUNO TECHNOLOGY

Constant regions: (C-Region / C-Domains)

Apart from the variable region, the remaining part (carboxyl terminal half of the immunoglobulin molecule) of the immunoglobulin is called the constant regions because the regions are not exhibiting any variation in the aminoacid sequence. But, there exist five different aminoacid sequence patterns in the constant regions of heavy chain, which determines the classes of immunoglobulins.

Classes of immunoglobulins:

Based on the aminoacid sequence in the heavy chain region, the immunoglubulins are classified into five classes as,

- Immunoglobulin G or IgG – possess gamma (γ) heavy chain
- Immunoglobulin M or IgM – possess mu (μ) heavy chain
- Immunoglobulin A or IgA – possess alpha (α) heavy chain
- Immunoglobulin E or IgE – possess epsilon (ε) heavy chain
- Immunoglobulin D or IgD – possess delta (δ) heavy chain

Each of these five different heavy chains is known as **Isotype**. Each isotype is encoded by a separate constant region genes and all the members of a species carry the same constant region genes. Within a species, each normal individual will express all isotypes in the serum.

The constant regions had two basic aminoacid sequences. In other words, the length of the aminoacid sequence in the constant region varies among the classes of immunoglobulins.

Example: The heavy chains of immunoglobulins such as IgG, IgA and IgD are composed of 330 aminoacids whereas the heavy chains of IgM and IgE are made up of 440 aminoacids.

Due to these variations in the length of the heavy chains, the light chains were recognized into two types as,

- Kappa (κ) light chain and
- Lambda (λ) light chain

Example: In humans, the light chain is 60% of kappa type and 40% of lambda type whereas in the case of mice 95% of kappa type and 5% of lambda type exist.

A single antibody molecule contains only one light chain types, either κ light chains or λ light chains, but both never exist.

Subclasses of heavy chains and light chains:

Minor differences in the aminoacid sequence of the α and δ heavy chains led to further classification of the heavy chains into sub-classes.

Example: Two subclasses of α heavy chains (α_1 and α_2) and four subclasses of δ heavy chains (δ_1, δ_2, δ_3 and δ_4) exist in the humans whereas the mice possess four subclasses of δ heavy chains (δ_1, δ_{2a}, δ_{2b} and δ_3).

The presence of different single aminoacids at 2 or 3 positions in the aminoacid sequence of λ light chains led to further classification of the light chain into subclasses.

Example: Four subclasses of λ light chains (λ_1, λ_2, λ_3 and λ_4) exist in the humans whereas the mice possess three subclasses of λ light chains (λ_1, λ_2 and λ_3).

Domains in the constant regions:

IMMUNO TECHNOLOGY

The constant regions of immunoglobulins such as IgG, IgA and IgD contain C_H1, Hinge, C_H2 and C_H3 domains whereas the constant regions of IgM and IgE has C_H1, C_H2 (Hinge-like domain), C_H3 and C_H4 domains.

Functions of C_H1 and C_L domains:

They help to hold the V_L and V_H domains together by virtue of the inter chain disulfide bonds between them. They also extend the Fab arms of the antibody molecule, thereby facilitating their interaction with the antigen.

Hinge region:

The heavy chains of γ, α and δ contain an extended peptide sequence between the C_H1 and C_H2 domains that has no homology with the other domains. This region is called the hinge region. There are two prominent aminoacids that are present richly in the hinge region. They are,

- Proline residues and
- Cysteine residues.

Proline residues:

The hinge region is highly rich in proline residues and is flexible giving IgG, IgA and IgD segmental flexibility. As a result, two Fab arms can assume various angles to each other **when antigen is bound.** This region can be cleaved with either pepsin or papain.

Cysteine residues:

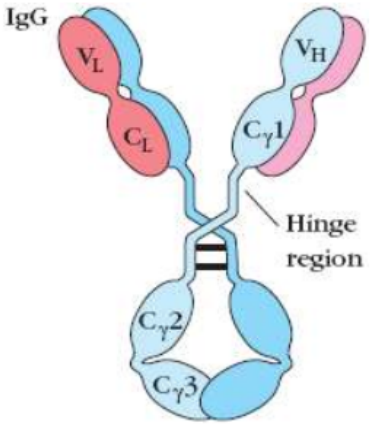
The cysteine residues in the hinge region form inter chain disulfide bonds that hold the two heavy chains together. The number of inter chain disulfide bonds in the hinge region varies considerably among different classes of immunoglobulins and between species.

Hinge-like domains:

The heavy chains of μ and ε lack a hinge region. They had an additional domain (C_H2 domain) of 110 aminoacids that has hinge-like features. The function of this extra domain has not yet been determined.

Effects of carbohydrate attachment to the constant region:

The two C_H2 domains of IgG, IgA and IgD and the two C_H3 domains of IgM and IgE are separated by oligosaccharide side chains. As a result, these two globular domains are much more accessible than the others to the aqueous environment. This accessibility is one of the elements that contributed to the biological activity of these domains in the activation of the complement attachment. It also affects the rate at which Igs are cleared from the serum by the liver. It probably increases the solubility of immunoglobulins.



Functions of Immunoglobulins

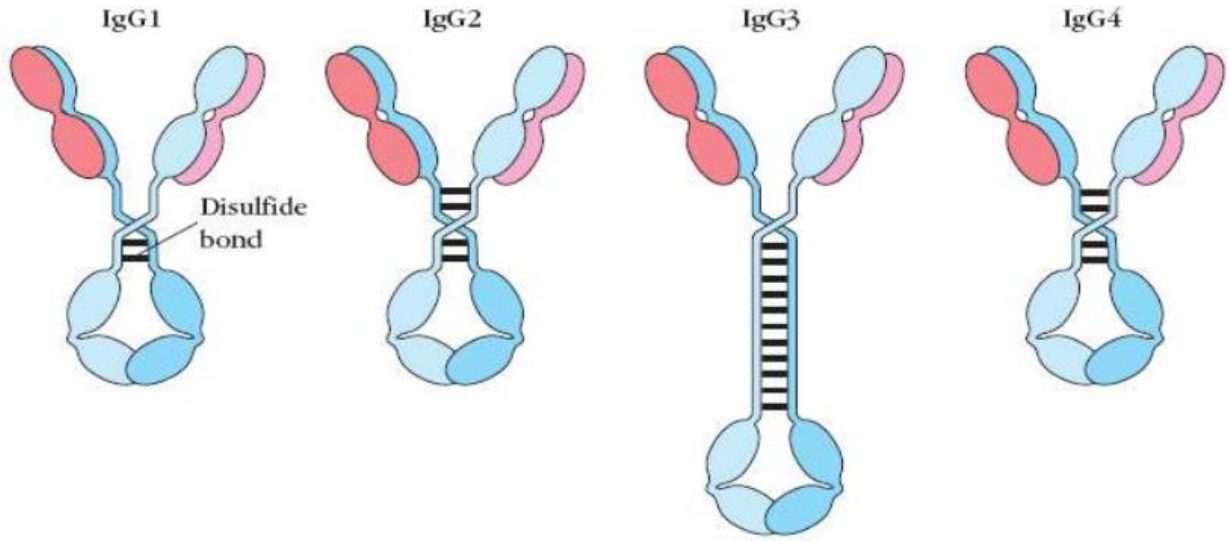
IgG

IgG, the most abundant class in serum, constitutes about 80% of the total serum immunoglobulin. The IgG molecule consists of two γ

heavy chains and two λ or κ two light chains. There are four human IgG subclasses, distinguished by differences in γ-chain sequence and numbered according to their decreasing average serum concentrations: IgG1, IgG2, IgG3, and IgG4 (see Table).

The amino acid sequences that distinguish the four IgG subclasses are encoded by different germ-line CH genes, whose DNA sequences are 90%–95% homologous. The structural characteristics that distinguish these subclasses from one another are the size of the hinge region and the number and position of the interchain disulfide bonds between the heavy chains. The subtle amino acid differences between subclasses of IgG affect the biological activity of the molecule:

- IgG1, IgG3, and IgG4 readily cross the placenta and play an important role in protecting the developing fetus.
- IgG3 is the most effective complement activator, followed by IgG1; IgG2 is less efficient, and IgG4 is not able to activate complement at all.
- IgG1 and IgG3 bind with high affinity to Fc receptors on phagocytic cells and thus mediate opsonization. IgG4 has an intermediate affinity for Fc receptors, and IgG2 has an extremely low affinity.



IgM

IgM accounts for 5%–10% of the total serum immunoglobulin, with an average serum concentration of 1.5 mg/ml. Monomeric IgM, with a molecular weight of 180,000, is expressed as membrane-bound antibody on B cells.

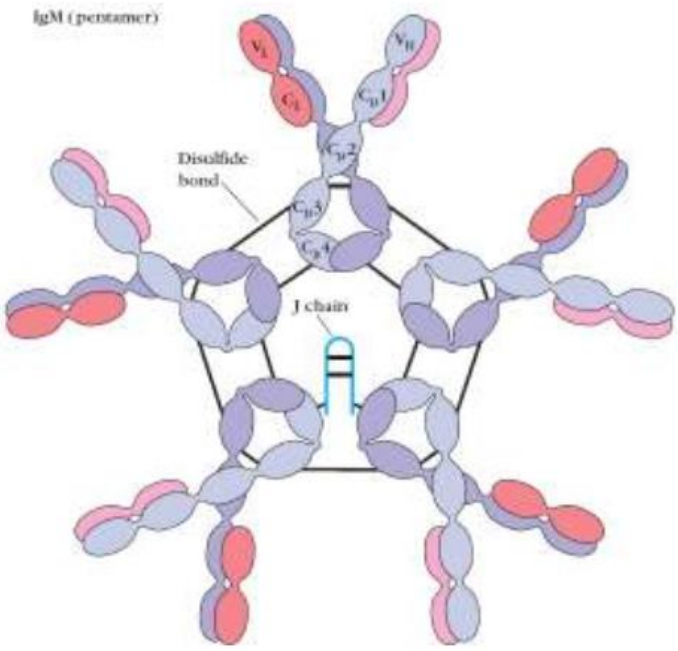
IgM is secreted by plasma cells as a pentamer in which five monomer units are held together by disulfide bonds that link their carboxyl- terminal heavy chain domains ($C_\mu4/C_\mu4$) and their $C_\mu3/C_\mu3$ domains (see Figure). The five monomer subunits are arranged with their Fc regions in the center of the pentamer and the ten antigen-binding sites on the periphery of the molecule.

Each pentamer contains an additional Fc-linked polypeptide called the **J (joining) chain,** which is disulfide-bonded to the carboxyl-terminal cysteine residue of two of the ten μ chains. The J chain appears to be required for polymerization of the monomers to form pentameric IgM; it is added just before secretion of the pentamer.

IgM is the first immunoglobulin class produced in a primary response to an antigen, and it is also the first immunoglobulin to be synthesized by the neonate. Because of its pentameric structure with 10 antigen-binding sites, serum IgM has a higher valency than the other isotypes.

IMMUNO TECHNOLOGY

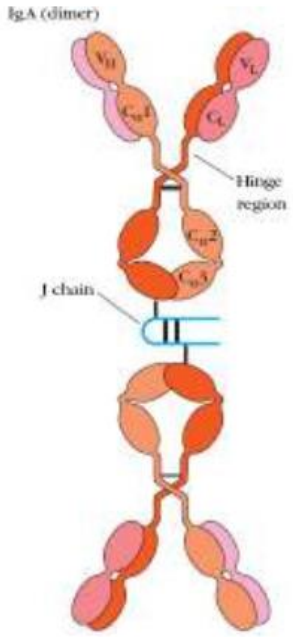
An IgM molecule can bind 10 small hapten molecules; however, because of steric hindrance, only 5 or fewer molecules of larger antigens can be bound simultaneously. Because of its high valency, pentameric IgM is more efficient than other isotypes in binding antigens with many repeating epitopes such as viral particles and red blood cells (RBCs). For example, when RBCs are incubated with specific antibody, they clump together into large aggregates in a process called agglutination. It takes 100 to 1000 times more molecules of IgG than of IgM to achieve the same level of agglutination. A similar phenomenon occurs with viral particles: less IgM than IgG is required to neutralize viral infectivity.



IgM is also more efficient than IgG at activating complement. Complement activation requires two Fc regions in close proximity, and the pentameric structure of a single molecule of IgM fulfills this requirement. Because of its large size, IgM does not diffuse well and therefore is found in very low concentrations in the intercellular tissue fluids. The presence of the J chain allows IgM to bind to receptors on secretory cells, which transport it across epithelial linings to enter the external secretions that bathe mucosal surfaces. Although IgA is the major isotype found in these secretions, IgM plays an important accessory role as a secretory immunoglobulin.

IgA

Although IgA constitutes only 10%–15% of the total immunoglobulin in serum, it is the predominant immunoglobulin class in external secretions such as breast milk, saliva, tears, and mucus of the bronchial, genitourinary, and digestive tracts.



In serum, IgA exists primarily as a monomer, but polymeric forms (dimers, trimers, and some tetramers) are sometimes seen, all containing a J-chain polypeptide. The IgA of external secretions, called **secretory IgA,** consists of a dimer or tetramer, a J-chain polypeptide, and a polypeptide chain called **secretory Component.**

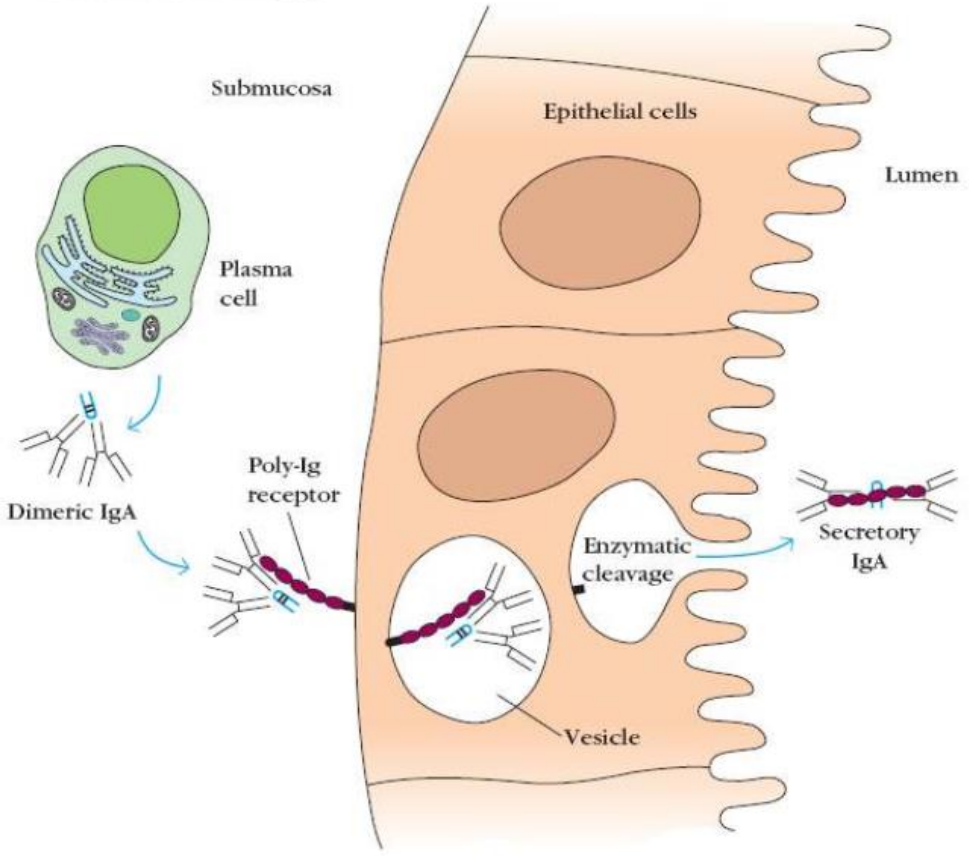
The J-chain polypeptide in IgA is identical to that found in pentameric IgM and serves a similar function in facilitating the polymerization of both serum IgA and secretory IgA. The secretory component is a 70,000-MW polypeptide produced by epithelial cells of mucous membranes. It consists of five immunoglobulin-like domains that bind to the Fc region domains of the IgA dimer. This interaction is stabilized by a disulfide bond between the fifth domain of the secretory component and one of the chains of the dimeric IgA.

IMMUNO TECHNOLOGY

Transcytosis – A mechanism by which dimeric IgA is formed

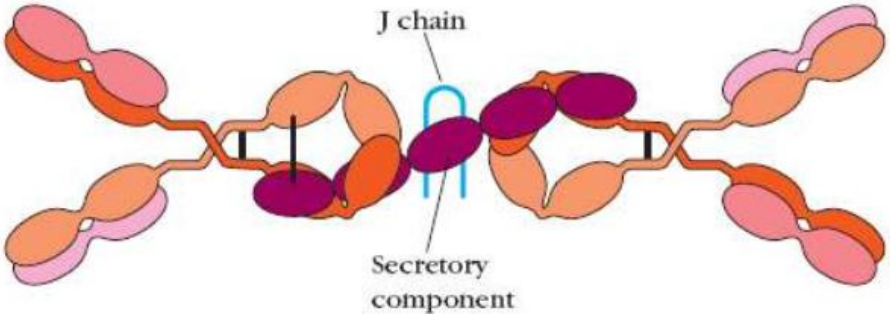
The plasma cells that produce IgA preferentially migrate to subepithelial tissue, where the secreted IgA binds tightly to a receptor for polymeric immunoglobulin molecules (see Figure).

Formation of secretory IgA



This **poly-Ig receptor** is expressed on the basolateral surface of most mucosal epithelia (e.g., the lining of the digestive, respiratory, and genital tracts) and on glandular epithelia in the mammary, salivary, and lacrimal glands. After polymeric IgA binds to the poly-Ig receptor, the receptor-IgA complex is transported across the epithelial barrier to the lumen. Transport of the receptor-IgA complex involves receptor-mediated endocytosis into coated pits and directed transport of the vesicle across the epithelial cell to the luminal membrane, where the vesicle fuses with the plasma membrane. The poly-Ig receptor is then cleaved enzymatically from the membrane and becomes the secretory component, which is bound to and released together with polymeric IgA into the mucous secretions.

Structure of secretory IgA



The secretory component masks sites susceptible to protease cleavage in the hinge region of secretory IgA, allowing the polymeric molecule to exist longer in the protease-rich mucosal environment than would be possible otherwise. Pentameric IgM is also transported into mucous secretions by this mechanism, although it accounts for a much lower percentage of antibody in the mucous secretions than does IgA. The poly-Ig receptor interacts with the J chain of both polymeric IgA and IgM antibodies.

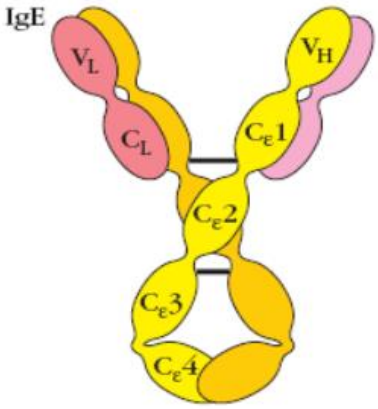
Production of IgA

The **daily production** of **secretory IgA** is **greater than** that of any other immunoglobulin class. IgA-secreting plasma cells are concentrated along mucous membrane surfaces. Along the jejunum of the small intestine, for example, there are more than **2.5×10^{10} IgA-secreting plasma cells**—a number that surpasses the total plasma cell population of the bone marrow, lymph, and spleen combined! Every day, a human secretes from **5 g** to **15 g** of **secretory IgA** into **mucous secretions**.

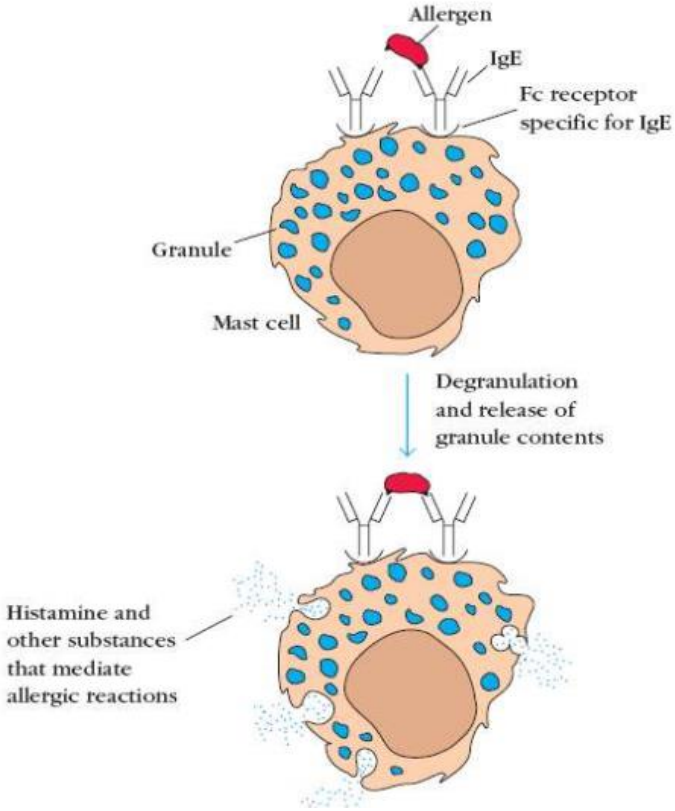
Effector functions of IgA

Secretory IgA serves an important effector function at mucous membrane surfaces, which are the main entry sites for most pathogenic organisms. Because it is polymeric, secretory IgA can cross-link large antigens with multiple epitopes. Binding of secretory IgA to bacterial and viral surface antigens prevents attachment of the pathogens to the mucosal cells, thus inhibiting viral infection and bacterial colonization. Complexes of secretory IgA and antigen are easily entrapped in mucus and then eliminated by the ciliated epithelial cells of the respiratory tract or by peristalsis of the gut. Secretory IgA has been shown to provide an important line of defense against bacteria such as ***Salmonella, Vibrio cholerae,*** and ***Neisseria gonorrhoeae*** and viruses such as **polio, influenza,** and **reovirus.** Breast milk contains secretory IgA and many other molecules that help protect the newborn against infection during the first month of life.

IgE

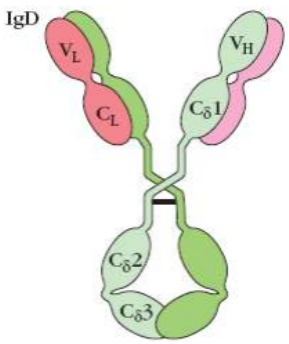


The potent biological activity of IgE allowed it to be identified in serum despite its extremely low average serum concentration (0.3 µg/ml) IgE binds to Fc receptors on the membranes of blood basophils and tissue mast cells. Cross-linkage of receptor bound IgE molecules by antigen (allergen) induces basophils and



mast cells to translocate their granules to the plasma membrane and release their contents to the extracellular environment, a process known as degranulation. As a result, varieties of pharmacologically active mediators are released and give rise to allergic manifestations (see Figure). Localized mast-cell degranulation induced by IgE also may release mediators that facilitate a buildup of various cells necessary for antiparasitic defense.

IgE antibodies mediate the immediate hypersensitivity reactions that are responsible for the symptoms of hay fever, asthma, hives, and anaphylactic shock.



IgD

IgD, has a serum concentration of 30 µg/ml and constitutes about 0.2% of the total immunoglobulin in serum. IgD, together with IgM, is the major membranebound immunoglobulin expressed by mature B cells, and its role in the physiology of B cells is under investigation.No biological effector function has been identified for IgD.

Biological properties of Ig classes and their subclasses

Property / activity	IgG_1	IgG_2	IgG_3	IgG_4	IgA_1	IgA_2	IgM	IgE	IgD
Molecular weight	150,000	150,000	150,000	150,000	150,000 - 600000	150,000 - 600000	900,000	190,000	150,000
Heavy chain component	γ	γ	γ	γ	α	α	µ	ε	δ
Invivo serum half life (Days)	23	23	8	23	6	6	5	2.5	3
Activates classical complement pathway	+	+ / -	++	-	-	-	+++	-	-
Crosses placenta	+	+ / -	+	+	-	-	-	-	-
Present on membrane of mature B cell	-	-	-	-	-	-	+	-	+
Binds to Fc receptors on phagocytes	++	+ / -	++	+	-	-	?	-	-
Mucosal transport	-	-	-	-	++	++	+	-	-
Induces mast cell degranulation	-	-	-	-	-	-	-	+	-
mIgs exist on mature B cell as	Monomer	Monomer	Monomer	Monomer	Monomer	Monomer	Monomer	Monomer	Monomer
sIgs released by plasma cell as	Monomer	Monomer	Monomer	Monomer	Monomer / Dimer /	Monomer / Dimer /	Pentamer	Monomer	Monomer

IMMUNO TECHNOLOGY

					Trimer / Tetramer	Trimer / Tetramer			

Note:

→ IgG, IgE, and IgD always exist as monomers; IgA can exist as a monomer, dimer, trimer, or tetramer. Membrane-bound

→ IgM is a monomer, but secreted IgM in serum is a pentamer.

→ IgM is the first isotype produced by the neonate and during a primary immune response.

IMMUNO TECHNOLOGY

Organization and Expression of Immunoglobulin Genes

Multigene Organization of Ig Genes

As cloning and sequencing of the light- and heavy-chain DNA was accomplished, even greater complexity was revealed than had been predicted by Dreyer and Bennett. The κ and λ light chains and the heavy chains are encoded by separate multigene families situated on different chromosomes (see Table).

Chromosomal locations of Ig genes in human and mouse

	Chromosome	
Gene	**Human**	**Mouse**
λ light chain	22	16
κ light chain	2	6
Heavy chain	14	12

In germ-line DNA, each of these multigene families contains several coding sequences, called **gene segments,** separated by noncoding regions. During B-cell maturation, these gene segments are rearranged and brought together to form functional immunoglobulin genes.

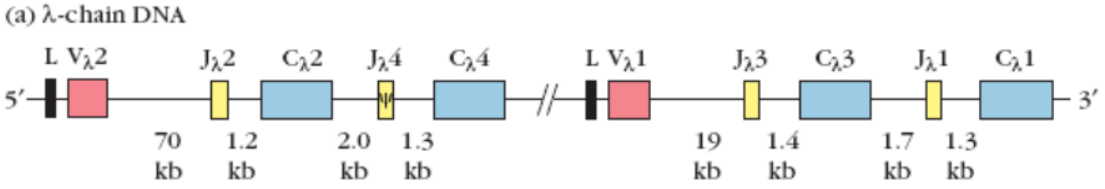
Each Multigene Family Has Distinct Features

The κ and λ light-chain families contain **V, J,** and **C gene segments;** the rearranged VJ segments encode the variable region of the light chains. The heavy-chain family contains **V, D, J,** and **C gene segments;** the rearranged VDJ gene segments encode the variable region of the heavy chain. In each gene family, C gene segments encode the constant regions. Each V gene segment is preceded at its 5' end by a small exon that encodes a short **signal** or **leader (L) peptide** that guides the heavy or light chain through the endoplasmic reticulum. The signal peptide is cleaved from the nascent light and heavy chains before assembly of the finished immunoglobulin molecule. Thus, amino acids encoded by this leader sequence do not appear in the immunoglobulin molecule.

λ-Chain Multigene Family

The first evidence that the light-chain variable region was actually encoded by two gene segments appeared when Tonegawa cloned the germ-line DNA that encodes the variable region of mouse λ light chain and determined its complete nucleotide sequence.

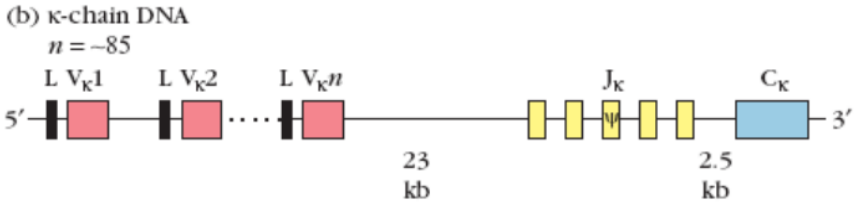
When the nucleotide sequence was compared with the known amino acid sequence of the λ- chain variable region, an unusual discrepancy was observed. Although the first 97 amino acids of the λ-chain variable region corresponded to the nucleotide codon sequence, the remaining 13 carboxyl-terminal amino acids of the protein's variable region did not. It turned out that many base pairs away a separate, 39-bp gene segment, called J for *joining,* encoded the remaining 13 amino acids of the λ-chain variable region.



Thus, a functional λ variable-region gene contains two coding segments—a 5' V segment and a 3' J segment— which are separated by a noncoding DNA sequence in unrearranged germ-line DNA. The λ multigene family in the mouse germ line contains three V_λ gene segments, four J_λ gene segments, and four C_λ gene segments (see Figure). The $J_\lambda 4$ is a **pseudogene,** a defective gene that is incapable of encoding protein; such genes are indicated with the psi symbol (ψ). Interestingly, $J_\lambda 4$'s constant region partner, $C_\lambda 4$, is a perfectly functional gene. The V_λ and the three functional J_λ gene segments encode the variable region of the light chain, and each of the three functional C_λ gene segments encodes the constant region of one of the three λ-chain subtypes (λ1, λ2, and λ3). In humans, the lambda locus is more complex. There are 31 functional V_λ gene segments, 4 J_λ segments, and 7 C_λ segments. In additional to the functional gene segments, the human lambda complex contains many V_λ, J_λ, and C_λ pseudogenes.

κ-Chain Multigene Family

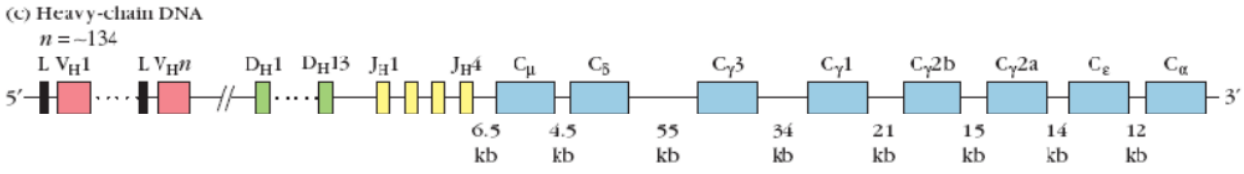
The κ-chain multigene family in the mouse contains approximately 85 Vκ gene segments, each with an adjacent leader sequence a short distance upstream (i.e., on the 5' side).



There are five Jκ gene segments (one of which is a nonfunctional pseudogene) and a single Cκ gene segment. As in the κ multigene family, the Vκ and Jκ gene segments encode the variable region of the κ light chain, and the Cκ gene segment encodes the constant region. Since there is only one Cκ gene segment, there are no subtypes of κ light chains. The κ-chain multigene family in humans, which has an organization similar to that of the mouse, contains approximately 40 Vκ gene segments, 5 Jκ segments, and a single Cκ segment.

Heavy-Chain Multigene Family

The organization of the immunoglobulin heavy-chain genes is similar to, but more complex than, that of the λ and κ light-chain genes. An additional gene segment encodes part of the heavy-chain variable region. The existence of this gene segment was first proposed by Leroy Hood and his colleagues, who compared the heavy-chain variable-region amino acid sequence with the V_H and J_H nucleotide sequences. The V_H gene segment was found to encode amino acids 1 to 94 and the J_H gene segment was found to encode amino acids 98 to 113; however, neither of these gene segments carried the information to encode amino acids 95 to 97. When the nucleotide sequence was determined for a rearranged myeloma DNA and compared with the germ-line DNA sequence, an additional nucleotide sequence was observed between the V_H and J_H gene segments. This nucleotide sequence corresponded to amino acids 95 to 97 of the heavy chain.



IMMUNO TECHNOLOGY

The heavy-chain multigene family on human chromosome 14 has been shown by direct sequencing of DNA to contain 51 V_H gene segments located upstream from a cluster of 27 functional D_H gene segments. As with the lightchain genes, each V_H gene segment is preceded by a leader sequence a short distance upstream. Downstream from the D_H gene segments are six functional J_H gene segments, followed by a series of C_H gene segments. Each C_H gene segment encodes the constant region of an immunoglobulin heavy-chain isotype. The C_H gene segments consist of coding exons and noncoding introns. Each exon encodes a separate domain of the heavy-chain constant region. A similar heavy chain gene organization is found in the mouse.

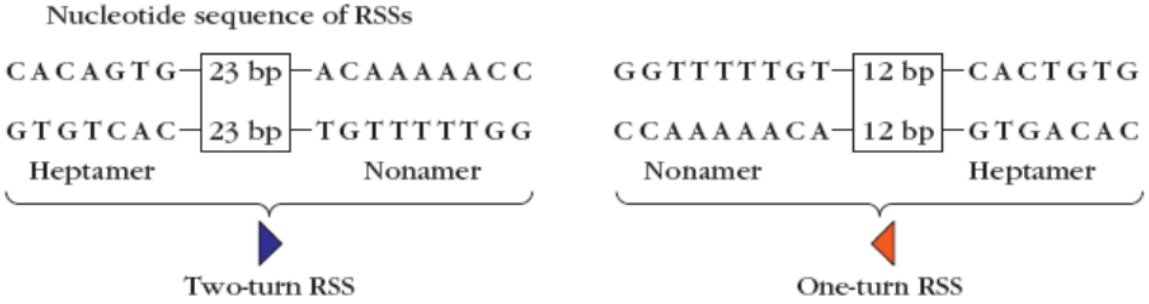
The conservation of important biological effector functions of the antibody molecule is maintained by the limited number of heavy-chain constant-region genes. In humans and mice, the C_H gene segments are arranged sequentially in the order C_μ, C_δ, C_γ, C_ε and C_α. This sequential arrangement is no accident; it is generally related to the sequential expression of the immunoglobulin classes in the course of B-cell development and the initial IgM response of a B cell to its first encounter with an antigen.

Mechanism of Variable-Region DNA Rearrangements

Recombination Signal Sequences

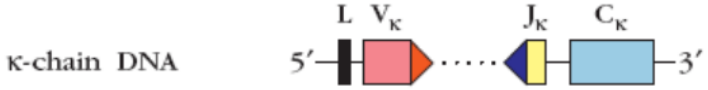
The discovery of two closely related conserved sequences in variable-region germ-line DNA paved the way to fuller understanding of the mechanism of gene rearrangements. DNA sequencing studies revealed the presence of unique **recombination signal sequences (RSSs)** flanking each germ-line V, D, and J gene segment. One RSS is located 3' to each V gene segment, 5' to each J gene segment, and on both sides of each D gene segment. These sequences function as signals for the recombination process that rearranges the genes.

Each **RSS** contains a **conserved palindromic heptamer** and a **conserved AT-rich nonamer** sequence separated by an **intervening sequence** of **12** or **23 base pairs** (see Figure). The intervening 12- and 23-bp sequences correspond, respectively, to **one** and **two turns** of the **DNA helix**; for this reason the sequences are called **one-turn recombination signal sequences** and **two-turn signal sequences.**

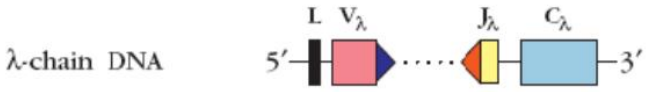


Location of RSS in the germ-line immunoglobulin DNA

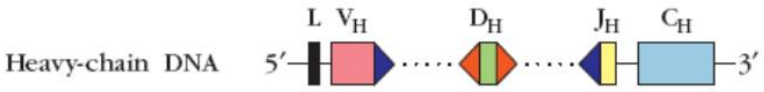
The V_κ signal sequence has a one-turn spacer, and the J_κ signal sequence has a two-turn spacer.



In λ light-chain DNA, this order is reversed; that is, the V_λ signal sequence has a two-turn spacer, and the J_λ signal sequence has a one-turn spacer.



In heavy-chain DNA, the signal sequences of the VH and JH gene segments have two-turn spacers, the signals on either side of the DH gene segment have one-turn spacers.



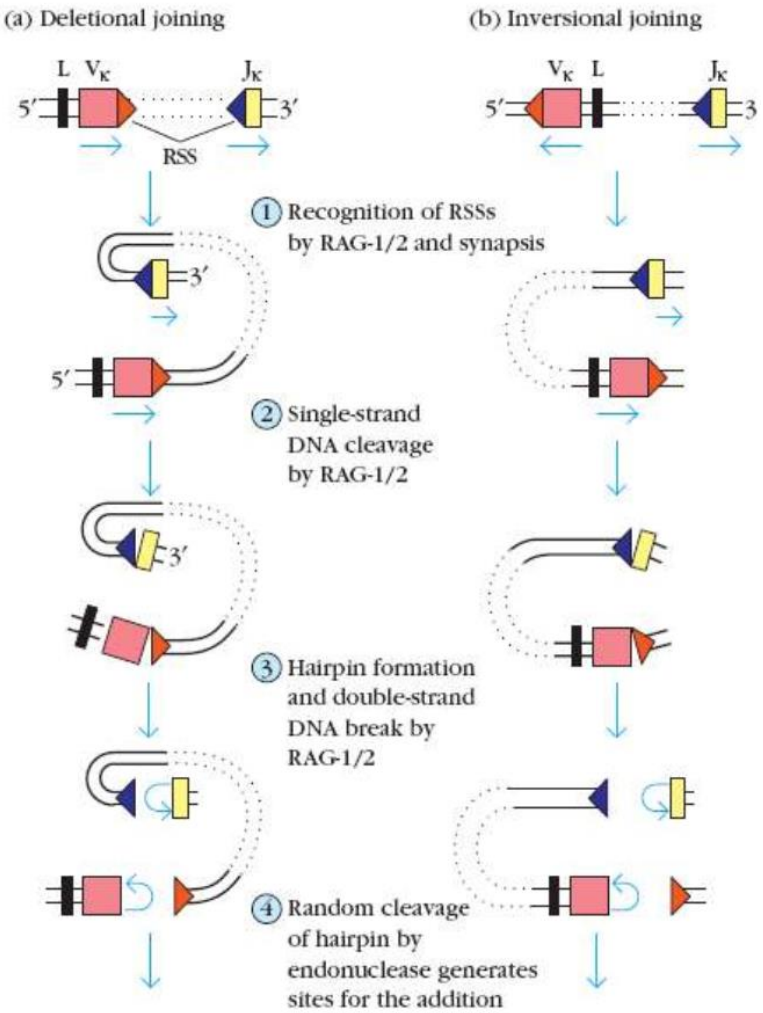
One turn two turn joining rule

Signal sequences having a one-turn spacer can join only with sequences having a two-turn spacer (the so called one-turn/two-turn joining rule). This joining rule ensures, for example, that a VL segment joins only to a JL segment and not to another VL segment; the rule likewise ensures that VH, DH, and JH segments join in proper order and that segments of the same type do not join each other.

V and J genes are joined by direct recombination

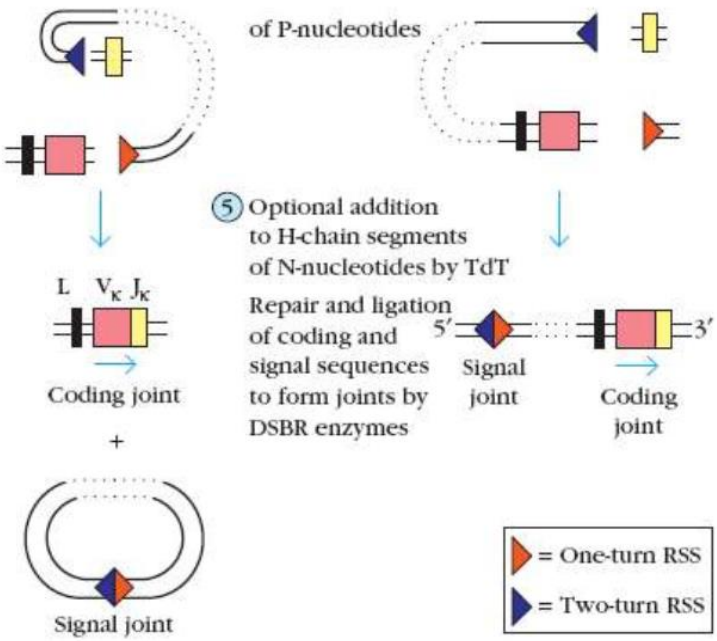
The recombination of variable-region gene segments consists of the following steps, catalyzed by a system of recombinase enzymes.

- Recognition of recombination signal sequences (RSSs) by recombinase enzymes, followed by synapsis in which two signal sequences and the adjacent coding sequences (gene segments) are brought into proximity
- Cleavage of one strand of DNA by RAG-1 and RAG-2 at the junctures of the signal sequences and coding sequences
- A reaction catalyzed by RAG-1 and RAG-2 in which the free 3'-OH group on the cut DNA strand attacks the phosphodiester bond linking the opposite strand to the signal sequence, simultaneously producing a hairpin structure at the cut end of the coding sequence and a flush, 5'-phosphorylated, double-strand break at the signal sequence
- Cutting of the hairpin to generate sites for the addition of **P-region nucleotides,** followed by the trimming of a few nucleotides from the coding sequence by a

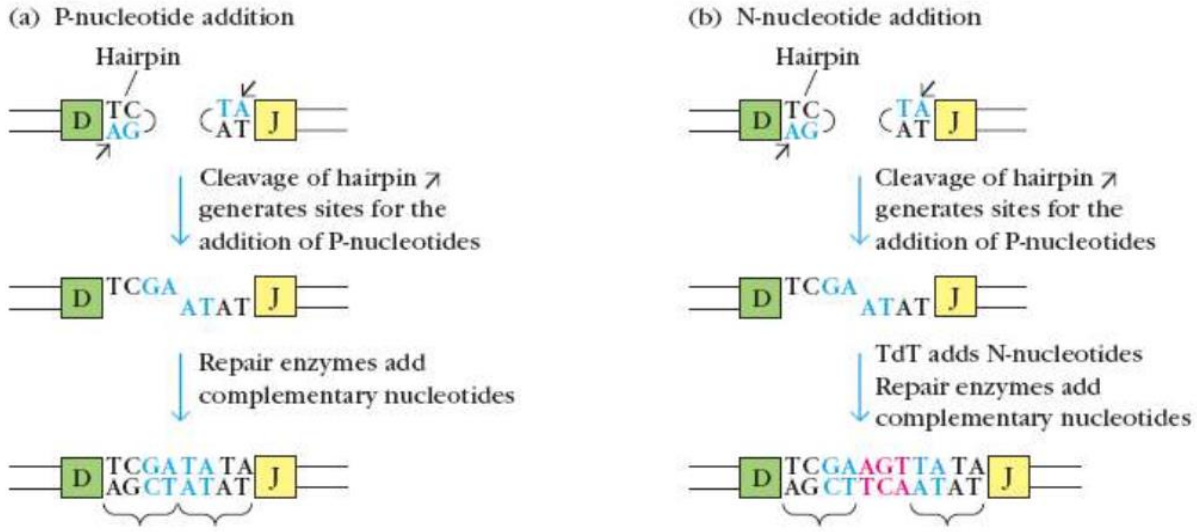


singlestrand endonuclease

- Addition of up to 15 nucleotides, called **N-region nucleotides,** at the cut ends of the V, D, and J coding sequences of the heavy chain by the enzyme terminal deoxynucleotidyl transferase
- Repair and ligation to join the coding sequences and to join the signal sequences, catalyzed by normal doublestrand break repair (DSBR) enzymes Recombination results in the formation of a **coding joint,** falling between the coding sequences, and a **signal joint,** between the RSSs.



The transcriptional orientation of the gene segments to be joined determines the fate of the signal joint and intervening DNA. When the two gene segments are in the same transcriptional orientation, joining results in deletion of the signal joint and intervening DNA as a circular excision product (see Figure). Less frequently, the two gene segments have opposite orientations. In this case joining occurs by inversion of the DNA, resulting in the retention of both the coding joint and the signal joint (and intervening DNA) on the chromosome. In the human κ locus, about half of the V_κ gene segments are inverted with respect to J_κ and their joining is thus by inversion.



Generation of antibody diversity

To date, seven means of antibody diversification have been identified in mice and humans:

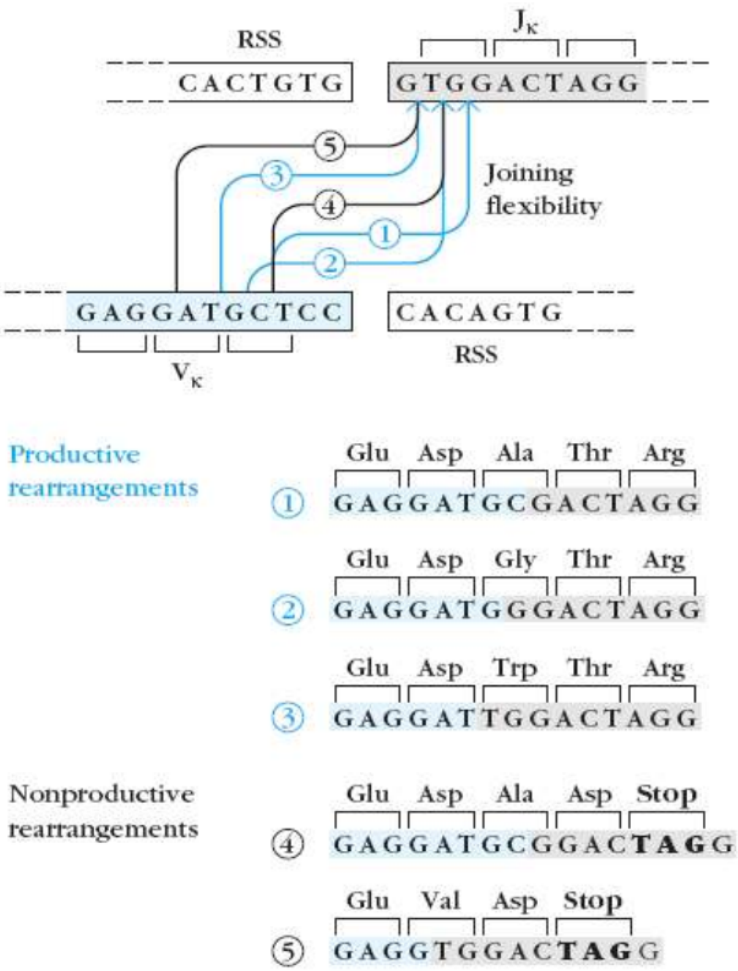
→ Multiple germ-line gene segments
→ Combinatorial V-(D)-J joining
→ Junctional flexibility
→ P-region nucleotide addition (P-addition)

→ N-region nucleotide addition (N-addition)
→ Somatic hypermutation
→ Combinatorial association of light and heavy chains

Although the exact contribution of each of these avenues of diversification to total antibody diversity is not known, they each contribute significantly to the immense number of distinct antibodies that the mammalian immune system is capable of generating.

Ig-Gene Rearrangements May Be Productive or Nonproductive

One of the striking features of gene-segment recombination is the diversity of the coding joints that are formed between any two gene segments. Although the double-strand DNA breaks that initiate V-(D)-J rearrangements are introduced precisely at the junctions of signal sequences and coding sequences, the subsequent joining of the coding sequences is imprecise. Junctional diversity at the V-J and V-D-J coding joints is generated by a number of mechanisms: variation in cutting of the hairpin to generate P-nucleotides, variation in trimming of the coding sequences, variation in N-nucleotide addition, and flexibility in joining the coding sequences. The introduction of randomness in the joining process helps generate antibody diversity by contributing to the hypervariability of the antigen-binding site. (This phenomenon is covered in more detail below in the section on generation of antibody diversity.) Another consequence of imprecise joining is that gene segments may be joined out of phase, so that the triplet reading frame for translation is not preserved. In such a **nonproductive rearrangement,** the resulting VJ or VDJ unit is likely to contain numerous stop codons, which interrupt translation (see Figure).

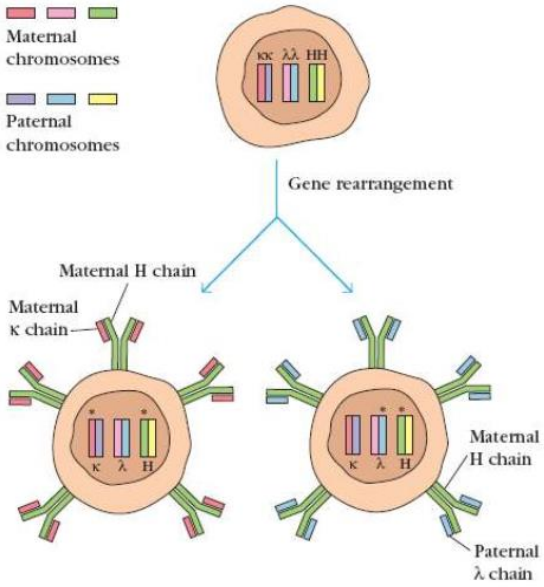


When gene segments are joined in phase, the reading frame is maintained. In such a **productive rearrangement,** the resulting VJ or VDJ unit can be translated in its entirety, yielding a complete antibody. If one allele rearranges nonproductively, a B cell may still be able to rearrange the other allele productively. If an inphase rearranged heavy-chain and light-chain gene are not produced, the B cell dies by apoptosis. It is estimated that only one in three attempts at VL-JL joining, and one in three subsequent attempts at V_H-D_HJ_H joining, are productive. As a result, less than 1/9 (11%) of the early-stage pre-B cells in the bone marrow progress to maturity and leave the bone marrow as mature immunocompetent B cells.

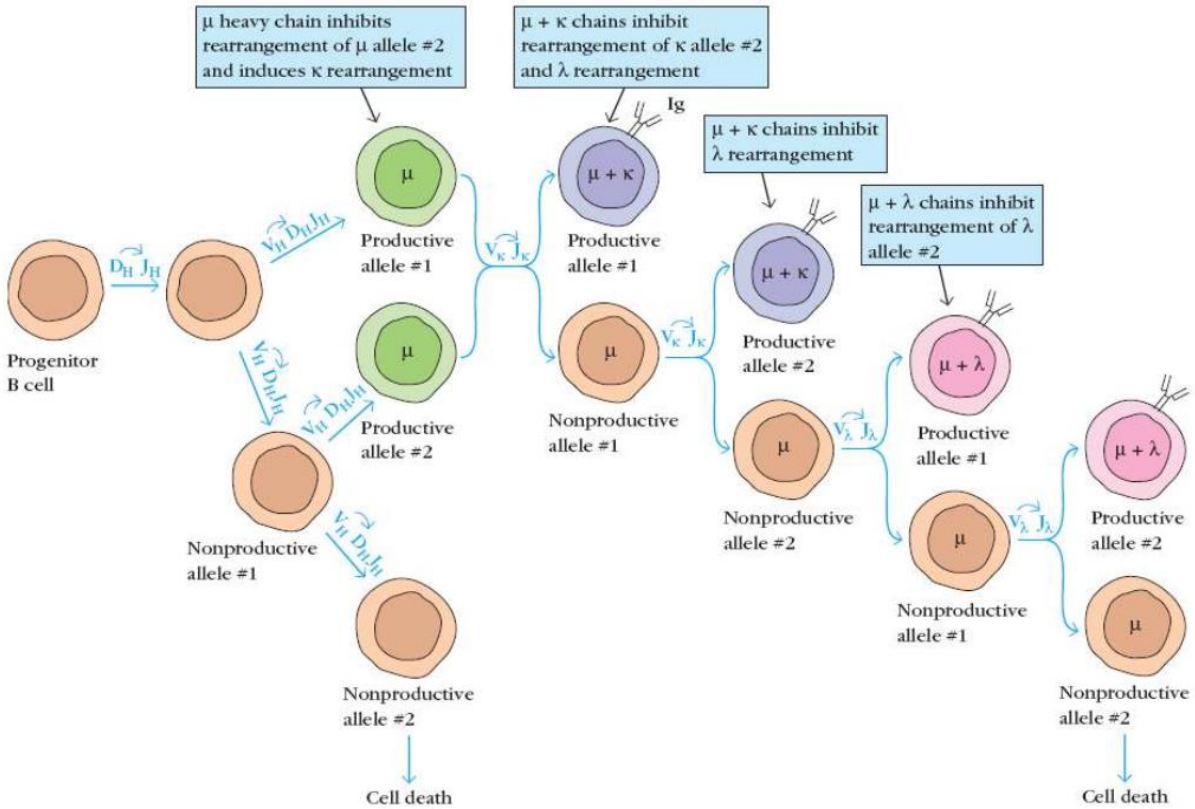
IMMUNO TECHNOLOGY

Allelic Exclusion Ensures a Single Antigenic Specificity

B cells, like all somatic cells, are diploid and contain both maternal and paternal chromosomes. Even though a B cell is diploid, it expresses the rearranged heavy-chain genes from only one chromosome and the rearranged light-chain genes from only one chromosome. The process by which this is accomplished, called **allelic exclusion,** ensures that functional B cells never contain more than one $V_HD_HJ_H$ and one V_LJ_L unit (Figure 5-10). This is, of course, essential for the antigenic specificity of the B cell, because the expression of both alleles would render the B cell multispecific. The phenomenon of allelic exclusion suggests that once a productive V_H-D_H-J_H rearrangement and a productive V_L-J_L rearrangement have occurred, the recombination machinery is turned off, so that the heavy- and light-chain genes on the homologous chromosomes are not expressed.



Because of allelic exclusion, the immunoglobulin heavy- and light-chain genes of only one parental chromosome are expressed per cell. This process ensures that B cells possess a single antigenic specificity. The allele selected for rearrangement is chosen randomly. Thus the expressed immunoglobulin may contain one maternal and one paternal chain or both chains may derive from only one parent. Only B cells and T cells exhibit allelic exclusion.

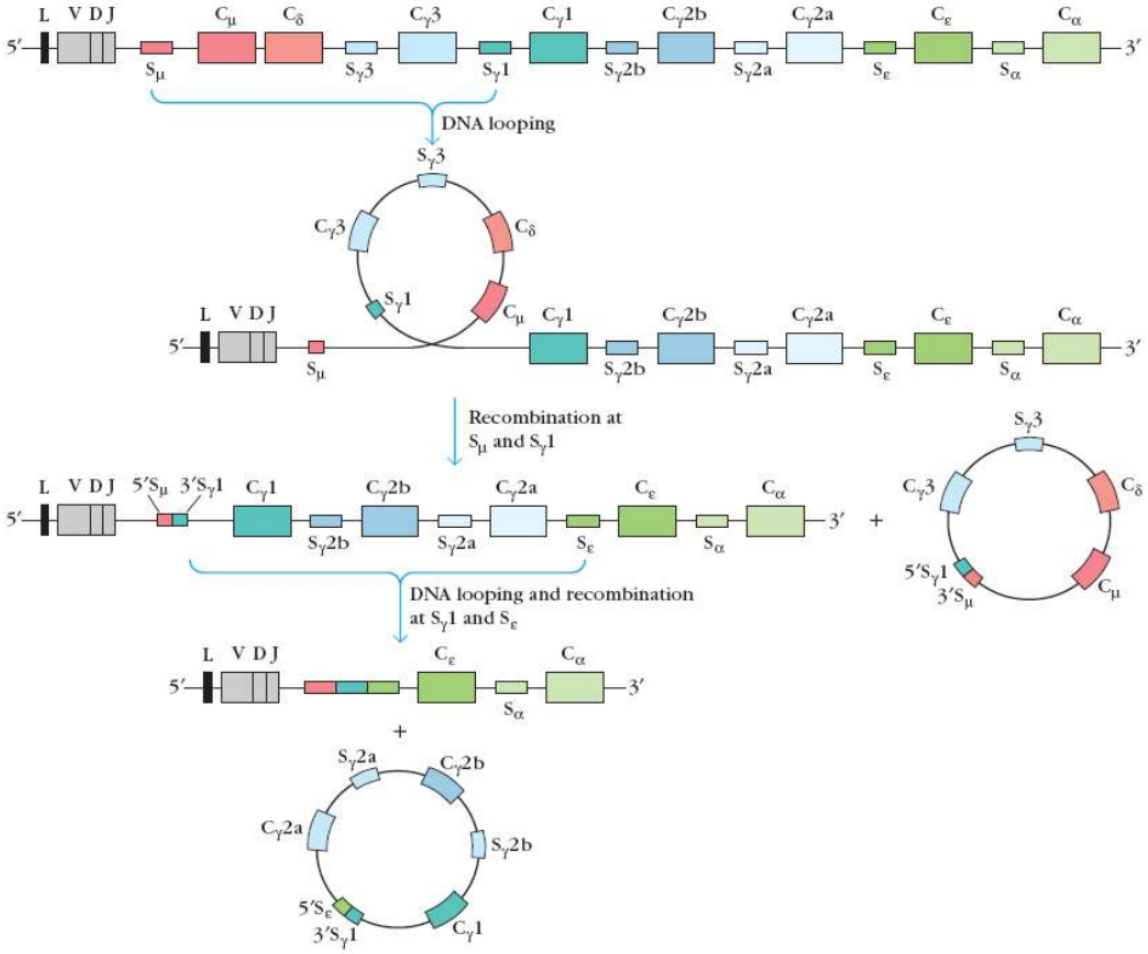


G.D.Yancopoulos and F.W.Alt have proposed a model to account for allelic exclusion (see Figure). They suggest that once a productive rearrangement is attained, its encoded protein is expressed and the presence of this protein acts as a signal to prevent further gene rearrangement.

According to their model, the presence of μ heavy chains signals the maturing B cell to turn off rearrangement of the other heavy-chain allele and to turn on rearrangement of the κ light-chain genes. If a productive κ rearrangement occurs, κ light chains are produced and then pair with μ heavy chains to form a complete antibody molecule. The presence of this antibody then turns off further light-chain rearrangement. If κ rearrangement is nonproductive for both κ alleles, rearrangement of the λ-chain genes begins. If neither λ allele rearranges productively, the B cell presumably ceases to mature and soon dies by apoptosis.

Class Switching among Constant-Region Genes

After antigenic stimulation of a B cell, the heavy-chain DNA can undergo a further rearrangement in which the VHDHJH unit can combine with any C_H gene segment. The exact mechanism of this process, called **class switching** or **isotype switching,** is unclear, but it involves DNA flanking sequences (called **switch regions**) located 2–3 kb upstream from each C_H segment (except C_). These switch regions, though rather large (2 to 10 kb), are composed of multiple copies of short repeats (GAGCT and TGGGG).



One hypothesis is that a protein or system of proteins that constitute the switch recombinase recognize these repeats and upon binding carry out the DNA recombinations that result in class switching. Intercellular regulatory proteins known as cytokines act as "switch factors" and play major roles in determining the particular immunoglobulin class that is expressed as a consequence of switching.

Interleukin 4 (IL-4), for example, induces class switching from C_μ to $C_\gamma 1$ to C_ε. In some cases, IL-4 has been observed to induce class switching in a successive manner: first from C_μ to $C_\gamma 1$ and then from $C_\gamma 1$ to C_ε (see Figure).

Examination of the DNA excision products produced during class switching from C_μ to $C_\gamma 1$ showed that a circular excision product containing C_μ together with the 5' end of the γ1 switch region ($S_\gamma 1$) and the 3' end of the μ switch region (S_μ) was generated. Furthermore, the switch from $C_\gamma 1$ to C_ε produced circular excision products containing $C_\gamma 1$ together with portions of the μ, γ, and ε switch regions. Thus class switching depends upon the interplay of three elements: switch regions, a switch recombinase, and the cytokine signals that dictate the isotype to which the B cell switches.

Proposed mechanism for class switching induced by interleukin 4 in rearranged immunoglobulin heavy-chain genes. A switch site is located upstream from each C_H segment except C_δ. Identification of the indicated circular excision products containing portions of the switch sites suggested that IL-4 induces sequential class switching from C_μ to $C_\gamma 1$ to C_ε.

Expression of Ig Genes

As in the expression of other genes, post-transcriptional processing of immunoglobulin primary transcripts is required to produce functional mRNAs. The primary transcripts produced from rearranged heavy-chain and light-chain genes contain intervening DNA sequences that include noncoding introns and J gene segments not lost during V-(D)-J rearrangement. In addition, as noted earlier, the heavy-chain C-gene segments are organized as a series of coding exons and noncoding introns. Each exon of a CH gene segment corresponds to a constant-region domain or a hinge region of the heavy-chain polypeptide. The primary transcript must be processed to remove the intervening DNA sequences, and the remaining exons must be connected by a process called RNA splicing. Short, moderately conserved splice sequences, or splice sites, which are located at the intron exon boundaries within a primary transcript, signal the positions at which splicing occurs. Processing of the primary transcript in the nucleus removes each of these intervening sequences to yield the final mRNA product. The mRNA is then exported from the nucleus to be translated by ribosomes into complete H or L chains.

Synthesis, Assembly, and Secretion of Immunoglobulins

Immunoglobulin heavy- and light-chain mRNAs are translated on separate polyribosomes of the rough endoplasmic reticulum (RER). Newly synthesized chains contain an amino-terminal leader sequence, which serves to guide the chains into the lumen of the RER, where the signal sequence is then cleaved. The assembly of light (L) and heavy (H) chains into the disulfide-linked and glycosylated immunoglobulin molecule occurs as the chains pass through the cisternae of the RER. The complete molecules are transported to the Golgi apparatus and then into secretory vesicles, which fuse with the plasma membrane.

The order of chain assembly varies among the immunoglobulin classes. In the case of IgM, the H and L chains assemble within the RER to form half-molecules, and then two half-molecules assemble to

form the complete molecule. In the case of IgG, two H chains assemble, then an H_2L intermediate is assembled, and finally the complete H_2L_2 molecule is formed. Interchain disulfide bonds are formed, and the polypeptides are glycosylated as they move through the Golgi apparatus.

If the molecule contains the transmembrane sequence of the membrane form, it becomes anchored in the membrane of a secretory vesicle and is inserted into the plasma membrane as the vesicle fuses with the plasma membrane. If the molecule contains the hydrophilic sequence of secreted immunoglobulins, it is transported as a free molecule in a secretory vesicle and is released from the cell when the vesicle fuses with the plasma membrane.