CLASSES OF IMMUNOGLOBULINS

Classes Of Immunoglobulins: Isotypes, Allotypes And Idiotypes

I. ISOTYPES

A. Definition

Isotypes are antigenic determinants that characterize classes and subclasses of heavy chains and types and subtypes of light chains.

If human IgM is injected into a rabbit the rabbit will recognize antigenic determinants on the heavy chain and light chain and make antibodies to them. If that antiserum is absorbed with human IgG the antibodies to the light chain determinants and any determinants in common between human IgM and IgG will be removed and the resulting antiserum will be react only with human IgM. Indeed, the antibodies will only react with the constant region of the μ chain. Antibodies to the variable region are rare perhaps because only a few copies of each different variable region are represented in the IgM and thus effective immunization does not occur. The determinants that are recognized by such antibodies are called isotypic determinants and the antibodies to those determinants are called anti-isotypic antibodies. Each class, subclass, type and subtype of immunoglobulin has its unique set of isotypic determinants.

B. Location

Heavy chain isotypes are found on the Fc portion of the constant region of the molecule while light chain isotypes are found in the constant region. The location of isotypic determinants is illustrated in Figure 1.

C. Occurrence

Isotypes are found in ALL NORMAL individuals in the species. The prefix Iso means same in all members of the species. Some individuals with immunodeficiencies may lack one or more isotypes but normal individuals have all isotypes.

D. Importance

Antibodies to isotypes are used for the quantitation of Ig classes and subclasses in various diseases, in the characterization of B cell leukemia and in the diagnosis of various immunodeficiency diseases.

II. ALLOTYPES

A. Definition

Allotypes are antigenic determinants specified by allelic forms of the Ig genes.

Allotypes represent slight differences in the amino acid sequences of heavy or light chains of different individuals. Even a single amino acid difference can give rise to an allotypic determinant, although in many cases there are several amino acid substitutions that have occurred.

Allotypic differences are detected by using antibodies directed against allotypic determinants. These antibodies can be prepared by injecting the Ig from one person into another. In practice however we obtain anti-allotype antisera from women who have had multiple pregnancies or from people who have received blood transfusions or from some patients with rheumatoid arthritis.

B. Location

In man the allotypic differences are localized to the constant region of the heavy and light chains as illustrated in the Figure 2.

C. Occurrence

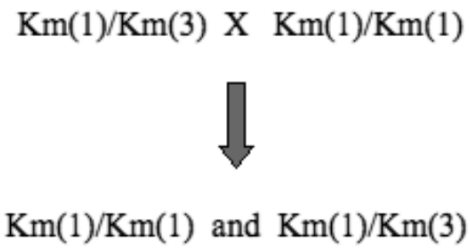
Individual allotypes are found in individual members of a species. All allotypes are not found in all members of the species. The prefix Allo means different in individuals of a species

D. Human Ig Allotypes

Nomenclature - Human Ig allotypes are named on the basis of the heavy or light chain on which it is located. Thus, an allotype on a Gamma 1 heavy chain is given the name: G1m(3). An allotype on a Kappa light chain is given the name: Km(1). Table 1 lists some human allotypes.

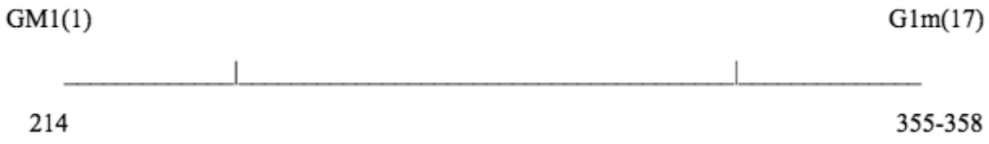
E. Genetics

1. Codominant autosomal genes - Allotypes that represent amino acid substitutions at the same position in a heavy or light chain (eg. G1m(3) and G1m(17) or Km(1) and Km(3) are inherited as codominant autosomal genes. e.g.



2. Allelic Exclusion - Although in a heterozygote both alleles are expressed, any individual Ig molecule will only have one allotype. This is because an individual B cell can only expresses one allele. This is called allelic exclusion. Allotypes that represent amino acid substitutions at different locations in a molecule (eg. G1m(1) and G1m(17)) can be found on the same molecule.

eg. In a G1m(1,17) individual both allotypes can be on the same heavy chain



F. Importance

1. Monitoring bone marrow grafts - Bone marrow grafts that produce a different allotype from the recipient can be used to monitor the graft.

2. Forensic medicine - Km and Gm allotypes are detectable in blood stains and semen and are useful in forensic medicine.

3. Paternity testing - The immunoglobulin allotypes are one of the characteristics used in legal cases involving paternity.

III. IDIOTYPES (Id)

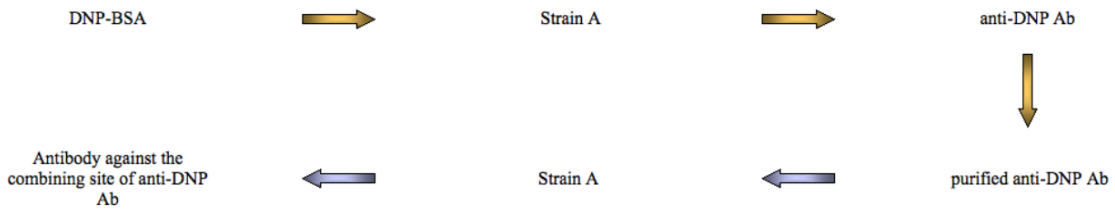
A. Definition - Unique antigenic determinants present on individual antibody molecules or on molecules of identical specificity.

Identical specificity means that all antibodies molecules have the exact same hypervariable regions.

Antigenic determinants created by the combining site of an antibody are called idiotypes and the antibodies elicited to the idiotypes are called anti-Id antibodies. Idiotypes are the antigenic determinants created by the

hypervariable regions of an antibody and the anti-idiotypic antibodies are those directed against the hypervariable regions of an antibody.

To understand what idiotypes are, it is helpful to understand how they are detected.



An antigen, in this case the hapten dinitrophenol, is injected into a mouse and antibodies (against DNP) are elicited. This antibody can be purified to homogeneity and injected into another mouse of the same strain. Most epitopes on the antibody will be seen by the second mouse's immune system as "self"; however, the epitopes that form the binding site to DNP (idiotopes - this is a term that is not often used and frequently is used interchangeably with idiotype) will be seen as foreign since the second mouse has not been injected with DNP-BSA. The second mouse will raise antibodies only against the idiotopes of the purified anti-DNP antibody. These are therefore anti-idiotypic antibodies

Antigenic determinants created by the hypervariable region of an antibody are idiotypes

B. Location

Idiotypes are localized on the Fab fragment of the Ig molecules as illustrated in Figure 3. Specifically, they are localized at or near the hypervariable regions of the heavy and light chains. In many instances the actual antigenic determinant (i.e. idiotype) may include some of the framework residues near the hypervariable region. Idiotypes are usually determinants created by both heavy and light chain HVR's although sometimes isolated heavy and light chains will express the idiotype.

C. Importance

1. V region marker - Idiotypes are a useful marker for a particular variable region.

2. Regulation of immune responses - there is evidence that immune responses may be regulated by anti-Id antibodies directed against our own Id's.

3. Vaccines - In some cases anti-idiotypic antibodies actually stimulate B cells to make antibody and thus they can be used as a vaccine. This approach is being tried to immunize against highly dangerous pathogens that cannot be safely used as a vaccine.^

4. Treatment of B cell tumors - Anti-idiotypic antibodies directed against an idiotype on malignant B cells can be used to kill the cells. Killing occurs because of complement fixation or because toxic molecules are attached to the antibodies.

Genetics Of Immunoglobulins - Light Chain Gene Families - Heavy Chain Gene Family - Mechanism of DNA Rearrangements - Origin Of Antibody Diversity - T Cell Receptor For Antigen

GENETICS OF IMMUNOGLOBULINS

I. HISTORY

Amino acid sequencing data revealed that a single C region could be associated with many different V regions. Also, it was shown that a single idiotype could be associated with different C regions (eg. IgM and IgG). To explain these data it was suggested that perhaps the two regions of the immunoglobulin molecule were coded for by separate genes and that the V and C region genes were somehow joined before an immunoglobulin molecule was made (i.e. there were two genes for one polypeptide). This was a revolutionary concept but with the advent of recombinant DNA technology, it has been shown to be the correct. The immunoglobulin heavy and light chains are coded for by three separate gene families each one on a separate chromosome - one for the heavy chain and one for each of the light chain types. Each of these gene families has several V region genes and one or more C region genes. The V and C regions genes are not however immediately adjacent to each other.

II. LIGHT CHAIN GENE FAMILIES

A. Germ line gene organization

The organization of the kappa and lambda light chain genes in the germ line or undifferentiated cells is depicted in Figure 1.

1. Lambda light chains - The lambda gene family is composed of 4 C region genes, one for each subtype of lamda chain, and approximately 30 V region genes. Each of the V region genes is composed of two exons, one (L) that codes for a leader region and the other (V) that codes for most of the variable region. Upstream of each of the C genes there is and additional exon called J (joining). The L, V, J and C exons are separated by introns (intervening non-coding sequences).

2. Kappa light chains - The kappa light chain gene family contains only one C region gene, since there is only one type of kappa light chain. There are many V region genes (approximately 250) each of which has a leader exon and a V exon. In the κ gene family there are several J exons located between the V and C genes. All of the exons are separated by introns.

B. Gene rearrangement and Expression

As a cell differentiates into a mature B cell that will make a light chain, there is a rearrangement of the various genes (exons) and the gene begins to be expressed as depicted in Figure 2.

As a cell commits to become a B cell making a light chain, there is a rearrangement of the genes at the DNA level such that one of the V genes is brought next to one of the J regions. This occurs by a recombination event which removes the intron between the V and J regions. The selection of which V gene is used is not totally random; there is some preference for the use of V genes nearest to the J regions. However, with time all V genes can be used so that all combinations of V genes and J regions can be generated.

A consequence of this DNA rearrangement is that the gene becomes transcriptionally active because a promoter (P), which is associated with the V gene, is brought close to an enhancer (E), which is located in the intron between the J and C regions. As transcription initiates from the promoter a pre-mRNA is made which contains sequences from the L, V J and C regions as well as sequences for the introns between L and V and between J and C (See Figure 2). This pre-mRNA is processed (spliced) in the nucleus and the remaining introns are removed. The resulting mRNA has the L, V J and C exons contiguous. The mRNA is translated in the cytoplasm and the leader is removed as the protein is transported into the lumen of the endoplasmic reticulum. The light chain is assembled with a heavy chain in the endoplasmic reticulum and the immunoglobulin is secreted via the normal route of secretory proteins. The region V region of the mature light chain is coded for by sequences in the V gene and J region and the C region by sequences in the C gene.

III. HEAVY CHAIN GENE FAMILY

A. Germ line gene organization

The organization of the heavy chain genes is depicted in Figure 3. In the heavy chain gene family there are many C genes, one for each class and subclass of immunoglobulin. Each of the C genes is actually composed of several exons, one for each domain and another for the hinge region. In the heavy chain gene family there are many V region genes, each composed of a leader and V exon. In addition to several J exons, the heavy chain gene family also contains several additional exons called the D (diversity) exons. All of the exons are separated by introns as depicted in Figure 3.

B. Gene rearrangements and expression As a cell differentiates into a mature B cell that will make a heavy chain, there is a rearrangement of the various genes segments (exons) and the gene begins to be expressed as depicted in Figures 4 and 5.

As a cell commits to become a B cell making a heavy chain, there are two rearrangements at the DNA level. First, one of the D regions is brought next to one of the J regions and then one of the V genes is brought next to the rearranged DJ region. This occurs by two recombination events which remove the introns between the V, D and J regions. As with the light chains the selection of the heavy chain V gene is not totally random but eventually all of the V genes can be used.

A consequence of these DNA rearrangements is that the gene becomes transcriptionally active because a promoter (P), which is associated with the V gene, is brought close to an enhancer (E), which is located in the intron between the J and Cmu regions. As transcription initiates from the promoter a pre-mRNA is made which contains sequences from the L, V, D, J Cmu and Cdelta regions as well as sequences for the introns between L and V, between J and Cmu, and between Cmu and Cdelta (Figure 4).

The pre-mRNA is processed (spliced) in the nucleus and the remaining introns, including those between the exons in the C genes, are removed (See Figure 5). The pre-mRNA can be processed in two ways, one to bring the VDJ next to the Cmu gene and the other to bring the VDJ next to the Cdelta gene. The resulting mRNAs have the L, V, D, J and Cmu or Cdelta exons contiguous and will code for a mu and a delta chain, respectively.

The mRNAs are translated in the cytoplasm and the leader is removed as the protein is transported into the lumen of the endoplasmic reticulum. The heavy chain is assembled with a light chain in the endoplasmic reticulum and the immunoglobulin is secreted via the normal route of secretory proteins. The region V region of the mature heavy chain is coded for by sequences in the V gene, D region and J region and the C region by sequences in the C gene.

IV. MECHANISM OF DNA REARRANGEMENTS

Flanking the V, J and D exons there are unique sequences referred to as recombination signal sequences (RSS), which function in recombination. Each RSS consists of a conserved nonamer and a conserved heptamer that are separated by either 12 or 23 base pairs (bp) as illustrated in Figure 6. The 12bp and 23 bp spaces correspond to one or two turns of the DNA helix.

Recombination only occurs between a 1 turn and a 2 turn signal. In the case of the λ light chains there is a 1 turn signal upstream of the J exon and a 2 turn signal downstream of Vlambda. In the case of the κ light chains there is a 1 turn signal downstream of the Vkappa gene and a 2 turn signal upstream of the J exon.. In the case of the heave chains there are 1 turn signals on each side of the D exon and a 2 turn signal downstream of the V gene and a 2 turn signal

upstream of the J exon. Thus, this ensures that the correct recombination events will occur.

The recombination event results in the removal of the introns between V and J in the case of the light chains or between the V, D, and J in the case of the heavy chains. The recombination event is catalyzed by two proteins, Rag-1 and Rag-2. Mutations in the genes for these proteins results in a severe combined immunodeficiency disease (both T and B cells are deficient), since these proteins and the RSS are involved in generating both the B and T cell receptors for antigen.

V. ORDER OF GENE EXPRESSION IN IG GENE FAMILIES

An individual B cell only produces one type of light chain and one class of heavy chain. (N.B. The one exception is that a mature B cell can produce both μ and δ heavy chains but the antibody specificity is the same since the same VDJ region is found on the μ and δ chains). Since any B cell has both maternal and paternal chromosomes which code for the immunoglobulin genes there must be some orderly way in which a cell expresses its immunoglobulin genes so as to ensure that only one type of light chain and one class of heavy chain is produced.

The order in which the immunoglobulin genes are expressed in a B cell is depicted in Figure 7 and 8.

Heavy chain (Figure 7) A cell first attempts to rearrange one of its heavy chain genes; in some cells the maternal chromosome is selected and in others the paternal chromosome is selected. If the rearrangement is successful so that a heavy chain is made, then no further rearrangements occur in the heavy chain genes. If, on the other hand, the first attempt to rearrange the heavy chain genes is unsuccessful (i.e. no heavy chain is made), then the cell attempts to rearrange the heavy chain genes on its other chromosome. If the cell is unsuccessful in rearranging the heavy chain genes the second time, it is destined to be eliminated.

Kappa light chain (Figure 8) When a cell successfully rearranges a heavy chain gene, it then begins to rearrange one of its kappa light chain genes. It is a random event whether the maternal or paternal kappa light chain genes are selected. If the rearrangement is unsuccessful (i.e. it does not produce a functional kappa light chain), then it attempts to rearrange the kappa genes on the other chromosome. If a cell successfully rearranges a kappa light chain gene, it will be a B cell that makes an immunoglobulin with a kappa light chain.

Lambda light chain (Figure 8) - If a cell is unsuccessful in rearranging both of its kappa light chain genes, it then attempts to make a lambda light chain. It is a random event whether the maternal or paternal lambda light chain genes are selected. If the rearrangement is unsuccessful (i.e. it does not produce a functional lambda light chain), then it attempts to rearrange the lambda genes on the other chromosome. If a cell successfully rearranges a lambda light chain

gene, it will be a B cell that makes an immunoglobulin with a lambda light chain.

The orderly sequence of rearrangements in the immunoglobulin gene families explains: 1) Why an individual B cell can only produce one kind of immunoglobulin with one kind of heavy and one kind of light chain. 2) Why a individual B cell can only make antibodies of one specificity. 3) Why there is allelic exclusion in immunoglobulin allotypes at the level of an individual immunoglobulin molecule but co-dominant expression of allotypes in the organism as a whole.

VI. ORIGIN OF ANTIBODY DIVERSITY

A. Background

Antibody diversity refers to the sum total of all the possible antibody specificities that an organism can make. It is estimated that we can make 107 - 108 different antibody molecules. One of the major questions in immunology has been how can we make so many different antibody molecules.

Theories which have attempted to explain the origin of antibody diversity fall into two major categories.

1. Germ line theory This theory states that we have a different V region gene for each possible antibody we can make. **2. Somatic mutation theory** This theory state that we have only one or a few V region genes and the diversity is generated by somatic mutations which occur in these genes.

B. Current Concepts

Our current thinking is that both the germ line and somatic mutation theories have some merit. It is thought that antibody diversity is generated by the following mechanisms.

1. A large number of V genes There are:

a) 30 lambda V genes b) 300 kappa V genes c) 1000 heavy chain V genes

2. V-J and V-D-J joining The region where the light chain V gene and J region or the heavy chain V gene and D and J regions come together is in the third hypervariable region. Since it is random which V and which J or D regions come together, there is a lot of diversity that can be generated by V-J and V-D-J joining.

3. Junctional diversity (Inaccuracies in V-J and V-D and D-J recombination) - (Figure 9)

Recombination between V-J and V-D-J is not always perfect and additional diversity can arise by errors that occur in the recombination event that brings

the V region next to the J or D regions or the D region next to the J region. It is estimated that these inaccuracies can triple the diversity generated by V-J and V-D-J joining. The diversity generated by this mechanisms is occurring in the third hypervariable region and thus, is directly affecting the combining site of the antibody.

4. N region insertion At the junction between D and J segments there is often an insertion of a series of nucleotides which is catalyzed by the enzyme terminal transferase. Terminal transferase catalyzes the random polymerization of nucleotides into DNA without the need for a template. This leads to further diversity in the third hypervariable region.

5. Somatic Mutation There is evidence that somatic mutations are occurring in the V gene, particularly in the place that codes for the second hypervariable region. Thus, somatic mutation probably contributes to antibody diversity to some extent.

6. Combinatorial Association Any individual B cell has the potential to make any one of the possible heavy chains and any one of the possible light chains. Thus, different combinations of heavy and light chains within an individual B cell adds further diversity.

7. Multispecificity Due to cross reactions between antigenic determinants of similar structure an antibody can often react with more than one antigenic determinant. This is termed multispecificity. Multispecificity also contributes to antibody diversity.

An example of how these mechanisms can generate a great deal of diversity is illustrated below:

	B Cell Receptor (Immunoglobulin)	
	Heavy	*Kappa*
V gene segments	1000	300
D gene segments	15	-
J gene segments	4	4
N region insertion	++	-
Junctional diversity	+++	+
Somatic mutation	+	+
Combinatorial association	V x D x J 1000 X 15 X 4	V x J 300 x 4
Total	6×10^4	1.2×10^3
Combinatorial association	7.2×10^7	

These calculations do not take into consideration the contributions of lambda light chains, somatic mutation junctional diversity, N region insertions or multispecificity.

The process of gene rearrangement of the heavy and light chains and the combinatorial association of these chains occurs during B cell development and is independent of antigen. Clones of B cells expressing all of the possible antibody specificities are produced during development and antigen simply selects those clones which have the appropriate receptor. The selected clones are then activated, proliferate and differentiate into antibody secreting plasma cells.

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