CHAPTER 6
ANTIBODY GENETICS: ISOTYPES, ALLOTYPES, IDIOTYPES

See APPENDIX: (3) OUCHTERLONY; (4) AFFINITY CHROMATOGRAPHY

Human immunoglobulins are made up of LIGHT and HEAVY chains encoded by a total of 14 CONSTANT REGION GENES organized into *three gene families*, namely ONE KAPPA gene, FOUR LAMBDA genes and NINE HEAVY CHAIN genes. Allelic variants exist for the *kappa locus* and six of the nine *heavy chain loci*.

The nine heavy chains define nine human CLASSES and SUBCLASSES of human immunoglobulin. Together with the five different light chains, these genes represent 14 distinct ISOTYPES which are present in *all* normal human sera. ALLOTYPES, or allelic variants within the constant regions, are known to exist for some of these isotypes, and are inherited in a Mendelian co-dominant fashion. IDIOTYPES consist of the unique combination of V_H and V_L which characterize a particular immunoglobulin's *combining sites.*

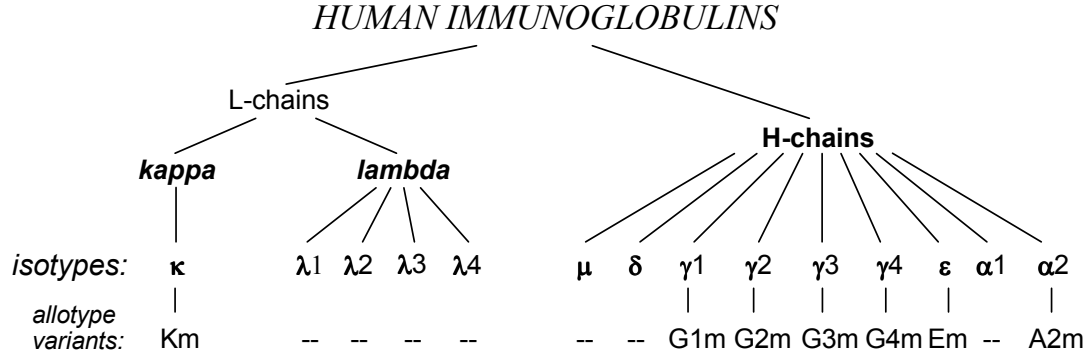

Figure 6-1

Immunoglobulins all have light and heavy chains, but the light and heavy chains of a particular Ig molecule may be different from those of other molecules. We have already seen that different kinds of heavy chains define the *class* (and *subclass*) of antibody to which they belong. We have also learned of the two varieties of light chains that exist, *kappa* and *lambda*. These and other structural differences between Ig polypeptides can be organized into three categories of differences, which we call *isotypic, allotypic*, and *idiotypic*. These are commonly distinguished serologically, *i.e.*, by the use of antibodies which recognize different specificities on target immunoglobulins.

ISOTYPES -- Distinct forms of light or heavy chains which are present in *all* members of a species, encoded at distinct genetic loci. *Kappa* and *lambda* are *isotypes* of light chains. Mu (μ), delta (δ), gamma-1 (δ1), *etc*. are *isotypes* of heavy chains. *All* isotypes can be readily found in *all normal sera*.

ALLOTYPES -- Genetic variants within the C-region sequences of particular isotypes that are inherited in an allelic manner (*"allelic type"*). Different members of a species will therefore *differ* from one another with respect to which particular alleles of a given isotype they received from their parents. Km1 and Km2 are *allotypes* of humans kappa chains; G1m(4) and G1m(17) are *allotypes* of human gamma-1 chains. The presence of particular allotypes, like isotypes, can be readily detected in those normal sera in which they are present.

IDIOTYPE -- An antigenic specificity (epitope) which distinguishes a particular *combination* of V_H and V_L (the antigen recognition site) from all others. Thus, a particular monoclonal immunoglobulin (a myeloma protein, for example) will bear an idiotype *different from any other*. Unlike isotypes or allotypes, particular *idiotypes* can generally be detected (with very rare exceptions) only in sera from myeloma patients. This is because any particular idiotype will be represented only at extremely low levels among the many thousands of kinds of combining sites present in serum immunoglobulin, even in specific immune responses.

Let's go through a series of experimental analyses of human myeloma proteins and Bence-Jones proteins. We will use classical serological approaches to demonstrate:

(1) The existence of two light chain isotypes (κ and λ);

(2) The presence of two κ chain allotypes (Km1 and Km2;

(3) The existence of two λ chain isotypes (λ1 and λ2).

EXPERIMENTAL DEFINITION OF LIGHT CHAIN ISOTYPES: KAPPA AND LAMBDA

We'll begin with a series of eight *purified Bence-Jones proteins*, each from a different patient (labeled A through H), and carry out an antigenic analysis to illustrate the properties of *isotypes* and *allotypes*. Let's take four of these proteins (A,B,C and E in this example) and use each them to immunize rabbits, resulting in four antisera. We will then take each of the four antisera (labelled "Ab") and test each of them by double diffusion in agarose (see APPENDIX 3, OUCHTERLONY analysis) against each of the eight original proteins. In the table below, a *plus* indicates that the antibody *does* show a line of precipitation with the particular B-J protein, and a *minus* that it *does not*.

Bence-Jones Proteins

Ab	A	B	C	D	E	F	G	H
Rab α A	+	+	—	+	—	+	+	—
Rab α B	+	+	—	+	—	+	+	—
Rab α C	—	—	+	—	+	—	—	+
Rab α E	—	—	+	—	+	—	—	+

Table 6-1

Clearly, while we have *four* different antisera, we see only *two* different patterns of reactivity, since antisera made against A and B show the same pattern as each other, and those against C and E are also the same. The results indicate that these Bence-Jones proteins can be separated into two "kinds": one group (A, B, D, F and G) reacts only with one pair of antibodies (αA and αB), the other group (C, E and H) reacts only with the other pair (αC and αE). We could call these two kinds of B-J proteins types I and II, or types *a* and *b*, but they have actually been named ***Kappa*** and ***Lambda*** after their discoverers, Korngold and Lipari.

Thus we find that *all* B-J proteins (and, in fact, all human immunoglobulin light chains) can be classified as either kappa or lambda type. Now we ask whether these two varieties represent *isotypes* or *allotypes* of light chains? To answer this question we must use our antisera to test a large number of normal human sera; when we do this we find that **every normal serum contains both kappa chains and lambda chains**. Therefore, these two types of light chains **fulfill the definition of isotypes**. This implies that there are two separate genetic loci which encode these two light chain types - they are *not* encoded by two different alleles at a single locus.

Kappa and lambda light chains are both found in immunoglobulins of all mammals. In humans about 60% of all light chains are of kappa type, and 40% are lambda. In mice and rats, on the other hand, about 95% of light chains are kappa type, while the vast majority of light chains of horses and cattle are lambda-type. The biological significance of these differences is not known.

The various *heavy chain classes and subclasses* which have been listed in Chapter 4 are all *isotypes* of human heavy chains. Unlike light chain isotypes, however, heavy chain isotypes are known to differ from one another in a variety of important biological functions -- IgM is the most efficient at complement fixation, IgA is most efficiently secreted into exocrine fluids, *etc*. (see Chapter 4). [It should be noted that different vertebrate species have different numbers (and kinds) of heavy chain isotypes. Rabbits, for example, have only a single kind of IgG (*i.e.* they have no gamma-chain subclasses), while the major serum immunoglobulin in chickens is termed IgY.]

EXPERIMENTAL DEFINITION OF KAPPA CHAIN ALLOTYPES (InV/Km)

Let's analyze this collection of B-J proteins further. We now take our Rabbit-anti-B (which is one of our anti-kappa reagents) and test it again in Ouchterlony against four of the kappa type chains, A, B, D and F. This time, however, we will put the four antigens in nearby wells to examine their antigenic relatedness (as opposed to simply testing each one independently for its ability to precipitate with the antibody). The results are shown below:

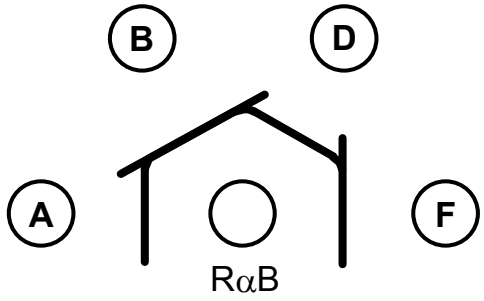

Figure 6-2

While Rabbit *anti-B* reacts with all four antigens, it clearly detects *more* epitopes on two of them, B and F, than on the other two. That is to say, in addition to detecting an epitope common to all of them, the antibody is detecting at least one epitope present on B and F which is *not* present on A and D.

In order to make the analysis clearer, we will **absorb** the antiserum, removing those antibodies which react with all four proteins and leaving only those antibodies reacting specifically with B and F. We do this by passing the antiserum over an "immunoadsorbent" column which contains antigen A covalently coupled to a matrix (see AFFINITY CHROMATOGRAPHY in APPENDIX 4). All those antibodies which can bind to B-J protein "A" will do so, all the others will pass through the column and will be recovered. The recovered antiserum (which passes through the column), we call RαB[abs A] . When we test this *absorbed* antiserum in the same manner as before, we see the results below:

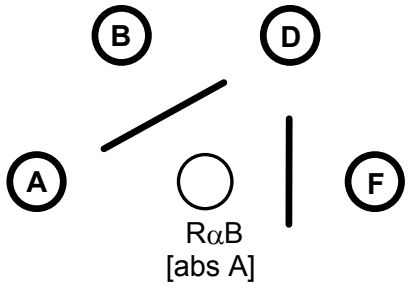

Figure 6-3

This absorbed antiserum now reacts *only* with B-J proteins *B* and *F*, and not at all with *A* and *D*. It therefore defines *two different forms of kappa chains*, which, when they were discovered, were originally called Inv^+ and Inv^-.

Now we need to ask once again, are these variants *isotypes* or *allotypes* of kappa chains? When we use this specific antiserum to test a large number of normal human sera, we find that *only some sera* show a reaction. Furthermore, we find that the presence or absence of this epitope (defined by Rab α B[abs A]) can be accounted for by the segregation of a pair of Mendelian alleles at a single locus (this information comes from family studies). This Inv factor is therefore *not* an isotype, but fulfills the definition of an *allotype*.

Thus, every individual human has kappa chains (an "*isotype*"), but the kappa chains in any individual's serum may exist as either or both of at least two allelic forms ("*allotypes*"); some people have only Inv^+ kappa chains, others have only Inv^- kappa chains, and some (who are heterozygotes) have both. Modern nomenclature replaces the name Inv with **Km** (for Kappa-marker), and a total of *three* allelic epitopes are now known to be defined by the Km locus, named Km(1), Km(2) and Km(3). Our anti-Inv antibody (Rab α B[abs A]) represents an *anti-Km(1)* antibody, and by a similar set of immunizations and absorptions we can produce specific antisera detecting *Km(2)* and *Km(3)*. Using these antibodies to study human populations, we can review the relationship between genotype and phenotype for the three alleles defined by this set of antisera, illustrated as follows:

Phenotype versus Genotype for Kappa Allotypes

Genotype	**Phenotype**
Km(1/1)	Km(1+)
Km(1/2)	Km(1+2+)
Km(1/3)	Km(1+3+)
Km(2/2)	Km(2+)
Km(2/3)	Km(2+3+)
Km(3/3)	Km(3+)

Since kappa chain alleles are *codominantly* expressed in all people (as is the case for *all* immunoglobulin genes), an individual who is heterozygous for the Km(1) and Km(2) genes will will always show a *positive* result when his serum is typed using either reagent. Thus, there are three alleles at the single *kappa* locus, and a total of six unique genotypes and phenotypes.

Many of the *human heavy chains* also show allelic variants. "Gm" is a general term for allotypes of human gamma chains (meaning "***G***amma ***m***arker"). "G1m", for example, specifies allelic markers of *gamma-1 heavy chains* which exist in several allelic forms, two of which are known as G1m(4) and G1m(17). Similarly, G3m specifies allelic markers of gamma-3 heavy chains, *etc*. As another example, while all normal human sera contain both IgA1 and IgA2 (which are therefore *isotypes*), the alpha-2 heavy chain present in IgA2 can exist as either of two allelic variants, which are known as A2m(1) and A2m(2).

Immunoglobulin allotypes have been known since the 1950's, with the discovery of rabbit allotypes by Oudin and of human allotypes by Grubb. Most of what we know of the remarkable organization of immunoglobulin genes, culminating in their characterization in the early 1980's by recombinant DNA techniques (which we will discuss in Chapter 8), was based on studies of the classical genetics of immunoglobulin allotypes in humans, rabbits and mice.

EXPERIMENTAL DEFINITION OF ISOTYPES OF HUMAN LAMBDA CHAINS (Oz)

Let's carry out for a third time the kind of analysis we just described for kappa chains, this time with the *lambda chain* Bence-Jones proteins in the panel above. We will use Rabbit *anti*-E (antiserum against one of the lambda chains) and test it against proteins C, E and H (all lambda chains), with the following results:

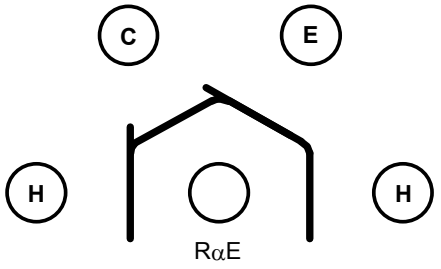

Figure 6-4

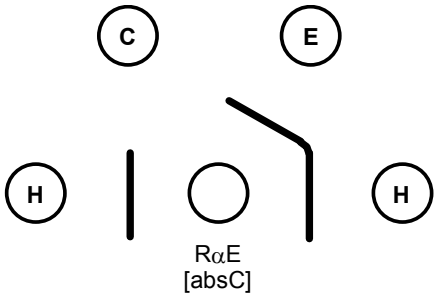

Figure 6-5

On the left is the pattern shown by the original antiserum, and on the right the pattern seen after the antiserum has been absorbed with protein C, as we had done before with the anti-kappa antiserum. We see that not all the lambda proteins are the same, *i.e.*, proteins E and H have at least one epitope that protein C lacks. We have thus defined a variant of human lambda chains which we call *Oz*; proteins E and H are Oz^+ while protein C is Oz^-.

Once again we ask if this represents an *allelic* variant of lambda chains, as Km is for kappa chains. When we test a large panel of normal human sera, we find that *every* serum contains Oz^+ lambda chains. (Every serum also contains Oz^- lambda chains, which we can determine by further analysis). Therefore we are not dealing with an allotype, but a *new isotypic variant*. While our earlier analysis had indicated the existence of at least two genetic loci for human light chains, one for kappa and one for lambda, we now have to hypothesize at least *two distinct loci* for lambda chains, one for each the two variants we have just defined (Oz^+ and Oz^-).

Since the original discovery of the Oz marker, two other isotypic serological markers have been found for human lambda chains, namely *Mcg* and *Kern*. These three specificities are found in four different combinations which define a total of four genetic loci encoding human lambda chains, named *lambda-1* through *lambda-4*. No *allelic* variants have yet been described for any of these four lambda isotypes, however.

EXPERIMENTAL DEFINITION OF IDIOTYPES: *INDIVIDUALLY SPECIFIC EPITOPES*

We will now examine the results of one additional set of analyses, this time following immunization of a rabbit not with a purified light chain, but with an entire *intact myeloma protein*. If we start (for example) with a protein which has a kappa light chain and a gamma-1 heavy chain (*i.e.,* a κG1 protein) we can predict the presence of specific antibodies to the *four distinct varieties of known epitopes* in the resultant antiserum:

anti-kappa. The rabbit should make antibodies which react with *all* kappa chains (anti-isotype), just as it did when we immunized with purified kappa chain.

anti-G1. We also expect to find antibodies which will react with all G1 heavy chains, defining the G1 isotype.

anti-Km. We might also expect to have anti-allotype antibodies. If the kappa chain happened to be Km(1), for instance, the rabbit should produce anti-Km(1), equivalent to the antibody we originally called "anti-Inv" in the example above.

anti-G1m. Similarly, we might expect the rabbit to recognize whatever G1m allotypic markers are present on the heavy chain. If the heavy chain happened to be G1m(17), for example, the rabbit could make antibodies to this epitope. (NOTE: While we need to consider this possibility, anti-Gm antibodies are not easily produced by heterologous immunizations, as in this example.)

Each of these categories of antibodies can be detected by Ouchterlony precipitation using an appropriate variety of myeloma proteins as antigens. Each of these four represents a *separate and distinct population of antibodies*, and each can be *removed* by appropriate absorption (as we have described above). What happens when we absorb (remove) all antibody activity to the four kinds of specificities listed above, then test the resultant absorbed antiserum against the original κG1 immunogen (as well as a variety of other myeloma proteins)? *Our prediction is that there should be no detectable remaining antibody, since we have removed antibodies to all those epitopes we know about.*

However, what we actually find is that **the absorbed antiserum still reacts with the original κG1**, although it does *not* react with any other myeloma protein (of *any* isotype or allotype composition). This antibody, therefore, defines an *idiotype*, or idiotypic epitope/specificity. It is "individually specific", reacting with the original immunogen but not with any other. Nor will it react with isolated light or heavy chains, even from the same protein, but it requires the presence of the *intact molecule*. Such an antibody is binding to an epitope created by the *combination of a particular V_H and V_L domain*, that is, *the unique antigen-combining site* of the immunizing myeloma protein. This idiotype will in general not be detectable in normal sera; even though the particular combination of V_H and V_L *may* be present on some normal serum Ig molecules, they will only be a few molecules among many thousands of different combining sites present in normal Ig and would be extremely difficult to detect.

CHAPTER 6, STUDY QUESTIONS:

1. Define and distinguish antibody ISOTYPE, ALLOTYPE and IDIOTYPE.

2. Given a newly produced antiserum directed against a human Bence-Jones protein, how would you determine if it recognized ISOTYPE or ALLOTYPE specificities?

3. How might you produce an *anti-G3*-specific antiserum? An *anti-Km(2)*-specific antiserum?

4. How would you produce an anti-IDIOTYPE antiserum?