Immunol.

THE UNIVERSITY OF TEXAS HEALTH SCIENCE
CENTER AT DALLAS

Department of Internal Medicine

MEDICAL GRAND ROUNDS

HLA-D REGION UPDATE

J. Donald Capra, M.D.
Professor of Microbiology
and Internal Medicine

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"Our hour is marked, and no one can claim a moment of life beyond what fate has predestined."

Napoleon Bonaparte

I. INTRODUCTION

The human major histocompatibility complex (MHC) or human leukocyte antigen (HLA) complex is located on the short arm of chromosome 6. Molecules encoded within the HLA complex have been implicated in the regulation of T cell and B cell differentiation, and in the ability of the host to mount a humoral and/or cell-mediated response against a myriad of antigens. Additionally, these molecules are thought to be involved in immunologic communication and cell-cell interactions that maintain the integrity of the immunologic system of an individual, including the ability to distinguish self from nonself.

At least three classes of molecules are controlled by the HLA region, each functioning in a distinct way to perform immunologic tasks. **The class I molecules,** HLA-A, -B, and -C, are the classic transplantation antigens. These molecules are responsible for graft rejection and regulate the killing of virus-infected cells. They are composed of two subunits, a 44,000 dalton heavy chain and a noncovalently associated 11,500 dalton light chain known as beta-2 microglobulin. The heavy chain is an intrinsic membrane glycoprotein which is HLA encoded and structurally polymorphic. Beta-2 microglobulin is an extrinsic, nonpolymorphic protein encoded on chromosome 15. The Class I molecules are expressed on all cell types except red blood cells.

The class II molecules, the HLA-D region antigens, are also composed of two subunits but unlike the class I molecules, both subunits are instrinsic membrane proteins and both subunits are encoded within the MHC. These subunits are noncovalently associated and consist of a heavy or alpha

chain of molecular weight 34,000 and a light or beta chain of molecular weight 29,000 (see Figure 1). The fact that both subunits of the class II molecule are encoded within the MHC is somewhat unusual. In most other cases in which multimeric proteins are made up of <u>different</u> subunits, the genes encoding the separate polypeptide chains are unlinked. Exceptions such as insulin and C4 arise when a precursor polypeptide is processed to form two or more subunits. The evidence is overwhelming that this form of processing does not occur for the class II antigens. While class I molecules have a ubiquitious tissue distribution, class II molecules are expressed most abundantly by B lymphocytes, activated T lymphocytes, and antigen-presenting cells including monocytes, macrophages, Langerhans' cells, and dendritic cells.

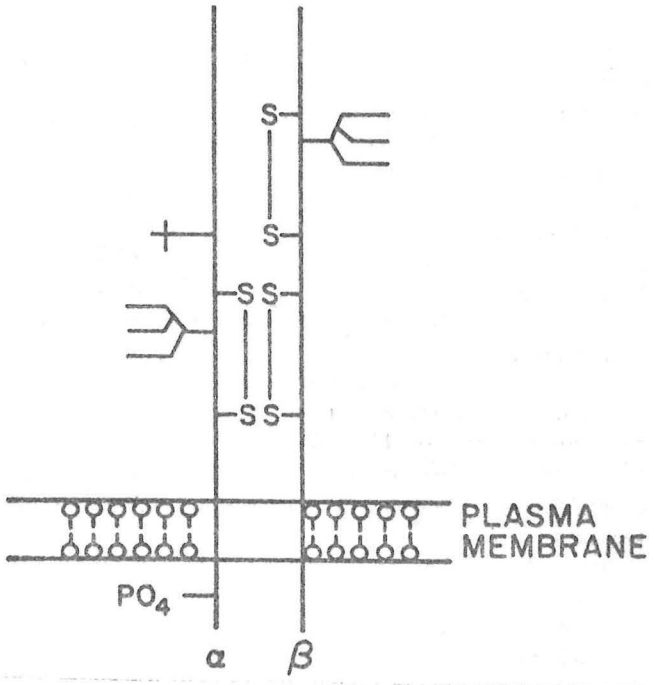

Figure 1. General structure of human class II molecules. S-S indicates disulphide bridges. The carbohydrate side chains are depicted as well as a possible phosphorylation site.

The early complement components encoded within or near the MHC are referred to as **class III molecules.** Collectively, they represent the C3 converting enzymes of the classical (C2 and C4) and alternative (Factor B) complement pathways (Carroll et al., 1984). These serum proteins participate in cell lysis and mediate inflammatory responses. Initially, it was thought that the class III molecules were not as polymorphic as the class I and II molecules but additional information indicates that they are at least as polymorphic. In addition to the complement components, two genes encoding adrenal cytochrome P-450 enzymes, specific for steroid 21-hydroxylation (21-OH) have recently been located within the "class III region" (White, et al., 1984) (see Figure 2). In humans, genetic defects in many of the steps of cortisol biosynthesis have been described although only in deficiency of cholesterol side-chain cleavage activity has a defective or deficient P-450 been documented. Of these inborn errors of metabolism, 21 hydroxylase deficiency is by far the most common, occuring in about 1/5000 individuals. It is inherited as a monogenic autosome recessive trait linked to the HLA gene complex. It is likely that this human disease is due to deficiencies or defects of one or both of these two 21-OH genes. Whether there are additional molecules encoded within the major histocompatibility complex remains to be seen although certainly there are suggestions of several others.

Figure 2 depicts our current concept of the genetic organization of the human major histocompatibility complex. The class II region, the HLA-D region has been divided into three "subregions." For simplicity, the subregions within the HLA-D region are shown to encode only a single molecule. However, in all instances, at least two polypeptide chains (an alpha

and a beta) are encoded within each subregion and in most instances multiple polypeptide chains are encoded within each subregion of HLA-D. The number of genes that are actually expressed is still a subject of controversy. The two "x's" in the figure indicate areas that may be recombinational hotspots as the vast bulk of recombinations that have been observed both within populations and families occur near these two points.

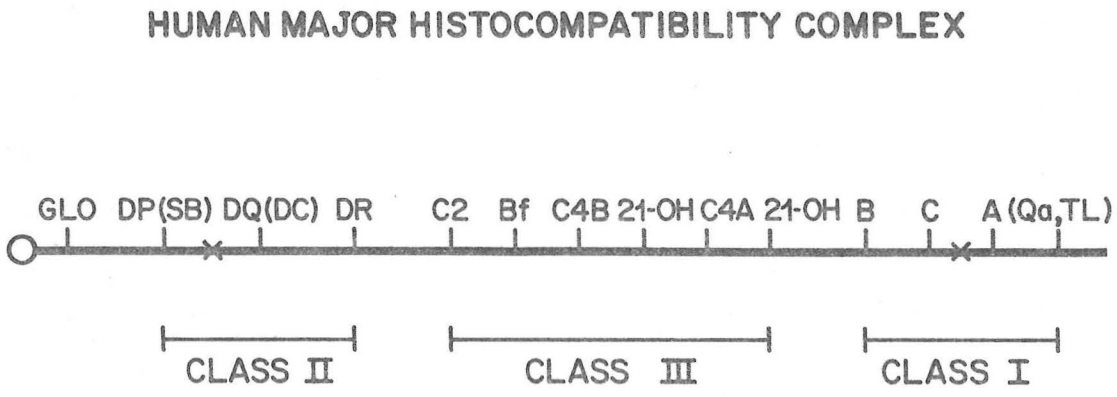

Figure 2. Genetic map of the human major histocompatibility complex. The precise order of some of these loci is not known. The centromere is located to the left.

Nomenclature has been and, at least for the near future, will continue to be a major problem in this area. Recently, at the Ninth International Histocompatibility Workshop, the nomenclature within the HLA-D region was changed considerably. The HLA-D region is now said to be comprised of three subregions referred to as HLA-DP (previously SB), HLA-DQ (previously DS, DC, MB) and HLA-DR (no change).

As our understanding of major histocompatibility complex-controlled immune responsiveness broadens and hybridoma and gene cloning technology advances, specific enhancement of desired immune responses and suppression

of deleterious ones will most likely become possible. The use of "state of the art" molecular biological techniques and the advent of highly discriminatory monoclonal antibodies have contributed to an explosion of information regarding the structure and function of this important family of molecules (Krangel, et al., 1980; Steinmetz and Hood, 1983; Kaufman, et al. 1984). Today's discussion will explore the complexity of the HLA-D region, with emphasis on the genetic organization, structure and function of the molecules.

II. HLA-DR SEROLOGY/HTC

All known human class II antigens are encoded in the genetic region centromeric to HLA-B and telomeric to the locus controlling the red cell antigen glyoxylase, Glo. This region is often loosely referred to as the "D" region. Recently, as is evident from Figure 2, three separate subregions of the HLA-D region have been defined. Historically the first phenotypic trait shown to be controlled by an HLA gene centromeric to HLA-B was its capacity to stimulate strong MLR in vitro. The trait was assumed to result from the product of a single gene designated HLA-D. However, at the present time, the exact contribution of the three separate subregions to the MLR is a matter of controversy. Indeed, many of these distinctions are just being worked out now that specific alloantisera and monoclonal antibodies are available for each of the subregions. It is likely, however, that the major MLR reactivity is due to disparity at the HLA-DR subregion and the majority of homozygous typing cell (HTC) reactivity will be subject to genes under the control of the HLA-DR subregion.

Tables 1 and 2 summarize our present understanding of some of the various serologic specificities extant in the HLA-DR region. Table 1 lists the Dw and DR specificities and gene frequencies from two classic papers. Table 2 indicates new provisional designations for HLA-DR specificities and, in particular, relates four new designations (DRw11-14) that were established at the Ninth Workshop and their previous equivalents.

TABLE 1

HLA CLASS II ANTIGENS

HLA-D		HLA-DR	
Allele	Frequency (%)	Allele	Frequency (%)
Dw1	7	DR1	7
Dw2	8	DR2	13
Dw3	8	DR3	11
Dw4	5	DR4	10
Dw5	6	DR5	10
Dw6	10	DRw6	2
Dw7	10	DR7	13
Dw8	3	DRw8	3
Dw9	1	DRw9	1
Dw10	3	DRw10	1
Blank	39	Blank	30

The HLA-D frequencies are from Dupont et al. (1980).
The HLA-DR frequencies are from Baur and Danilovs (1980).

TABLE 2

NEW PROVISIONAL DESIGNATIONS FOR HLA-DR SPECIFICITIES

New	Previous equivalents
DRw11	LB5
DRw12	LB5x8, DR5 short, FT23
DRw13	6.6, 6.1, 6Z
DRw14	6.9, 6.3, 6X, 901
DRw52	MT2
DRw53	MT3

At the present time there are still approximately 20-30% HLA-DR blanks in the Caucasian population and, therefore, it is likely that new specificities exist. Based, however, on what is known at the present time the following compilation of DR specificities is proposed:

Based on the most recent evidence, DR1, 3, 5, 7, w8, and w10 presently exist as single serologic entities; DRw9 as two; and DR2, 4, and w6 as three. This makes a total of seventeen serologic specificities that are likely to achieve status as DR alleles.

Obviously a goal of modern HLA genetics is to understand the structural basis for each of these specificities and while much of this information is available we are still ignorant on a number of different issues. Most of these specificities are likely (although not exclusively) encoded in the HLA-DR subregion which encodes a single alpha chain (non-polymorphic) and probably three beta chains. It has been exceedingly difficult to assign specific beta chain gene products to these polymorphisms. However it seems quite clear that the beta chains are the polymorphic component of class II antigens as no variation in alpha chains has been observed to explain any of these specificities. Whether differences in reactivity are explicable based on different beta chain gene products is still a matter of controversy.

Finally, since approximately 30% of the population are officially listed as "DR blank" it is likely that many additional specificities will be discovered. Recently, Moller, et.al. (1984) have described by restriction fragment length polymorphism three additional groupings by examining individuals who are homozygous DR blank/blank. It is likely, with the rapid advances being made utilizing this approach to HLA-DR typing that these 30% HLA-DR blank phenotypes will be dissected in the near future.

III. HLA-DQ SEROLOGY

As discussed previously many of the early studies which revealed the complexity of the HLA-D region were the result of serological analyses at the cellular level of alloantigenic specificities found on HLA-D region products. By the 7th Histocompatibility Workshop not only had clusters of alloantisera defining the HLA-D alleles been described, additional clusters of alloantisera demonstrated defined patterns of cross-reactivity, with each cluster of antisera encompassing two or more of the HLA-DR alleles. By the 8th Workshop, additional clusters of cross-reactive alloantisera had been described. Due to their ability to recognize several HLA-DR allelic products, these antisera were classified as recognizing "supertypic specificities." Those recognized to date include the MB, MT, DC, LB, BR and Te series (see Table 3). Each of these specificities is found in linkage

TABLE 3

SUPERTYPIC SPECIFICITIES

MB series			MT series		
	Associated DR specificities	Equivalent specificities		Associated DR specificities	Equivalent specificities
MB1	DR1, 2, w6, w8[a], w10[a]	DC1, Te21, MT1[b]	MT1	DR1, 2, w6, w10[a]	DC1, Te21, MB1[b]
MB2	DR3,7	DC3,Te24	MT2	DR3, 5, w6, w8[a]	BR3
MB3	DR4, 5, w9[a]	DC4,Te22,MT4	MT3	DR4, 7, w9, w10[a]	BR4

disequilibrium (associated strongly) with a number of DR specificities. For example, in the MB series, MB1 is associated with DR1, 2, w6, w8 and w10; MB2 with DR3 and 7; and MB3 with DR4, 5 and w9. Members of a given supertypic series were thought to represent allelic products since they segregate from one another in family studies and, particularly in the case of the MB series, are in Hardy-Weinberg equilibrium. Until recently the relationship between these independently defined supertypic series has not been

tionship between these independently defined supertypic series has not been clear. For example, on the one hand, many MB, DC, LB and Te specificities appear to have similar, if not identical, DR-associated distributions (i.e. MB1, DC1 - see Table 3) suggesting that they may represent the same segregant series. On the other hand, the MB and MT series (with the exception of MB1 and MT1) have very different DR-associated distributions and probably represent different series. With this in mind a simplified scheme relating the supertypic specificities to each other is presented in Table 3 (taken from Hurley et al., 1983a). In this way, the multiple supertypic series can be condensed into two series of alloantigenic specificities, MB and MT. The DC, LB, and Te series are similar to the MB series, and the BR series is similar to the MT series.

Controversy has revolved around whether these supertypic specificities represent shared (public) determinants on the associated HLA-DR molecules or specificities in linkage disequilibrium present on molecules distinct from DR. The first real breakthrough occurred when Tosi et al (1978) described the specificity DC1 which they argued was on a molecule distinct from DR. This was the first real evidence suggesting the existance of a second HLA-D region locus, although the structural basis of the DC1-bearing molecule was not determined for several years and the crucial nature of the discovery was not widely appreciated at the time (Tosi et al., 1982, 1984).

During the 8th Workshop a new polymorphic B cell system, the MT ("multi-specific") system, was defined (Park, et al., 1980). Attempts to understand and reconcile the MT system with MB (DC) led to much of the confusion concerning the second locus and the molecular localization of the supertypic specificities. The studies with DC1 and the MB specificities

strongly argued for a second locus, but because DC1, MB1, and MT1 were assumed to be identical specificities (see Table 3) and because many believed that the MT series represented an allelic series, the data regarding MB (DC) vs MT became almost impossible to reconcile. Not until studies on the biochemical structure of the second locus product were performed by several laboratories (especially using monoclonal antibodies) did it become apparent that MB (DC) was a specificity on a molecule distinct from HLA-DR. Even with this revelation regarding MB (DC), the molecular basis for the MT (BR) supertypic specificity was not apparent.

The second locus as defined by Tosi et al. (1978) and Duquesnoy et al. (1979) has been called by a variety of names since its discovery. Probably the most widely accepted was the DC terminology (named for Dora Centis who was instrumental in its discovery in the Tosi laboratory). Each specificity was designated as DC followed by the number of the first DR specificity in strong linkage disequilibrium. For example, DC4 was the specificity controlled by the DC locus in linkage disequilibrium with DR4 and DR5.

In some laboratories the locus was referred to as MB (for "More B" or "Milwaukee Blood") or LB (for "Leiden B") and in still others as DS (for "Secondary D") (Goyert, et al., 1982). It is now clear that molecules bearing the DC (MB) specificities show considerable variation among the DR alleles (Goyert and Silver, 1983, Giles, et al., 1984a, b, c). An HLA nomenclature committee met following the 9th Workshop and the designation "HLA-DQ" was adopted for the subregion. Specificities associated with this locus were given the designation HLA-DQ followed by "w" to indicate the designation is provisional and appropriate numbers, usually in sequence (i.e. HLA-DQw1, etc.) (see Table 4).

Table 4

NEW PROVISIONAL DESIGNATIONS FOR HLA-DQ SPECIFICITIES

New	Previous equivalents
DQw1	MB1, DC1, MT1, LB-E12, Te21
DQw2	MB2, DC3, Te24, LB-E17
DQw3	MB3, DC4, MT4, Te22

As several biochemical studies have demonstrated that both the DQ alpha and beta loci are polymorphic (Giles, et al., 1984c), the question arises as to why DQ allele-specific alloantisera have not been readily available. One plausible explanation is that any such alloantisera which do exist would have been designated as "DR-specific alloantisera." In fact, recent evidence suggests that such alloantisera do exist, either as solely DQ-specific sera or in conjuction with DR allele-specific alloantisera (Stastny, et al., 1984). Using fluorescence inhibition with monoclonal antibodies and lysostrip experiments, several alloantisera which recognize "private" specificities on DQ molecules (refered to by the authors as "DS private" or "DSP" specificities) have been described. Thus, in addition to the broad supertypic specificities DQw1 (MB1), DQw2 (MB2) and DQw3 (MB3), the DQ molecules also carry allele-specific specificities.

The role of the HLA-DQ products in primary and secondary MLR has been difficult to assess due to the extremely high linkage disequilibrium with HLA-DR. These studies are possible now that "subregion specific"

monoclonal antibodies and alloantisera can be defined. For example, Stastny's group (Stastny, et al., 1984) demonstrated that many antisera that are referred to as DR typing reagents actually have specificities for the HLA-DQ subregion products. These specificities are not MB (DC) super-typic specificities for if they were the antisera from the beginning would have been considered MB rather than DR typing sera. However, it appears that the product of each DQ allele is biochemically distinct from every other. Thus, it is not surprising that many antisera have now been found that recognize, for example, the DQ molecule which is generally associated with DR4 and not the DQ molecule which is generally associated with DR5. By careful dissection of antisera that, for example, recognize the DQ molecule associated most commonly with DR4 but not the DR molecule in DR4 cell lines, Stastny's group has been able to demonstrate specific contributions of DR subregion products versus DQ subregion products in several systems. These are largely done by blocking reactivities in the MLR with highly defined alloantisera or monoclonal antibodies specific for the products of each of the subregions. By this means the generalization which has emerged is that the vast majority of reactivities are, indeed, due to the HLA-DR subregion product disparities but that in several specific instances, the DQ subregion products can contribute significantly and, indeed, be the sole reactors in an MLR. Similar circumstances pertain for antigen presentation and other reactivities that had previously been referred to as "HLA-D." In general SB gene products (now termed DP) do not appear to significantly contribute to these reactivities. However, in certain rare instances clear reactivity due to DP differences can be correlated with MLR.

IV. HLA-DP: SEROLOGY/HTC

The primed lymphocyte test (PLT) has been used to identify an additional series of polymorphic HLA-D region antigens recently referred to as HLA-DP (formerly known as SB or FA). In this test, responder lymphocytes are selectively sensitized to stimulator lymphocytes which differ from the responder by a few, or a single, stimulatory determinant(s). Such sensitized cells are then restimulated and a "secondary MLR" is performed. Shaw used this technique to demonstrate a "secondary B" (SB) cell system, HLA-DP, which has been shown to consist of at least six alleles (Shaw et al. 1980, 1982, Pawelec et al., 1982a, b, c, 1983). A recombination between HLA-DR and HLA-DP and two recombinations between HLA-DR and the enzyme marker GLO1 mapped the HLA-DP locus centromeric to HLA-DR and telomeric to GLO1 (Shaw et al. 1981). Kavathas et al. (1981) also demonstrated that HLA-DP was a distinct locus using HLA deletion mutants. From a series of gamma-ray induced mutant B cell lines which had lost the expression of HLA-DR, -A, and -B, they found two mutants that were not capable of inducing a DR specific stimulation, but were capable of HLA-DP specific stimulation.

Attempts to define HLA-DP products serologically have been relatively difficult. The DP molecule is apparently a poor immunogen compared to DR and DQ, and alloantisera defining DP are rare. Monoclonal antibodies with polymorphic specificity for DP have also been rather scarce. One DP specific antibody, I-LR1 (Nadler, et al. 1981), has been shown to react with certain DP allelic products (HLA-DPw2, w3, and some DPw5 molecules). The initial biochemical characterization of an HLA-DP molecule

was performed using this monoclonal antibody. More recently another monoclonal, B7/21, has been described which also recognizes the HLA-DP product (Royston et al., 1981). Unlike I-LR1, B7/21 is apparently monomorphic, reacting with the product of all known HLA-DP alleles. The antibody, also known as anti-FA, is specific for an antigen which maps identically to DP in HLA haplotype loss mutants. Most convincingly, the anti-FA antibody has been used successfully to recognize L cells which have been transfected with the HLA-DP alpha and beta genes (Bodmer, W.F., personal communication).

As will be described later, there is growing evidence that there are two HLA-DP gene products expressed in B cells (particularly B lymphoblastoid cell lines). It has not yet been determined whether any or all of these monoclonal antibodies react with one or both of these gene products. Additionally, if both gene products are expressed, it is not known whether one or both are polymorphic.

V. HLA-DR BIOCHEMISTRY

The early studies on human class II molecules involved bulk isolation of molecules and the antigens were often isolated from cell lines that were not class II homozygous. Considerable confusion existed as to the relationship between the isolated molecules and the allotypic specificity expressed by the cell line. The major breakthrough was the development of monoclonal antibodies. The alloantisera that had been used were simply too weak or not available in enough quantity for the isolation of enough material which reacted with specific antisera of defined characteristics to be of general use. The monoclonal antibodies were extremely important not only in sorting out the genetic complexity of the HLA-D region but also in isolating molecules for biochemical analysis. By and large early studies used monoclonal antibodies that, in retrospect, were very poorly characterized. Relatively few monoclonal antibodies are truly subregion specific and even fewer are specific for the products of specific alleles. In addition, the extraordinary complexity of the region was not appreciated; that is, while most workers anticipated a human counterpart of the murine I-A and I-E subregion encoded molecules, few expected that the I-E counterpart, the HLA-DR subregion, would encode multiple beta chains and none had predicted the presence of the HLA-DP locus which had not been defined in the mouse.

Be that as it may, significant progress occurred in the period between 1980 and 1982 as monoclonal antibodies were applied to the problem. Kaufmann, et al. (1980) and Walker et al. (1980) performed peptide mapping

and Charron and McDevitt (1980) two dimensional gel electrophoresis analysis of several different cell lines with a panel of monoclonal antibodies. The results were fairly conclusive in illustrating that the heavy chains of different HLA-DR allospecificities were virtually identical in all cell lines examined but the light chains of different HLA-DR allospecificities differed and, therefore, must bear the alloantigenic determinant. These studies effectively put to rest the controversy as to which of the two chains bore the polymorphic determinants. Later investigators would detail that additional beta chains were encoded in the HLA-DR subregion and would more fully document the extent of variability between different beta chains that arose from different alleles (Kaufman and Strominger, 1982). Recently, Hurley et al. (1984) and Giles et al. (1984b) have isolated HLA-DR beta chains from two different homozygous cell lines of each HLA-DR haplotype (DR1-7). The chains were isolated using the DR-reactive monoclonal antibodies L203 and/or IIIE3. Two cell lines of each DR haplotype were used to insure that any variation demonstrated represented an allelic difference and not a random point mutation. Allelic polymorphism was found among the DR beta chains examining only amino-terminal tyrosine sequences.

The next important development occurred when a group of investigators began to appreciate that the complexity of either sequences (Hurley, et al., 1982a, 1983b), or two dimensional gels (Schackelford and Strominger, 1980, deKretser et al., 1982, 1983) was greater than could be anticipated from a single alpha and beta chain in the HLA-DR subregion. There were at least three interpretations of these data. First, that variable glycoscylation patterns explained much, if not all, of the heterogeneity.

Secondly, that there were multiple beta chain loci within the HLA-DR subregion and finally, that some of the molecules were encoded in a separate subregion, possibly analagous to the murine I-A subregion.

Few investigators fully appreciated the extraordinary complexity of the whole HLA-D region or, indeed, the additional complexities that were introduced by the heteroantibodies, even though they were monoclonal. Important insights then began to derive from monoclonal antibodies to HLA-D region products that were characterized as being either a) monomorphic for the HLA-DR subregion alone and lacked activity for any other subregion product (these studies were greatly facilitated by studies with deletion mutants), or b) polymorphic particularly the development by Johnson et al (1982), and Radka et al. (1983) of monoclonal antibodies that behaved with HLA-DR subregion products precisely as alloantisera. Finally, around 1982 it was appreciated that despite the advantages of having cell lines which grew well, a far more crucial determinant was homozygosity and the wide distribution of these cell lines (particularly through the international workshop route) lessened the confusion considerably. In addition, at this time complete structural analysis of DR alpha and beta chains emerged from the biochemical analysis from the Hilschmann laboratory (Yang, et al., 1982; Kratzin, et al., 1983) and from cDNA clones from Lee, et al., (1982), Korman, (1982a, 1982b), Wiman et al. (1982a, b), Gustafsson et al., (1982), and Larhammar et al., (1982a, b), such that the general structural features of the molecules, as well as allelic variation began to be appreciated.

The overwhelming evidence at the present time is that the HLA-DR

subregion encodes a single nonpolymorphic alpha chain and two or three extensively polymorphic beta chains (Figure 3). The reason for hedging on a number of beta chain loci largely derives from the very real possibility that the number of beta chain loci may differ in different haplotypes.

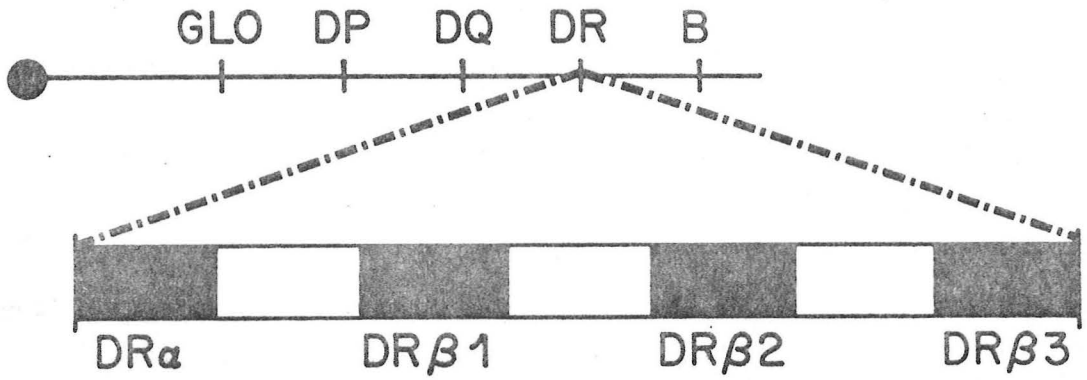

Figure 3. Schematic representation of the HLA-DR subregion derived from biochemical studies. The order of the genes is unknown. The alpha chain is not polymorphic. Probably all three beta chains are polymorphic although this point is not certain.

The structure of an HLA-DR alpha chain cDNA is shown in Figure 4. This amino acid sequence was also deduced by Kratzin, et al. (1983). Five laboratories have reported complete alpha sequences and while there are a few discrepancies they are likely of little consequence. The alpha chain is composed of 229 amino acids of which 191 are exposed on the outside of the plasma membrane. The membrane imbedded portion of the chain is thought to consist of 23 hydrophobic amino acids. The succeeding 15 amino acids form the cytoplasmically localized hydrophylic tail. The extracellular portion with carbohydrate moieties linked to Asn^{78} and Asn^{118} seems to be

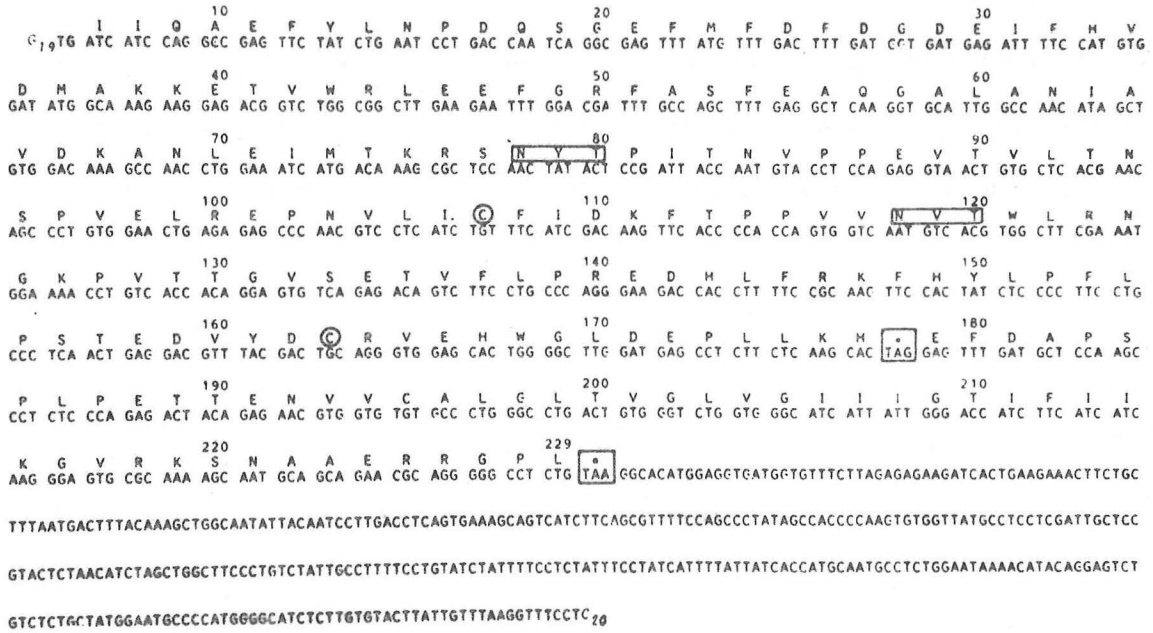

Figure 4. Nucelotide sequence and predicted amino acid sequence of a DR alpha chain cDNA. Residues in boxes denote the attachment sites for N-linked carbohydrates; c, cysteine residues.
From Larhammar et al., 1982a, b.

organized into two domains. The second domain (which contains the only disulphide bond of the alpha chain) displays amino acid sequence homology to immunoglobulin constant regions as well as to the second domain of the beta chain of class II antigens (see below), to the third domain of heavy chains of class I molecules and to beta-2 microglobulin. These observations were made virtually simultaneously by Larhammar, et al. (1981, 1982a, b, 1983b), Korman, et al. (1982b), Yang, et al. (1982) and Lee, et al. (1982).

The structure(s) of the beta chains has been somewhat more difficult to obtain but four different laboratories have obtained complete beta chain sequences. The protein sequence was derived by Kratzin, et al. (1980) and cDNA clones were obtained by several laboratories. The amino

acid sequence has 229 amino acid residues. It has two immunoglobulin-like disulphide loops and a 21 amino acid residue membrane integrated segment. Ten amino acid residues reside on the cytoplasmic side of the plasma membrane. The single asparagine-linked carbohydrate moeity is attached to Asn^{19}. The amino terminal 91 residues of the beta chain are homologous to the corresponding region of HLA-A, -B, and -C antigen heavy chains. Residues 92 to 192 of the beta chain display statistically significant homology to members of the immunoglobulin family, beta-2 microglobulin, and the immunoglobulin-like domains of HLA-A, -B, and -C antigen heavy chains.

A model depicting the general overall structure of class II molecules which was originally described for the HLA-DR subregion by Korman et al., (1982b) is shown in Figure 1. It illustrates the general structure of the molecule showing the heavy and light chains (alpha and beta) to be similar in size despite their apparent differences by SDS gels. These differences are due to the fact that the alpha or heavy chain has two carbohydrate attachment sites whereas the beta chain has but a single site (Shackelford and Strominger, 1983). The alpha chain is depicted to be phosphorylated at its hydrophilic carboxyl terminus (Kaufman and Strominger, 1979), although there is evidence that most if not all phosphoylation of class II molecules occurs on the gamma chain (P. Peterson, personal communication). Figure 5 shows a comparison of the amino acid sequence of the alpha II domain of the heavy and light chain of HLA-DR and HLAB7, beta-2 microglobulin and the C gamma-3 domain of immunoglobulin G, illustrating the homology between these structures.

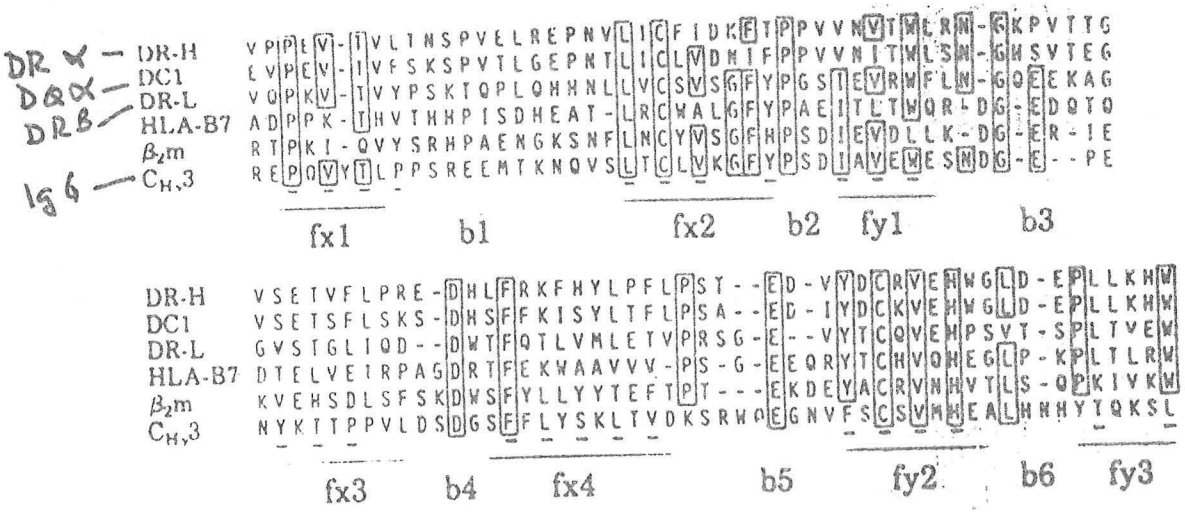

Figure 5. Amino-acid sequence comparisons (one-letter code) of the second domain of DR alpha with the sequence of the second domain of DR beta, the third domain of HLA-A, HLA-B and HLA-C, beta 2-microglobulin, the constant domain of the kappa light chain (C_L) and the three constant regions of an IgG1 heavy chain (C_H1, C_H2 and C_H3). Boxes: residues shared by the sequence of the DR alpha chain and any of the other sequences.
From Larhammar et al., 1982b.

VI. HLA-DQ BIOCHEMISTRY

Several studies using a variety of techniques have been responsible for definitively proving the existence of a second locus in the HLA-D region. Shortly after their initial findings, Corte et al. (1981) used a monoclonal antibody (BT3/4) to demonstrate by two-dimensional peptide mapping that the molecule which bears the DQw1 specificity was biochemically distinct in both its alpha and beta subunits from those that carry the DR specificities. Shackleford et al. (1981a) used human alloantisera and the monoclonal antibody Genox 3.53 (anti-DQw1) to demonstrate that the supertypic specificity DQw1 was carried on a two-chain molecule, which as judged by two-dimensional gel electrophoresis, was distinct from the DR molecule.

Although many of the early reports demonstrating a second locus suggested possible homology of this locus with the murine I-A locus final proof of this hypothesis awaited amino acid sequence analysis of the DQ molecule. Goyert, et al. (1982) first demonstrated homology of the DQ molecule with the murine I-A molecule by amino-terminal amino acid sequence analysis of both the alpha and beta chains. Using a monoclonal antibody SG171, which recognizes DR and DQ in DR7 cell lines, and also a rabbit antiserum Rb03, which reacts solely with DQ in all cell lines tested, they described the presence of an I-A homologue in at least two DR haplotypes. Bono and Strominger (1982, 1983) confirmed that the DQw1 specificity resided on the DQ molecule. Amino terminal sequence analysis of the DQw1 bearing molecule isolated using the monoclonal antibody Genox 3.53 (anti-

DQw1) revealed homology of the alpha chain to the murine I-A alpha chain. The beta chain isolated in this study appeared to be blocked at the amino terminus. Subsequently, Giles et al. (1983) using the monoclonal antibody IVD12, demonstrated that the supertypic specificity DQw3 (MB3, DC4) also resides on an HLA-DQ molecule. These data confirmed that the DQw1 and DQw3 supertypic specificities reside on HLA-D region molecules which represent allelic products of the HLA-DQ subregion. There is good evidence suggesting that the DQw2 (MB2, DC3) specificity also resides on a DQ molecule (Karr et al., 1983, 1984) further strengthening the original argument that the MB-bearing molecules represent an allelic series (Duquesnoy, et al., 1979).

In addition to the serological and structural variation mentioned previously for the DQ molecules bearing the DQw specificities (i.e., DQw1 vs DQw3), there is a growing body of evidence that molecules bearing the same supertypic specificity are distinct from one another. The first example of this distinction was reported by Shackelford et al. (1983), who demonstrated by two-dimensional gel electrophoresis that the beta subunit of a DQw1-bearing molecule isolated from a DR2 cell line was distinct from the beta subunit of a DQw1-bearing molecule from a DRw6 cell line. Goyert and Silver (1983) showed by two-dimensional gel electrophoresis that DQ beta chains varied in electrophoretic mobility depending upon the haplotype from which the chains were derived. More recently, Giles et al. (1984c) determined the amino-terminal tyrosine sequences for DQ molecules which bear the DQw3 determinant isolated from two DR4 and two DR5 homozygous cell lines and showed that although the distribution of the amino-terminal tyrosine residues in the alpha chains was identical, differences existed bet-

ween DQ beta chains isolated from the cell lines of differing DR specificities. This work has since been extended to include amino terminal tyrosine sequences of DQ molecules isolated from two cell lines of each DR haplotype (DR1-7). (Giles, et al., 1984b). By this limited analysis, a minimum of three allelic forms of DQ alpha and five allelic forms of DQ beta were found. When examining appropriate combinations of DQ alpha and beta chain molecules, six out of seven haplotypes examined could be distinguished from each other. These data demonstrate at the primary structural level allelic polymorphism of both the alpha and beta chains of the HLA-DQ molecule.

Shortly after the demonstration of allelic polymorphism of murine class II molecules, evidence was presented for the formation of hybrid molecules in F_1 animals providing a possible molecular mechanism for the phenomenon of gene complementation (Silver et al., 1980, Cook et al., 1980). This trans gene complementation increases the alloantigen repertoire in heterozygotes.

Recently Giles et al. (1984b) examined two DQ heterzygous cell lines and demonstrated the formation of hybrid DQ molecules within these cells. The DQw3-specific monoclonal antibody IVD12, which by Western blotting analysis reacts with isolated DQ beta chains from DQw3 positive cells, was used to isolate ^{3}H-tyrosine labeled DQ molecules from two cell lines typed as DQw2/DQw3 heterozygotes. Since the amino-terminal tyrosine sequences of DQw3.4 and DQw3.5 alpha chains are distinct from DQw2.7 alpha chains (DQw3.4 and DQw3.5 alpha chains possess a tyrosine at position 25, whereas the DQw2.7 alpha chain does not) it was possible by

subjecting the separated chains of IVD12-reactive molecules to amino-terminal amino acid sequence analysis to compare the results between homozygous and heterozygous cell lines in order to test directly for trans complementation.

In addition to the polymorphism found associated with the DQ molecules, further complexity in this family of molecules might exist through the expression of multiple DQ molecules within a single DR homozygous cell line. Although recent findings demonstrate the presence of multiple DQ-like alpha and beta chain genes within the genome of a single cell (Auffray, et al., 1983), it is not known how many of these genes are expressed. Several studies have attempted to show that at least two DQ molecules are expressed in a cell line.

If, indeed, there are two DQ alpha and two DQ beta chains expressed in a homozygous cell, the possibility obviously exists for combinatorial associations leading to a maximum of four distinct molecules in a homozygous cell line (alpha 1 beta 1, alpha 1 beta 2, alpha 2 beta 1, alpha 2 beta 2) and if full trans association occurs in the HLA-DQ subregion, heterozygous individuals could generate as many as 16 distinct molecules.

At the time of this writing there are four complete sequences of DQ alpha chains available and two nearly complete sequences of DQ beta chains. Like the DR situation, one set of these structures was developed in the Hilshmann laboratory by classical protein chemical techniques. The structure of the alpha and beta chains of DQw1 bearing molecules was derived in this manner by Gotz, et al. (1983). The majority of the structures that

are available for both the alpha and beta chains of the HLA-DQ subregion are from cDNA and genomic sequences (largely the former). It is important at the outset in describing these structures to appreciate that at the time of this writing it has not been definitively established a) which of these chains are conclusively expressed, and b) in each instance, whether the two structures being compared are allelic; that is, as has been mentioned above, there is the likelihood that there are a minimum of two expressed DQ alpha chains and two DQ beta chains. There is some evidence that one set of these molecules bears the supertypic specificities DQw1, 2, and 3. It is possible, although not conclusively shown that the so-called DX alpha and beta chain represents the second DQ product. It is equally possible that these represent products of a separate subregion. When investigators isolate molecules by bulk isolation, or when cDNA or genomic clones are sequenced, it is not known which of the products is being compared as this would require extensive serologic and functional analysis. Thus it is possible that one laboratory's cDNA clone of an alpha chain of the DQ subregion isolated, for example, from a DR4 homozygous cell line is not the true allele of the DQ alpha chain cDNA isolated from a DR1 homozygous cell line. One of these chains may represent DQ alpha 1 and the other represent DQ alpha 2. The complete nucleotide sequence and predicted amino acid sequence of the DQ alpha cDNA clone determined by Schenning, et al. (1984) is shown in Figure 6.

Figure 6. Nucleotide sequence and predicted amino acid sequence of a DQ alpha cDNA clone. Cysteines involved in the intramolecular disulfide bond and the attachment sites for N-linked carbohydrates are within boxes. From Schenning et al., 1984.

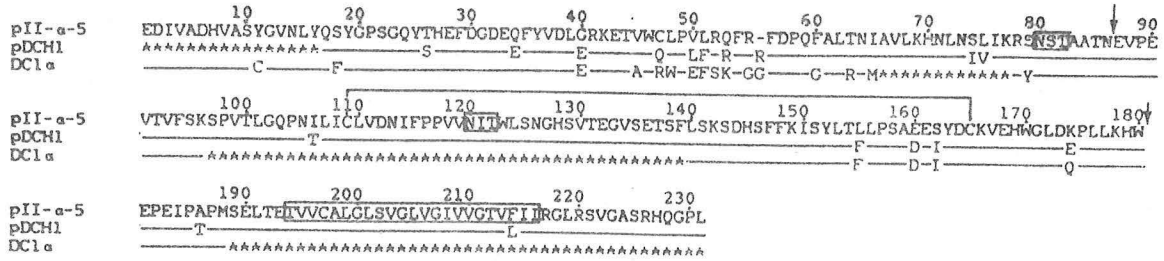

Figure 7. Comparison of three available DQ alpha amino acid sequences. The pII-alpha -5 sequence is derived from a cDNA clone of the DR3,w6 cell line Raji, and pDCH1 is from a cDNA clone of a DR4,w6 cell line (Auffray et al., 1982). DC1 alpha is a protein sequence from a DR2,2 cell line (Gotz et al., 1983). Stars denote amino acid residues not available for comparison. Arrows mark exon boundaries. Sites for addition of N-linked carbohydrate and the membrane-spanning segment are within boxes.

A comparison of this sequence with a DQ alpha chain derived from a cDNA clone of a DR3/w6 cell line (Raji) and a second DQ alpha chain from a cDNA clone of a DR4w6 cell line (Auffray et al, 1982) and the DQ alpha sequence derived from a DR2 homozygous cell line by Gotz et al. (1983) is shown in Figure 7. The striking structural homology is evident. The first noteworthy observation is that the three chains are decidedly different in structure. Recall that DR alpha chains are essentially identical. Indeed in the DR alpha chain, only a single variant position has been found although DR alpha sequences from six different sources have been described. Although the majority of the DQ alpha chain sequences are incomplete (either incomplete cDNA clones or incomplete amino acid sequence data), alignment of the sequences is easy and the majority of the polymorphism appears to be in the N-terminal or first domain (alpha 1 domain) - residues 1-86. There is approximately 15% difference between the three chains in this area. The second domain, residues 87-180, shows only four or five differences depending upon the comparison and even these differences tend to be clustered. Recall the second domain of class II molecules is remarkably homologous to immunoglobulin and in this region not only are the DQ alpha chains more similar to each other but, as we will discuss later, they are more similar between DR and DQ alpha. The three sequences show considerable homology through the transmembrane and cytoplasmic regions although the data here are less complete due to the amino acid sequence being incomplete. Peterson's group has pointed out the possibility that the second domain and transmembrane domains may be crucial in the interaction between alpha and beta chains of the different heterodimers and, therefore, have been allowed to diverge less (Schenning et al., 1984). Conversely, the

difference between the various types of alpha and beta chains respectfully may have to be large enough to prevent formation of hybrid antigens such as DQ alpha with DR beta. This region of the molecule would be a likely source for this kind of difference.

Figure 8 shows the cDNA sequence and derived amino acid sequence for a DQ beta chain; cystines and the attachment sites for n-linked carbohydrate are within boxes. Arrows mark exon boundaries inferred from the DQ beta gene. Figure 9 compares the amino acid sequence of four nearly complete DQ beta chains derived from different cell lines of different DR type. Again, the bulk of the variation between these molecules appears in their first domains. However, three minor clusters of "hypervariability" can be seen between positions 52 and 57, 70 and 77, and 84 and 90. In no position do all four of the DQ beta chains have a different amino acid. However three different amino acid residues occur at 7 positions, 6 of which are located in the amino terminal domain. This is likely to be far more variation than is due to chance alone and it is likely that the the amino terminal domain of the beta chain of the DQ molecule is the seat of allelic polymorphic variation that results in various functional allospecificities. These data also suggest that the variation is not without limit and that only certain positions may exhibit variation.

Figure 8. Nucleotide sequence and predicted amino acid sequence of a DQ beta cDNA clone. Cysteines and the attachment site for N-linked carbohydrate are within boxes. Arrows mark exon boundaries inferred from a DC beta gene. From Larhammar et al., 1983b.

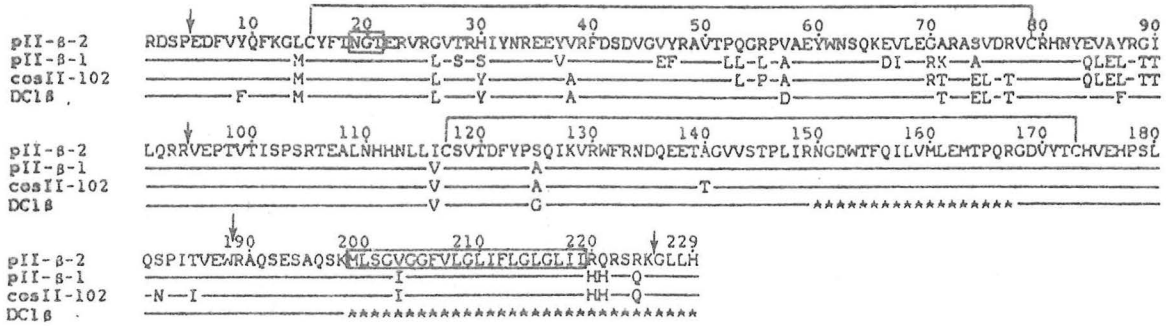

Figure 9. Comparison of DQ beta amino acid sequences. The pII-beta-2 and pII-beta-1 sequences are derived from cDNA clones of the DR3,w6 cell line Raji (Larhammar et al., 1982b) and cosII-102 from a gene of a DR4,4 individual (Larhammar et al., 1983b). DC1 beta is a protein sequence of a DR2,2 cell line (Gotz et al., 1983). Stars denote amino acid residues not available for comparison. Arrows mark exon boundaries. The site for addition of N-linked carbohydrate and the membrane-spanning segment are within boxes. From Schenning et al., 1984).

VII. BIOCHEMISTRY HLA-DP

The discovery of the monoclonal antibody, I-LR1, shown to be reactive with some of the allelic products of HLA-DP led to the initial biochemical characterization of the DP molecule. Using this antibody, the molecule isolated was shown to consist of two chains resembling the alpha and beta chains of the DR antigens in molcular weight as measured by sodium dodecyl sulfate gel electrophoresis (Nadler, et al., 1981). Partial amino-terminal amino acid sequence analysis has been performed on I-LR1-reactive molecules (Hurley, et al., 1982b, Hurley, et al., 1983b). These data were the first to demonstrate at the primary structural level that the alpha and beta chains of the DP antigens were distinct from the alpha and beta chains of the DR and DQ antigens. These sequence data were also crutial in allowing others to verify potential cDNA and genomic clones as DP equivalents.

Unlike HLA-DR and -DQ where biochemical analysis has been performed on "cold" material, the only information presently available derived from HLA-DP molecules comes from that reported by Hurley et al. (1982b) using radiolabelled material. Only a single cDNA sequence of DP alpha has published (Auffray et al. 1984) and it is shown in Figure 10. Note, as indicated by stars in the DP alpha sequence, the concordance of the sequence to the report of Hurley et al. (1982b).

Three separate laboratories have reported the isolation of cDNA clones comprising the bulk of the DP beta chain. Unfortunately, however,

none of the cDNA clones provide a complete beta chain sequence as one begins at amino acid position 60, one at position 50 and one at position 6. Thus, the information that is available at the present time is lacking in the amino terminal portion of the DP beta chain. Gustafsson et al. (1984a) have isolated a cDNA clone of DP beta as well as a genomic clone. The composite is shown in Figure 11. Comparison of the three available (none is complete) sequences of DP beta (Gustafsson et al., 1984a, Long et al., 1984, Roux-Dosseto et al., 1983) is complicated for the same reasons as in DQ alpha and beta comparisons - that is - allele vs. locus cannot be adequately addressed. However, assuming these are allelic, they are remarkably similar with greater than 95% homology in the regions that can be compared.

As will be evident later, it is likely that there are two DP alpha and two DP beta genes that are closely homologous. Which of the alpha/beta pairs reacts with monoclonal antibodies such as ILR1 and B7/21 and which have been isolated by cDNA cloning are subjects of future investigation. At the present time, there is no firm data that both of these genes are expressed and there is some data that one DP alpha gene may be nonfunctional.

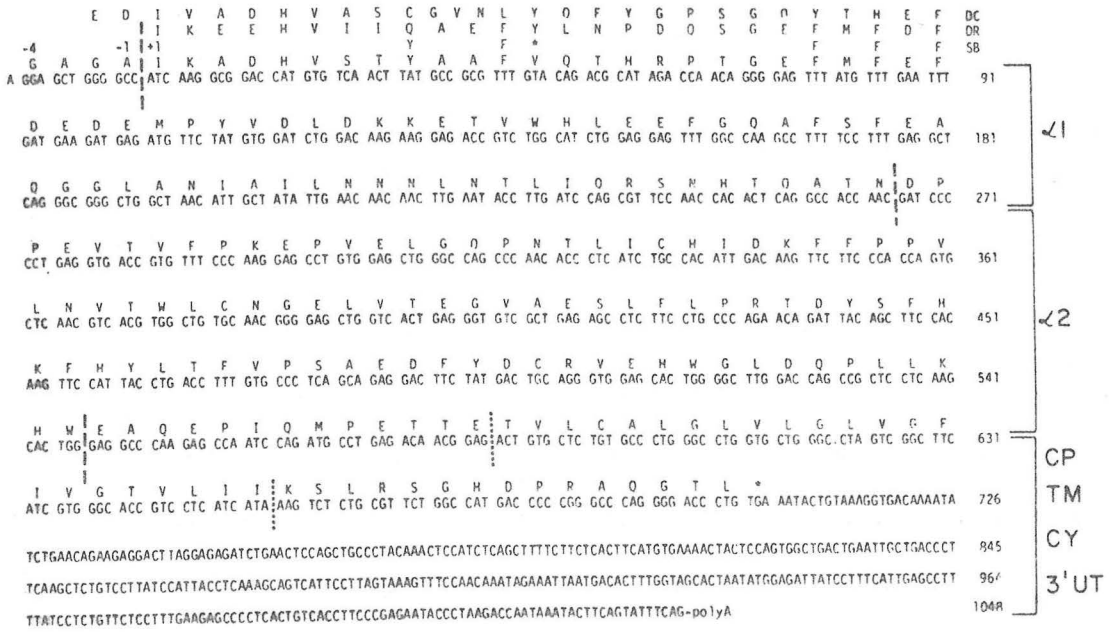

Figure 11. Sequence of cDNA clone of the DP alpha chain. The domains are indicated to the right. From Auffray et al., 1984.

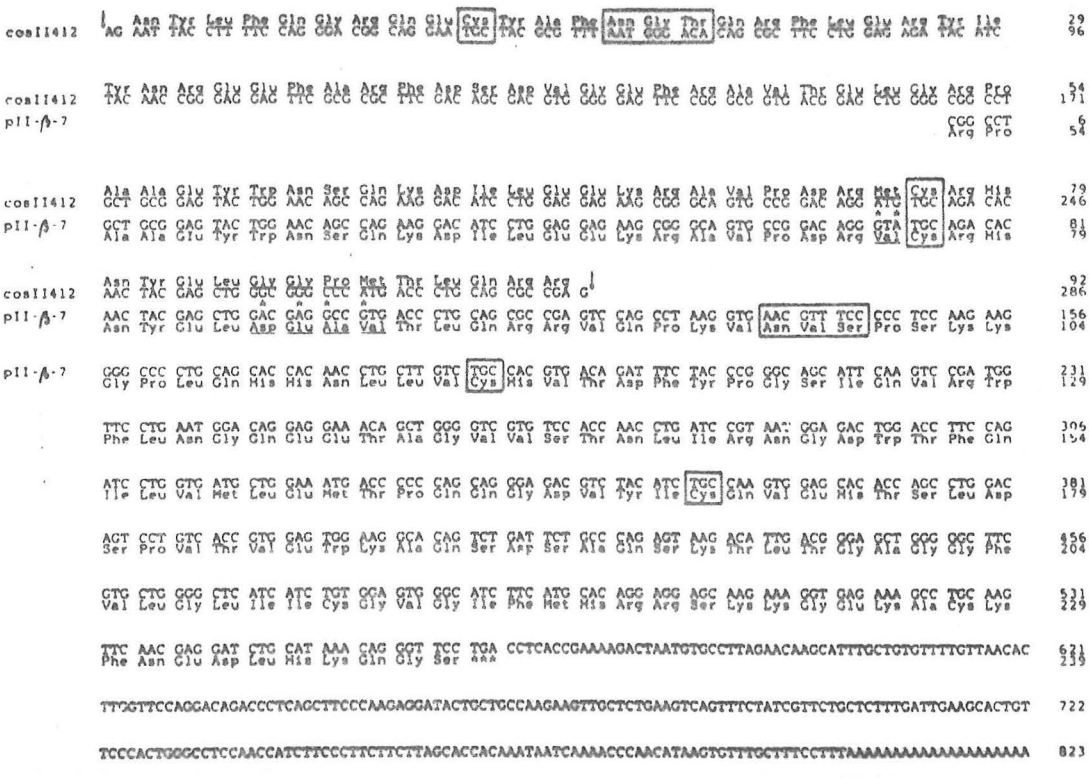

Figure 12. The nucleotide and predicted amino acid sequence of a DP beta cDNA clone and of the first domain exon of a genomic DP beta clone (cosII-412). Asterisks denote nucleotide substitutions between the two sequences. Amino acid replacements are underlined. Cysteine residues are boxed, as are two putative attachments sites for N-linked oligosaccharides at Asn 19 and Asn 98. From Gustaffson et al., 1984a.

VIII. SUPERTYPIC SPECIFICITY LOCALIZATION

The molecular bases of each of the supertypic specificities has been an active area of investigation during the last five years. This section attempts to summarize a comtemporary view on localizing the supertypic specificities to one or more of the three groups of class II molecules, DR, DQ and DP.

As previously discussed, the supertypic specificities may be most logically grouped into two series, MB and MT (see Table 3). The MB series has been well documented to reside on DQ molecules (Goyert, et al., 1982, Giles, et al. 1983, Tanigaki, et al., 1983a). In the cases of DQw1 and DQw3, monoclonal antibodies have been utilized to isolate molecules whose amino acid sequences are clearly DQ (Giles, et al., 1983). Although the DQw2-bearing molecule has not been verified by primary structural analysis as being DQ, several laboratories have presented evidence suggesting this is the case (Karr, et al., 1984). Confirmation of DQw2 localization on DQ awaits the description of a monoclonal antibody specific for DQw2 which can be used for isolation of the molecule for primary structural analysis.

Recently, Hurley, et al. (1984) have analyzed the biochemical bases of the MT2 and MT3 serologic specificities using two monoclonal antibodies with MT-like specificity. These monoclonal antibodies, I-LR2(DRw52-like) and 109d6(DRw53-like), were used to define the molecules bearing these specificities from a set of homozygous cell lines. I-LR2- and 109d6-reactive molecules were compared to DR, DQ, and in some instances DP mole-

cules isolated from the same cell line by inhibition of cell surface fluorescence or cytotoxicity, as well as amino acid sequence analysis and peptide mapping. Partial amino-terminal amino acid sequences of DR (203-reactive), DRw52(ILR-2-reactive), and DRw53(109d6-reactive) molecules were determined and the data show that the major populations of molecules bearing MT2 and MT3 (DRw52 and DRw53) determinants are indistinguishable from DR molecules.

The BR molecules have been shown to exhibit homology with DR molecules and could represent one of the multiple DR subsets. Sequence analysis of the BR molecules should establish their relationship to the DR molecules and definitively establish their relationship to the DR molecules. A general model which relates DR and MT specificities is shown in Figure 12.

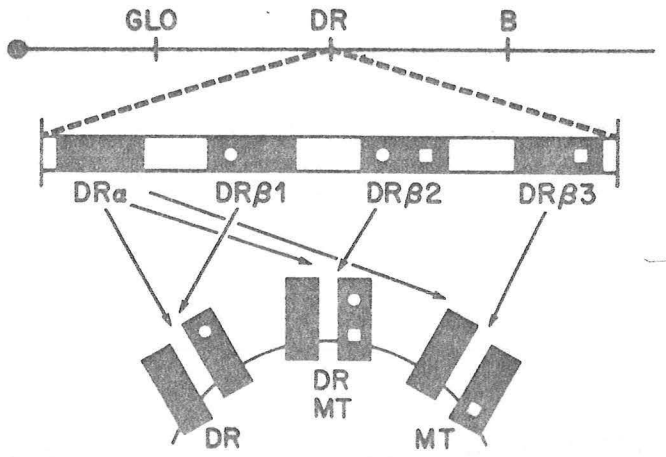

Figure 12. Schematic diagram of the possible genetic organization of the HLA-DR subregion. Evidence presented to date suggests that the DR molecules are encoded by a single alpha chain gene and multiple beta chain genes within a haplotype. Some but not all of these species bear MT (DRw52 and DRw53) serologic determinants. Some of the molecules also bear DR serologic determinants. All three of these subsets may not exist in all cells. From Hurley et al., 1984.

IX. INVARIANT (GAMMA) CHAIN

Class II antigens are associated intracellularly with a family of invariant polypeptides which were first demonstrated by two-dimensional gel electrophoresis (Jones et al., 1978, Owen et al., 1981, Kvist, et al., 1982, Machamer and Cresswell 1982). These polypeptides arise through various processing events and are encoded by a single gene which has recently been mapped to human chromosome 5 (Claesson-Welsh et al, 1984). The invariant (or gamma) chain, a transmembrane protein, is noncovalently associated transciently with class II antigens during their transport to the cell surface (Claesson et al., 1983). A fraction of these invariant chains are thought to integrate into the plasma membrane independently of the class II molecules.

The exact function(s) of the invariant chain is undetermined. It may regulate intracellular transport of class II antigens, or it may prevent the formation of molecules composed of alpha and beta subunits encoded within different subregions (e.g., DR alpha with DQ beta) (Sung and Jones, 1981, Claesson & Peterson, 1983). Recently Peterson has proposed a role for gamma chain in the genesis of the high mannose carbohydrate moiety on class II alpha chains. The nucleotide sequence of the cDNA clone which corresponds to the entire translated portion of the invariant chain demonstrates (1) that the amino-terminus of the gamma chain resides on the cytoplasmic side of the membrane (Figure 13) and (2) that the invariant chain lacks an amino terminal signal sequence (Claesson et al, 1983, Long et al., 1983). These data together with the distribution of carbohydrate moities suggest that the invariant chain has a reversed membrane orienta-

tion as compared to class II molecules. The proposed membrane orientation of alpha, beta and gamma chains is shown in Figure 13.

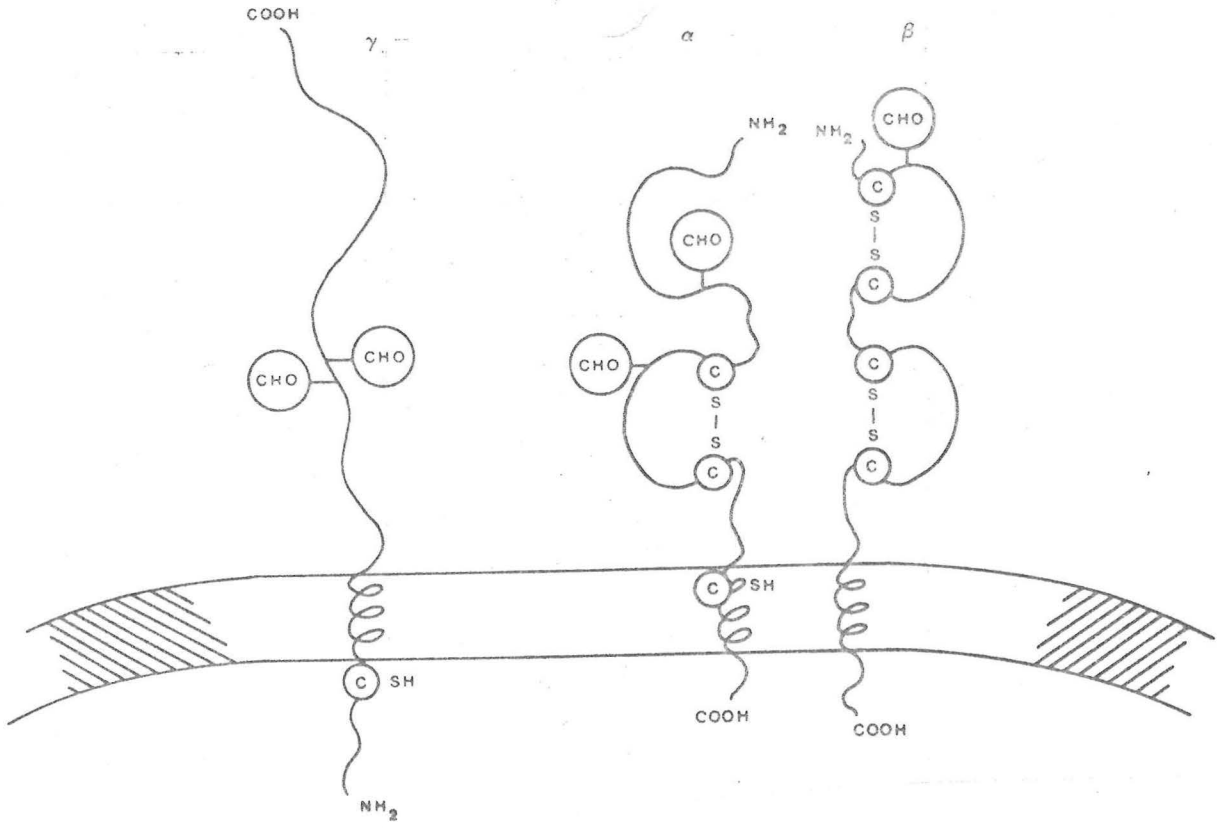

Figure 13. Proposed membrane orientation of alpha, beta and gamma chains of class II molecules. Cysteines (C) and asparagine-linked carbohydrate moieties (CHO) are indicated. The nonglycosylated tails reside on the cytoplasmic side of the membrane. From Claesson et al. (1983).

X. GENES

The utilization of molecular biological approaches in the area of class II genetics and biochemistry has had a monumental impact in a relatively short period of time. The isolation of cDNA and/or genomic clones encoding HLA-D region molecules, coupled with Southern filter hybridization analysis of human DNA, has contributed greatly to our current understanding of the number and organization of the genes. In addition, DNA sequence studies have been our major source of information concerning the primary structures of class II molecules.

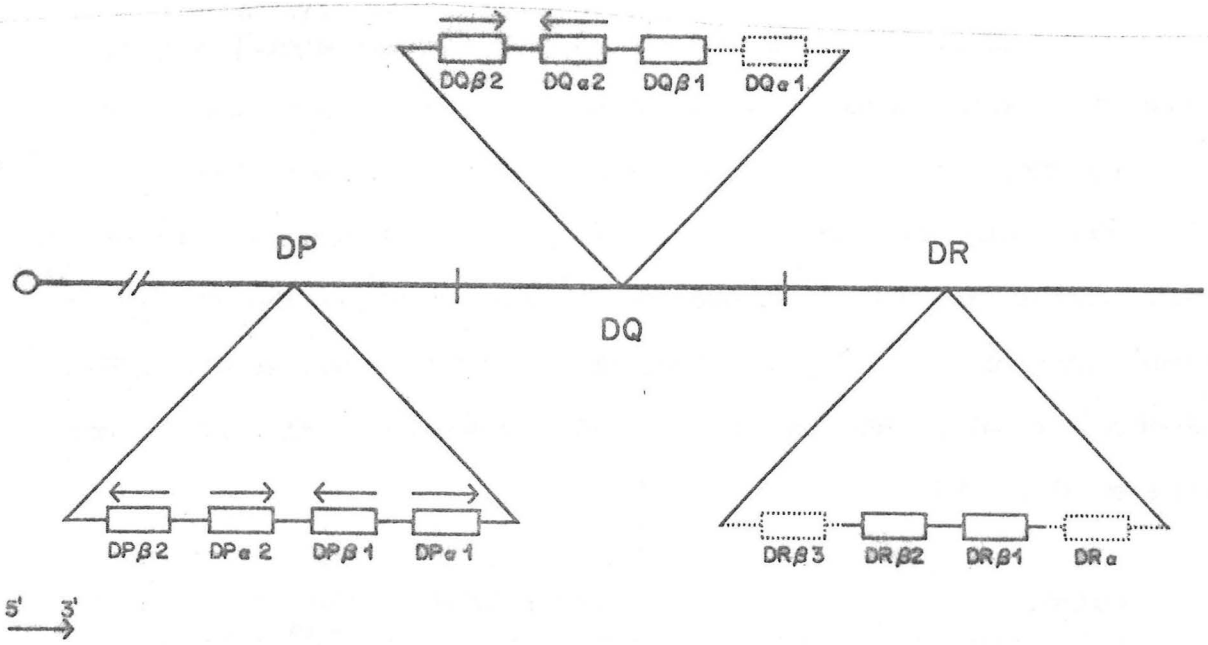

Figure 14. Genomic organization of the human HLA-D region. The arrows indicate direction of transcription. Where genes are indicated in dotted rectangles they have not been linked by overlapping DNA fragments.

The organization of the human class II genes is presented in Figure 14. Both the alpha and beta subunit products of each subregion have

been shown to be encoded by genes which map to the short arm of the sixth chromosome (Lee, et al., 1982, Trowsdale, et al., 1983, Auffray et al., 1983, Bohme et al., 1983, Morton, et al., 1984). The exact order of these blocks of genes (subregions) comes from studies involving deletion mutants and recombinations within members of a family. Mapping HLA-DP centromeric to HLA-DR/DQ was firmly established using a series of haplotype loss mutant B cell lines Mapping HLA-DQ in relationship to HLA-DR is less certain, but recent studies of Moller, et al. (1984) in their analysis of a family in which one member possesses a rare crossover event suggests that HLA-DQ is centromeric to HLA-DR. In almost all cases the order of the genes within each subregion may be reversed with regards to the centromere.

In addition to these (and other) cDNA clones, several genomic clones have been isolated from either phage or cosmid libraries of human DNA. The cDNA clones have the advantage of looking only at those loci in the genome which are likely expressed (i.e., not pseudogenes). On the other hand, genomic clones have allowed for the elucidation of the exon/intron organization of class II genes (Lee, et al., 1982, Gorski et al., 1984, Schamboeck et al., 1983, Das et al., 1983, Larhammar et al., 1983b, Trowsdale et al., 1984).

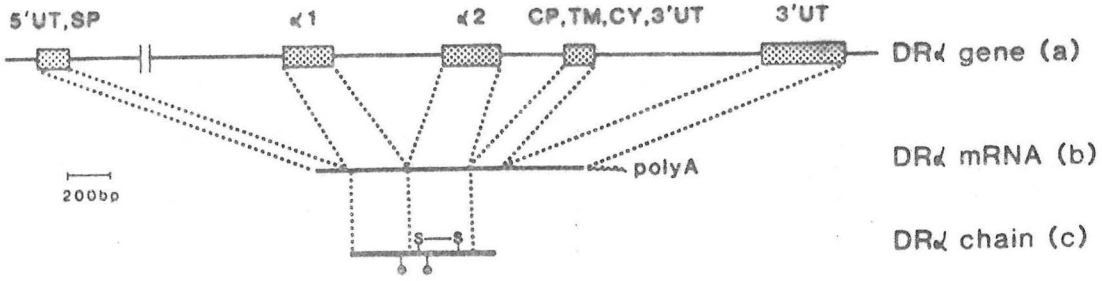

Figure 15. Organization of a prototypic human class II gene. From Das et al., 1983.

Shown in Figure 15 is the organization of a prototypic class II gene. The structure is very reminiscent of the exon/intron organization of class I genes suggesting an evolutionary relationship between the two. The first exon corresponds to the 5' untranslated and signal sequences. The second and third exons encode the two extracellular domains, while the fourth exon encodes a hydrophilic connecting peptide, the hydrophobic transmembrane region, the intracytoplasmic carboxyterminus, and a few nucleotides of the 3' untranslated region. The rest of the 3' untranslated region is present in the fifth exon.

Several cosmid clones containing DP-like genes have also been identified and studied. Recently it was reported that one cosmid clone of about 30kb of DNA that contains two DP alpha genes and one DP beta gene in the order of alpha-beta-alpha. Larhammer et al. (1984) have similar results with two overlapping cosmids. Likewise Trowsdale et al. (1984), have identified three overlapping cosmid clones which contain coding sequences for two DP alpha and two DP beta genes. In general, within a subregion, the class II genes are about 10 kb apart. The subregions, themselves, have not been formally linked so the distance between, for example, DP and DQ is not known.

Comparison of the nucleotide and predicted amino acid sequences of these class II genes allows for an analysis of their evolutionary relationships. In particular, the predicted amino acid sequences of the alpha chains from each of these subregions can be compared to one another and to the murine I-E and I-A alpha chains (Table 5). This kind of comparison

clearly demonstrates that the DR and DQ alpha chains are the human equivalents of the murine I-E and I-A alpha chains, respectively. However, in a similar comparison the DP alpha subunit appears to be equally similar to each of the other alpha chains (54-61% homologous). A domain by domain comparison of DP alpha with each of the other alpha chains does not allow for a simple conclusion as to the evolutionary relationship of DP alpha to these other molecules. An excellent review of many of these comparisons is given by Gustafsson et al. (1984b).

Class I genes of mouse and human have been successfully transfected into mouse L cells. (Goodenow et al. 1983; Lemonnier et al. 1983). In these studies the product of a single transfected gene after association with a second chain, beta-2-microglobulin, already present in the mouse L cells, was expressed on the surface of the L cells and was able to function as a restriction element in T-cell-specific cytotoxic responses against viruses. Likewise, class II genes of both species have been successfully transfected into mouse L cells (Rabourdin-Combe and Mach, 1983; Malissen et al., 1983). Although these expressed molecules were recognized by appropriate monoclonal antibodies they were apparently not able to serve as restricting elements in antigen-specific T-cell proliferation assays. Expression of functional class II molecules has recently been accomplished by transfection of murine class II genes into B lymphoma cell lines (Germain et al., 1983; Ben-Nun et al., 1984). However, similar attempts to obtain expression of functional HLA-D region molecules following transfection has been more difficult. Recently, Gillies et al. (1984) have identified a cell type-specific transcriptional enhancer element associated with the mouse I-E beta gene which is likely to play an important role in the regu-

lated expression of class II genes. It is likely that similar elements exist in the human HLA-D region genes.

Despite these difficulties, expression of human class II genes in appropriate cell types promises to simplify current attempts to correlate HLA-D region gene products with their functional roles in the immune response. Coupled with the techniques of exon shuffling and site directed mutagenesis, transfection of HLA-D-region genes into appropriate cells should provide the necessary approaches to localize allodeterminants, functional domains, and potential epitopes which may be directly involved in increased susceptibility to certain diseases.

TABLE 5

COMPARISON OF THE AMINO ACID SEQUENCES OF HUMAN AND MURINE CLASS II ALPHA CHAINS

	% HOMOLOGY			
	DQ	I-A	DR	I-E
DP	60	63	65	57
DQ		75	64	53
I-A			59	52
DR				78

From Auffray et al; 1984

XI. RESTRICTION FRAGMENT LENGTH POLYMORPHISM

The technique of "DNA typing" by examining restriction enzyme fragment length polymorphism (RFLP) has generated considerable attention in many laboratories. This technique examines structural polymorphism of DNA by Southern filter hybridization with class II-specific DNA probes. In general, the use of a battery of restriction enzymes and the appropriate HLA-D region probes permits the identification of haplotype-specific patterns (Wake, et al., 1982b, Auffray, et al., 1983, Bohme, et al., 1983). Under conditions of "low stringency" essentially all alpha genes and all beta genes cross hybridize. However, conditions can be found such that probes will only detect genes in a single subregion. There is only approximately 50-60% homology between subregions but 90-95% homology within a subregion. Thus, a DQ alpha probe will detect DQ alpha 1 and DQ alpha 2 but not DP alpha or DR alpha if the conditions are appropriate.

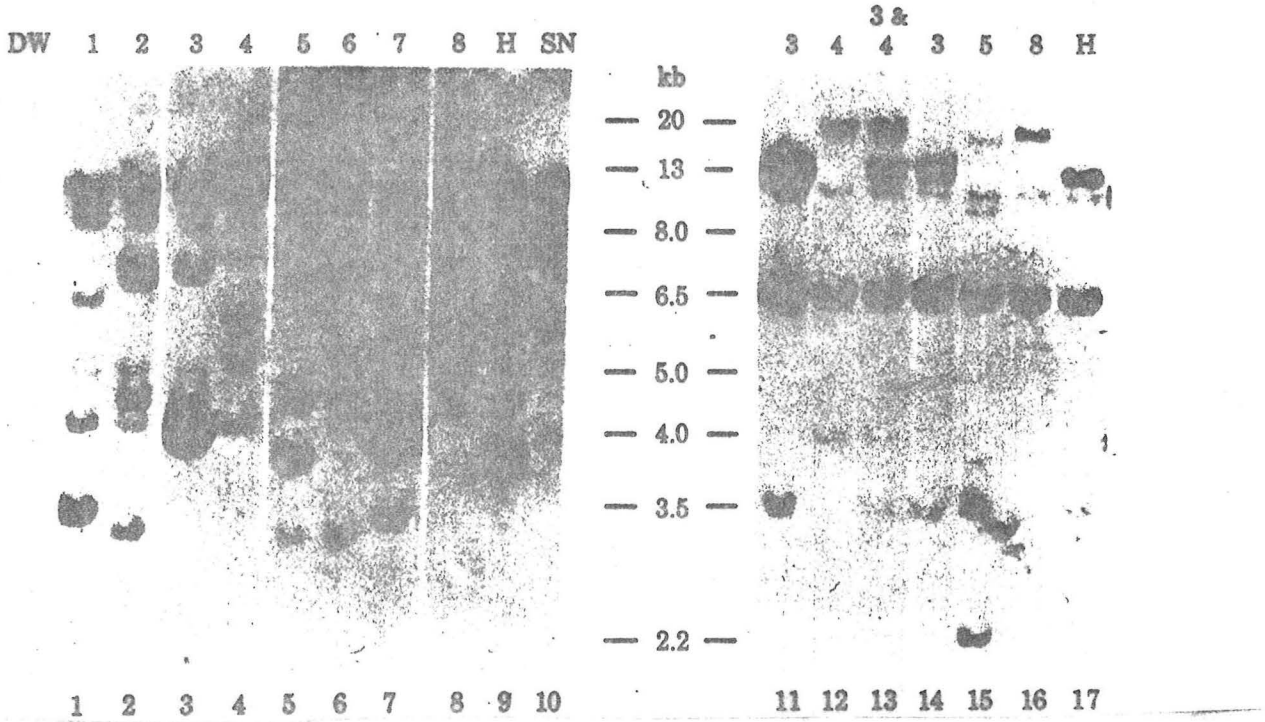

Figure 16. Autoradiogram of blot hybridization analysis of homzygous HLA-Dw typing cells with an HLA-DQ beta probe. From Owerbach et al., 1983.

An example of the approach is shown in Figure 16. The probe used is HLA-DQ beta. The number of restriction fragments seen indicates that there must be 2-5 beta genes in the genome. Recall this experiment does not address the number of these genes that are expressed. Nonetheless, note that each DRw type (shown across the top of the autoradiogram) has a distinct pattern when both enzyme digests are examined. For example, while Dw4 and Dw8 have an indentical pattern with EcoRI (lanes 12 and 16), they are different with BamHI (lanes 4 and 8). Lane 13 contains DNA digested with EcoRI from a 3/4 heterozygote. Note that the pattern is the sum of lanes 12 and 14.

Results of "DNA typing" indicate that in some instances genotypic polymorphism of class II genes is greater than phenotypic variation as evidenced by serological analysis. For example, in one study the DNA from DR3/3 genetically homozygous individuals could be split into two groups. It is anticipated that this technique will aid in the classification of those individuals which are untypeable by present techniques, and will also identify categories of additional polymorphisms which may be biologically relevant.

A distinct advantage of "DNA typing" over serological or cellular typing is the ability of this analysis to detect not only polymorphic restriction sites located <u>within the coding region</u> (which may or may not result in the expression of distinct molecules), but also polymorphic sites located <u>within flanking sequences or introns</u>. This kind of polymorphism is better appreciated when examining DNA from numerous individuals with a probe to a nonpolymorphic molecule such as the DR alpha chain. Erlich et

al. (1983) have hybridized Bgl II-digested DNA with a cDNA probe specific for DR alpha. Their analysis has revealed three allelic restriction fragment lengths which map near the 3' end of the HLA-DR alpha gene. They propose to utilize these polymorphic restriction sites as genetic markers for the analysis of genetic predisposition to HLA-associated diseases.

Caution should be taken in interpretation of data obtained from RFLP analyses as they relate to location of serological specificities and/or "disease genes." For example Spielman et al. (1984) have reported RFLP patterns using the retriction enzyme EcoRl and the DQ alpha chain cDNA probe that "corresponds with the HLA-DR cross-reactive serotypes that are associated with variation at the HLA-DQ locus." Although the patterns obtained do indeed associate with the MTl, MT2 (DRw52), and MT3 (DRw53) specificities, it is now known that only the MTl (DQwl) specificity is localized to the HLA-DQ subregion. The MT2 (DRw52) and MT3 (DRw53) specificities have been localized to variations at the DR beta loci (Hurley et al., 1984). Correlations such as DQ alpha polymorphism with the MT specificities must be attributed to close linkage disequilibrium. These same precautions should be taken when attempting to correlate RFLP's with "disease genes." Nevertheless, the technique of "DNA typing," coupled with the current serological approaches for HLA typing, should prove to be very beneficial, and appears to be the wave of at least the "near" future for tissue typing laboratories.

XII. FUNCTION

HLA-D region molecules have been shown to act as primary and secondary stimulators in mixed lymphocyte reactions, as targets for cytotoxic T cells, and as controlling elements in antigen presentation. In the mouse, the immune response to some antigens is restricted to determinants on I-E molecules, while to other antigens the response is restricted to determinants on I-A molecules. Similar delineations of function probably exist among the multiple HLA-D region molecules as well.

Products of all three of the currently identified subregions of the HLA-D region can provide the major stimulus for lymphocyte activation in the mixed lymphocyte reaction and in the secondary proliferation of the primed lymphocyte typing assay (Termijtelen et al, 1982). In PLT's the responding lymphocytes have been shown to recognize DR antigens (Bach, et al., 1979) MT determinants (Zeevi, et al., 1982), DQ determinants (Zeevi, et al., 1982), DP antigens (Shaw et al, 1980); and HLA-D determinants distinct from currently defined serologic specificities (Zeevi, et al, 1983; Eckels & Hartzman, 1981).

Human class II molecules were shown to be a cytotoxicity target in a mouse anti-human xenogeneic system by Engelhard et al. (1980). Two studies have supported this finding using cell-mediated lysis between HLA-A, B-matched, HLA-D mismatched individuals to demonstrate that HLA-D region antigens can serve as targets for CTL (Feighery and Stastny, 1979; Albrechtesen et al, 1979). Krensky et al, 1982 subsequently demonstrated that

Daudi, a cell line that expresses DR antigens but no class I antigens, could be used to generate long-term DR-specific human CTL lines. Their findings not only suggested that DR could serve as a target antigen for long-term allogenic CTLs, but that these antigens activated OKT4 + cytotoxic cells rather than the traditional OKT8 + effector cells activated by class I molecules.

The HLA-D region also encodes restriction determinants utilized by T cells when responding to foreign antigens (Bergholtz and Thorsby, 1977, 1979). A number of investigators have described experiments in which HLA-DR molecules served as restricting elements in antigen-specific T cell responses (Thorsby et al., 1982). Several groups of investigators have presented evidence suggesting that additional HLA-D region encoded specificities may serve as restriction elements in antigen-specific T cell proliferation. Berle and Thorsby (1982), and Ball and Stastny (1984a) have all demonstrated that T cells responsive to viral antigens appear to utilize MT-bearing molecules as restriction elements. Eckels et al. (1983) found that some T-cell clones utilized HLA-DP-encoded restriction elements in response to certain viral antigens. Ball and Stastny (1984b) have provided evidence that HLA-DQ molecules serve as restricting elements in antigen-specific T cell responses. They chose to study polypeptide antigens that in the mouse are recognized by T cells using I-A-subregion encoded restriction determinants (Schwartz et al, 1976). One human T cell line specific for GAT utilized a restriction determinant on DQ molecules as evidenced by blocking studies with DQ specific monoclonal antibodies. The epitopes on the DQ molecules recognized by the T cell line appeared to be distinct from alloantigenic determinants currently defined by serology.

XIII DISEASE ASSOCIATIONS

The initial description in 1967 of a significant association between certain HLA antigens and susceptibility to disease generated a new level of enthusiasm surrounding the basic question of why some people get certain diseases and others do not. Early studies of disease association showed a strong association of certain diseases with certain alleles of the class I molecules. Upon the discovery of the HLA-D-region, several of these diseases were shown to be more closely associated with certain alleles of HLA-DR. With the recent identification of additional HLA-D region products it might be predicted that some of these diseases may be even more strongly associated with these newly defined loci rather than HLA-DR. In fact, recent evaluations of several diseases have shown them to be more closely related to specificites found on HLA-DQ molecules. For example, certain malignancies have been associated with the DQw3 (MB3) specificity. While these malignancies show weak association with particular DR alleles, the association with DQw3 is striking. Although it is possible that the DQw3-bearing molecule is directly involved with increased susceptibility to CLL, it is also likely that the "disease gene" is in linkage with the DQw3 specificity and may be a gene whose product has yet to be identified serologically or biochemically.

The varied functional roles played by the HLA-D-region molecules in the control of immune responsiveness may be important in determining susceptibility to particular diseases. In fact, one group of diseases shown to have an increased association with certain HLA-DR alleles is characte-

rized by aberrant immunologic reactions. Included in this group of diseases are multiple sclerosis, thyroditis, Sjogren's syndrome, Graves' disease, myasthenia gravis, and insulin dependent diabetes mellitus (IDDM), to name a few. Recently, IDDM has received increased attention mainly due to the description of a suitable animal model, the BB rat. These animals display an increased incidence of diabetes similar to type 1 diabetes of man. The rats demonstrate pancreatic beta cell destruction followed by hypoinsulinemia and hyperglycemia, and finally ketoacidosis and death if not treated with exogenous insulin. At least two genes have been demonstrated as being necessary for the development of diabetes in these rats. One of these genes controls a T-cell lymphopenia found in the BB rat, while the second gene is associated with the rat MHC, RT1. Restriction fragment length polymorphism was examined in 50 BBN rats (non-diabetes prone) and 22 BB rats using a ^{32} P-labeled cloned I-A alpha gene probe. This analysis revealed four chromosome types present in the BBN rats while only one chromosome type was present in the BB rats. The possibility of two gene control in human diabetes has not been excluded. IDDM has been associated with individuals with two DR alleles: DR3 (relative risk of 3.3) and DR4 (relative risk of 6.4). In homozygous individuals the relative risk increases to 10 for DR3 and 16 for DR4. Interestingly, the highest relative risk is found for individuals who are DR3/4 heterozygous (relative risk of 33). If the increased susceptibility to IDDM is, in fact, more closely related to alleles of HLA-DQ rather than alleles of HLA-DR, the formation of hybrid DQ molecules in DR3/4 heterozygotes and the attendant implications of these particular heterodimers in the immune response process might be a plausible explanation for the increased relative risk for DR3/4 heterozygotes.

XIV. CONCLUSION

The human HLA-D region controls the expression of cell-surface antigens involved in communication between lymphoid cells. This communication appears to be critically important in immune responsiveness as suggested by the linkage of disease susceptibility in humans to particular HLA-D-region alleles or specificities. In order to understand how these HLA-D-region molecules function in cellular collaboration and antigen presentation, it is important to elucidate the assortment of HLA-D-region molecules found on the surface of immune response related cells. At least three structurally distinct HLA-D-region molecules, DR, DQ and DP have been isolated from a single cell line. Coupled with information at the DNA level, this effectively divides the HLA-D region into at least three subregions encoding a minimum of six protein chains. It is very likely that up to twelve subunits are actually expressed. Much recent work has defined the biochemical basis for the serologically and functionally defined antigens bearing allospecificities.

The number of loci already described in the HLA-D region provide a large repertoire of cell-surface molecules which can be used in communication between immune response related cells. Transassociation of chains within a subregion has been shown to generate additional molecules. Additional mechanisms which generate polymorphism probably exist. The association between susceptibility to particular diseases and certain allelic products of HLA-D subregions suggests that roles played by the multiple HLA-D-region molecules in cellular collaboration and antigen presentation are varied.

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