THE UNIVERSITY OF TEXAS HEALTH SCIENCE
CENTER AT DALLAS

Department of Internal Medicine

GRAND ROUNDS

HLA-D REGION ANTIGENS
STRUCTURE, FUNCTION AND GENETICS

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

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INTRODUCTION

At one time, leukocyte antigens were a tangle of cell-surface markers, many of which could be identified only in one laboratory; they were then organized into a system with the appearance of a well regulated and orderly series of membrane antigens of little interest outside transplantation biology. With little warning in the mid-70's, HLA became one of the most precise of all tools for the anthropologist, and now a new horizon of biologic functions and involvements in disease is opening up. Like Topsy, it has "just growed" and like Pandora's box, it continues to offer unlooked for and sometimes unwanted surprises.

The most complete early description of human lymphocyte antigens was given by Van Rood in a Journal of Clinical Investigation article in 1963 (1) based on his doctoral thesis (2). Table I lists some of the significant events in the history of HLA and shows that in roughly 25 years, the system has grown and become ever more precise, particularly in the last five years, as the complete primary structures of many of the components of this major multigene family in man and other animals has been elucidated.

Interest in the human MHC has been intensified and broadened in appeal particularly to the field of medicine with the remarkable association of particular HLA antigens with certain diseases. More recently, innovative methods have been developed for the study of the biochemistry of these antigens as integral membrane components. Antigens controlled by the MHC are implicated in the regulation of T cell and B cell differentiation;

TABLE I

SIGNIFICANT EVENTS IN THE HISTORY OF HLA

1953	Leukocyte-agglutinating antibodies are found in the sera of multitransfused patients and many multiparous women.
1958	The first human lymphocyte antigen (now HLA-A2 and A28) is described.
1962	The first allelic lymphocyte antigenic system is described.
1963	The mixed lymphocyte culture (MLC) assay is developed as an *in vitro* example of primary immune recognition.
1964	Miniaturization of the lymphocytotoxicity test and the introduction of rabbit complement establishes a rapid, reproducible microtechnique, which replaces leuko-agglutination.
1964	First evidence of the relevance of leukocyte antigens to skin transplantation and the use of HLA matching within families for kidney transplants.
1968	Two HLA loci are established, one called the First or LA locus (now HLA-A) and the Second or Four locus (now HLA-B).
1969	First demonstration of a third locus, HLA-C, which is also detectable by lymphocytotoxicity.
1970	First demonstration that a fourth locus, now HLA-D, controls immune recogition in the MLC.
1972	Discovery of HLA-DR antigens.
1974	Linkage between HLA and the complement component Bf noted; later C2 and C4 are linked
1979	First complete sequence of an HLA molecule.
1981	First complete sequence of a DR molecule.

other MHC-linked genes govern the ability to mount a humoral or cellular immune response against bacteria, viruses, or transplanted allogeneic cells or organs. The products of the MHC, both cell membrane molecules and plasma proteins, are involved in immunologic communication and cell-cell interactions which maintain the integrity and well being of the immunologic system of an individual and possibly represent a major mechanism for self-recognition (3). Much of the structure, genetics and serology of the three major transplantation antigens of the human MHC, HLA-A, -B and C, has been reviewed here before. Today I would like to focus on the "fourth" locus of the human MHC, the HLA-D region, also the subject of a recent Grand Rounds (4). A few comments about the whole gene complex are in order before we proceed.

The human major histocompatibility complex, called the HLA region, is located on the short arm of chromosome six (Figure 1). To date, this region is known to code for about ten cell surface proteins and at least three serum complement components. However, by its sheer size, the HLA region could accommodate the structural genes for many more proteins. The HLA-controlled cell surface antigens are highly polymorphic, both serologically and structurally. This polymorphism is thought to be essential for the function of these molecules.

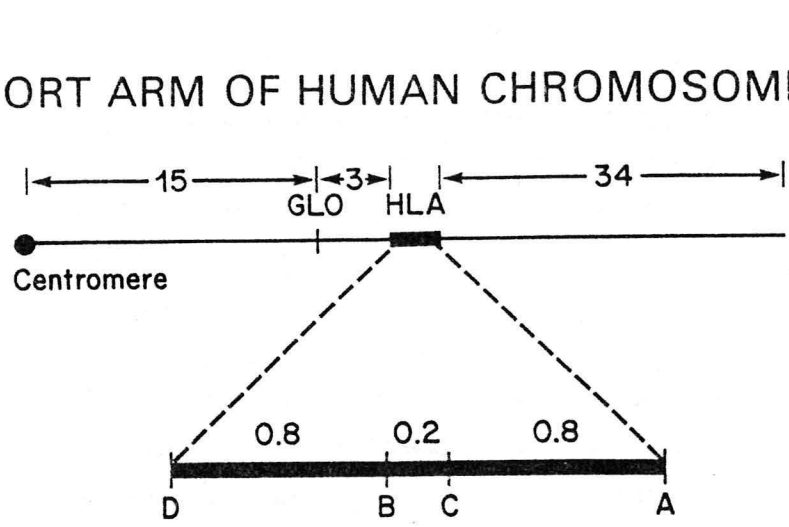

Figure 1. The region of the human sixth chromosome containing the major histocompatibility complex. The subject of this Grand Rounds is the futher complexity of the D region. The distances are based on recombination frequencies and are referred to as centimorgans.

The HLA-A, -B and -C region antigens, referred to as Class I molecules, are composed of two subunits. The 44,000 dalton heavy chain is an intrinsic membrane glycoprotein which is MHC encoded and structurally polymorphic. It is associated noncovalently with beta-two microglobulin, an extrinsic, invariant protein which is encoded on chromosome 15. In the mouse, where a similar gene complex has been studied, the chromosome locations are 17 and 2, respectively. The HLA-A and -B antigens are analagous in structure and function to the murine H-2D and H-2K antigens. Class I molecules are expressed on all cell types except red blood cells. The complete amino acid squence of several of these proteins has been determined.

The HLA-D region antigens, referred to as Class II molecules, are also composed of two subunits, both of which are intrinsic membrane proteins. They are expressed primarily on lymphoid tissue. The structure of these molecules and the complexity of the HLA-D region are the subject of this Grand Rounds.

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 (B) complement pathways.

REVIEW OF THE HLA-A, -B AND -C ANTIGENS (CLASS I MOLECULES)

The complete primary structure of several histocompatibility antigens has become available within the past four years (5-10). These structures were accomplished by amino acid sequencing and recently by analysis of the DNA itself (11,12). A schematic depiction of the structure of these molecules is shown in Figure 2. They are composed of two chains: the large glycoprotein subunit encoded on the sixth chromosome has a size of 44,000 daltons and traverses the cell membrane; the small, non-covalently associated subunit, beta-two microglobulin is encoded on chromosome 15, and has a size of 11,500 daltons. The large chain can be divided into five regions:

1. An N-terminal region (termed alpha-1) with a molecular weight of about 13,500 which bears the single glycan moiety of the molecule but has no disulfide loops;
2. A second region (termed alpha-2) with a molecular weight of about 10,500 which has an internal disulfide bridge;
3. A third region (termed alpha-3) which also has a molecular weight of about 10,500 and also has an internal disulfide bridge (the first three regions are outside the cell membrane);
4. A fourth region (termed Tm) of about 3,000 daltons which is the hydrophobic, intramembraneous portion of the molecule, and finally
5. A small hydrophilic region of about 3,500 daltons which is intracytoplasmic and may interact with cytoskeletal elements.

The extracellular regions are probably folded into domains with a beta pleated sheet structure, as is beta-two microglobulin. Several molecules of both the HLA-A and HLA-B loci have been sequenced and they differ one from the other in about 10% of their amino acid residues. The human molecules are about 75% homologous to the analogous murine antigens.

There are certain regions of all transplantation antigens (particularly in the alpha-1 domain) that are particularly variable and are thought to be responsible for the alloantigenic sites. Similar regions exist in similar positions in the murine H-2 molecules.

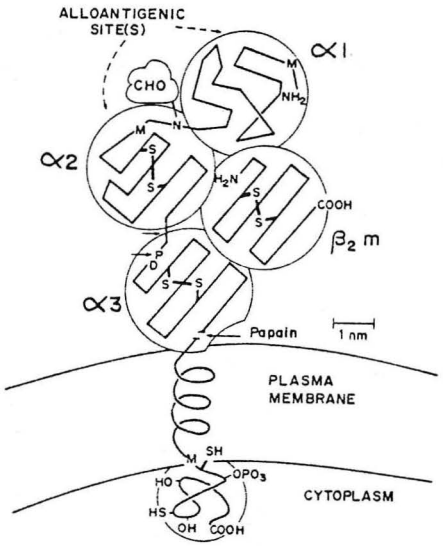

Figure 2. Schematic representations of major transplantation antigens. The heavy chain is MHC encoded, while the smaller subunit, beta-2 microglobulin is encoded on a different chromosome. (from reference 13)

Studies had revealed a number of similarities between The HLA-A and -B antigens and immunoglobulins, a point of considerable evolutionary interest in view of the possible role of histocompatibility antigens as cell-bound defense molecules and of immunoglobulins as circulating defense molecules. Once the complete amino acid sequence of these molecules was available, it was possible to compare their sequences to all sequenced proteins using a computer program designed to detect such evolutionary relationships. Only the region containing the second disulfide loop (the so-called alpha-three region) shows significant sequence homology to other proteins, that is, they have highly significant sequence homology to immunoglobulins. It has been predicted that this region folds into a beta pleated sheet similar to immunoglobulin domains and is probably the region of the HLA molecule that interacts with beta-2 microglobulin.

Biosynthetic studies of these molecules both in man and mouse have demonstrated conclusively that they are intrinsic membrane proteins. Work at the DNA level in both species has now reached the point where we should soon have available the genomic structures of virtually all of the relevant molecules.

THE HLA-D REGION

I. Introduction

The human MHC also controls the expression of a series of antigens controlled by the HLA-D region, which is the subject of this Grand Rounds. These antigens and the analogous murine Ia antigens are found primarily on B lymphocytes and monocytes and, thus, they are of particular interest to immunologists. Normal resting T cells express little or no HLA-D antigens, but on activation they synthesize them. The murine I region is known to encode at least two Ia antigens (14) and evidence now suggests that in man there are not two but at least three and probably four distinct loci which encode these crucial membrane glycoproteins.

II. Characterization of the DR Complex (Class II Molecules)

A. Subunit Composition

The DR antigen is composed of two noncovalently associated subunits, a heavy (alpha) chain with a molecular weight of 34,000 and a 29,000 molecular weight light (beta) chain (Figure 3). Cross-linking studies have established that the stoichiometry of the subunits of the DR complex is 1:1. There is no evidence for the formation of dimers of the same chain or of tetramers (Figure 4).

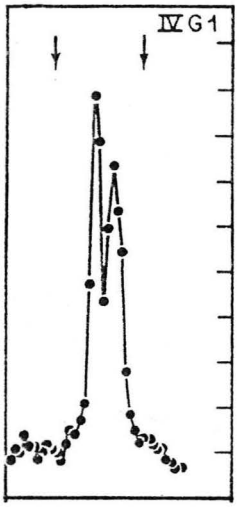

Figure 3. SDS polyacrylamide gel electrophoresis of immunoprecipitated radiolabeled DR antigens from a human EBV-transformed lymphocyte cell line. Molecular weight markers (immunoglobulin heavy and light chains) are indicated by the arrows. The chains migrate with a molecular weight of approximately 34,000 and 29,000. (from Hurley et al., reference 15).

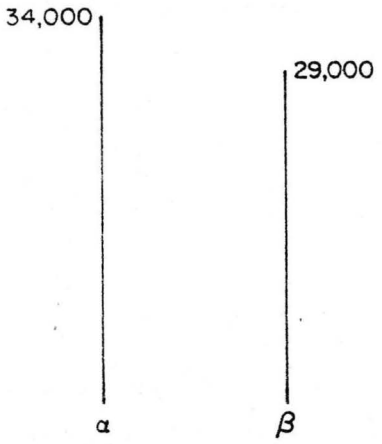

Figure 4. Minimal model of the HLA-DR molecule. The two chains are of 34,000 and 29,000 molecular weight. They are non-covalently associated as a single complex. Neither dimers nor tetramers have been found.

B. Association with the Membrane

The DR alpha and beta chains are intrinsic membrane proteins. Vectorial labeling of inside/out membrane vessicles with lactoperoxidase has demonstrated that both chains span the lipid bilayer (16). Several experiments have established that it is the carboxyterminal portion of each chain which is membrane associated. If enzymes such as papain are utilized to isolate DR antigens, only their amino terminal portions are recovered. However, when non-ionic detergents such as NP40 are utilized to isolate DR antigens the carboxyterminal portions of the molecules are recovered as well (17). The C-terminal region of the molecule has been shown to be extremely hydrophobic further suggesting that this is the region of each chain which is embedded in the membrane. This is further supported by the observation that the DR alpha and beta chains are labeled with the photo-activatable lipophilic reagents adamantane diazirine and hexanoyldiiodo-N-(4-azido-2-nitrophenyl) tyramine. These reagents partition into the lipid bilayer and upon photoactivation will covalently label the membrane embedded portion of a protein. The label is lost from both chains after the penultimate peptides are removed with papain.

The DR alpha chain can be labeled in vivo with ^{32}P orthophosphate in this same C-terminal region of the molecule. This indicates that phosphorylation of the portion of the DR alpha chain exposed to the cytoplasm occurs. However, unlike the HLA-A and -B heavy chains which are essentially quantitatively phosphorylated, only about 10-20% of the DR alpha chains are labeled with phosphate. There is no evidence that the subpopu-

lations of DR molecules which I will discuss below are preferentially phosphorylated. A small fraction of the DR alpha chains can also be labeled with ^{3}H-palmitate as can HLA-A and -B heavy chains. Similar post-translational modifications occur in a variety of intrinsic membrane proteins but their significance is not known.

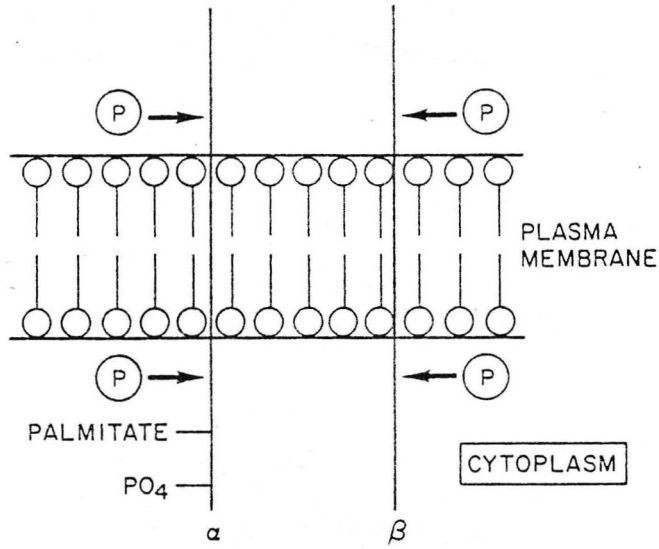

Figure 5: The presumed arrangement of the HLA-DR alpha and beta chains near the membrane. A portion of each chain traverses the membrane and a portion of each chain extrudes into the cytoplasm. The alpha but not beta chain can be phosphorylated as well as labeled with palmitate. Ⓟ refers to sites of cleavage by the enzyme papain. Adapted from reference 16.

C. Carbohydrate Moieties

Both chains of the DR molecule are glycosylated. The alpha chain has one high mannose and one complex N-linked oligosaccharide. The beta chain has one complex N-linked oligosaccharide. The location of the carbohydrate moieties on each chain is shown in Figure 6. Note that each of the complex carbohydrates is located within a disulfide loop while the high mannose moiety, located on the alpha chain, is not.

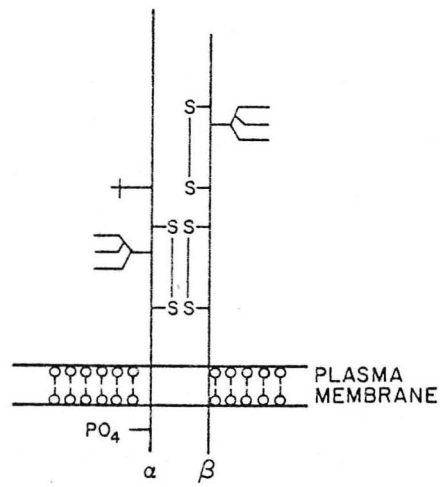

Figure 6. Schematic representation of the DR molecule showing the two-chain structure, carbohydrate moieties, the disulfide bonds and its relationship with the plasma membrane and cytoplasm. Note the phosphorylation site on the alpha chain within the cytoplasm. There is also a free sulfhydryl group within the hydrophobic portion within the plasma membrane. Adapted from reference 16.

III. Biochemical Nature of DR Polymorphism

It was known from genetic analyses that there were a minimum of ten alleles that mapped to the D region of the human major histocompatibility complex. When it became apparent that the DR antigen was a two chained molecule, it was not known whether both chains or only one was encoded within the complex nor was the kind or extent of polymorphism known. In general, there are two types of polymorphism that can be described in proteins. In what is referred to as simple polymorphism, the allelic products differ by one or at most a very few amino acids and typically the variations are located in a particular region of the molecule. The vast majority of known polymorphic systems are of this type. A more recently described type of polymorphism is referred to as complex polymorphism and the histocompatibility antigens and the DR antigens (particularly the beta chain) are of this type. That is, their amino acid sequence variation is extensive from allelic product to allelic product and the variations typically extend throughout the molecule.

As mentioned above, the HLA-DR locus has at least ten well defined alleles. The structural basis of their serologically defined polymorphism has been investigated by two-dimensional (2-D) gel electrophoresis, peptide mapping and more recently by complete amino acid sequence and/or DNA sequence analysis (19-26). These studies have demonstrated that DR alpha chains are largely invariant although recent evidence indicates they are encoded within the major histocompatibility complex.

DR beta chains are highly polymorphic in the complex fashion described above, that is, there are multiple amino acid differences between the different allelic beta chains. An example of peptide map differences between DR beta chains is shown in Figure 7 which shows that the radiolabeled tryptic peptides of the DR beta chains derived from two different cell lines expressing DR molecules of differing alleles have virtually no coincident peptides.

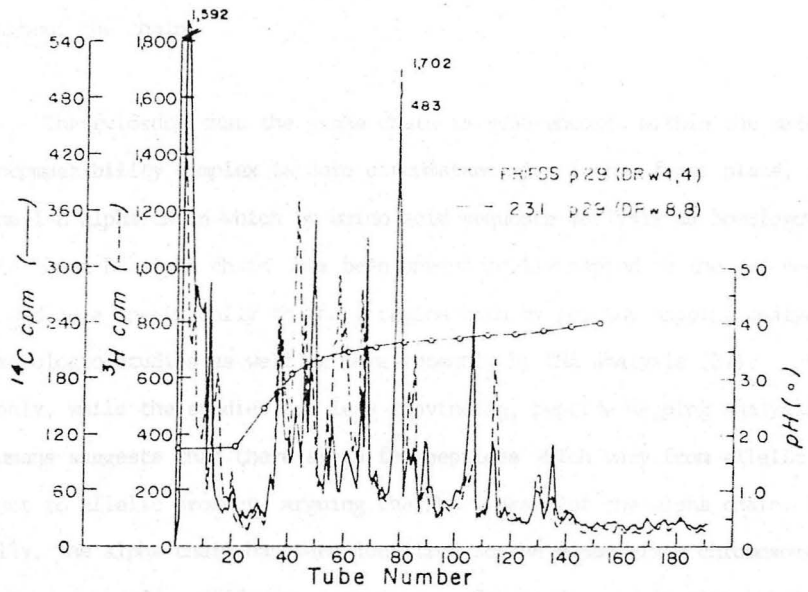

Figure 7. Ion exchange chromatography of tryptic digests of the mixture of beta chains from ^{3}H - lysine-labeled PRIESS (DR4) and ^{14}C lysine labeled 23.1 cells (DR8). Virtually none of the peptides are coincident in this comparison (from reference 25).

When such comparisons were made between all possible combinations of DR beta chains from different alleles (that is, for example, DR1 vs. DR2), it was found that essentially all the beta chains varied, while, when they were compared within alleles (that is, for example, DR1 vs. DR1) the peptide patterns were identical. This supports the view that the allelic products of the beta chain locus are encoded by the major histocompatibility complex (that is, the region of chromosome six previously defined by the serologic specificities, "DR", and the MLC reactivity - "D"). The limited amino acid sequence data available at present suggests that the variation (as predicted by the peptide mapping data) extends throughout the chain.

The evidence that the alpha chain is also encoded within the major histocompatibility complex is more circumstantial. In the first place, the murine I-E alpha chain which by amino acid sequence analysis is homologous to the human DR alpha chain, has been unequivocally mapped to the H-2 complex and more specifically to the I region both by peptide mapping analysis and serologic studies as well as more recently by DNA analysis (27). Secondly, while the studies are less convincing, peptide mapping analysis in humans suggests that there are a few peptides which vary from allelic product to allelic product, arguing the HLA linkage of the alpha chain. Finally, the alpha chain has been localized to the human sixth chromosome by two groups using different techniques. One study used in situ hybridization and actually located the DR alpha chain centromeric to the HLA-A heavy chain (Strominger, personal communication).

IV. Primary Structure of the DR Antigen

A. Structure of the DR alpha chain

The complete amino acid sequence of a DR alpha chain has been determined by both protein and DNA analysis (28-32). At the present time, there is no definitive evidence that DR alpha chains vary from individual to individual. The sequence is about 60% homologous to that of the murine I-E alpha chain. The alpha chain has one disulfide bond in the region referred to as the alpha-two domain of the HLA-DR heavy chain. The amino acid sequence in this domain is highly homologous to constant regions of immunoglobulins as well as to the alpha-three domain of HLA antigens. Figure 8 shows a schematic view of the alpha chain.

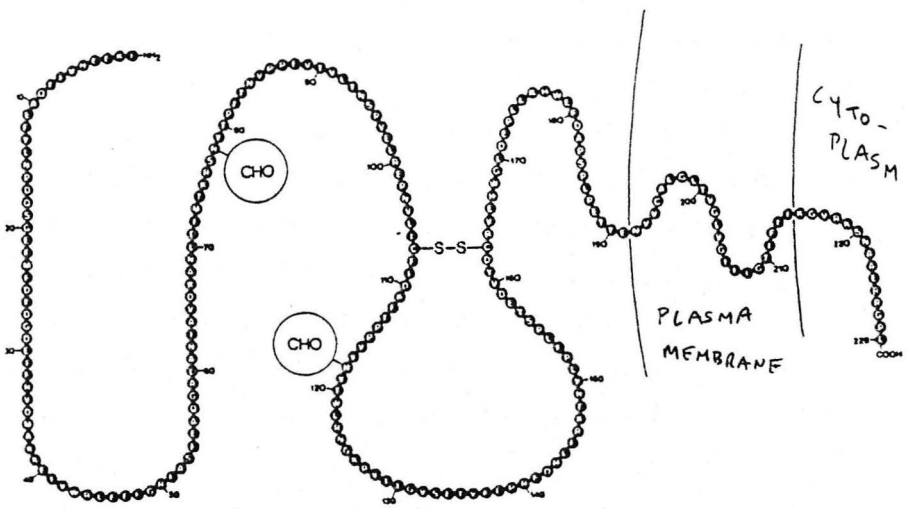

Figure 8. Amino acid sequence of the DR alpha chain shown schematically in the one letter code. CHO: Carbohydrate moiety. S-S: Disulfide bond. (from reference 31).

B. Structure of the DR beta chain

The complete amino acid sequence of the beta chain of DR antigens has been achieved in several laboratories both by protein and DNA sequencing (33-35). The beta chain contains four cysteines which form two disulfide bridges. The external domains (the portions of the chain outside the cell) are referred to as alpha-one and alpha-two. The alpha-two domain has been shown to have amino acid sequence homology to immunoglobulins and beta-2 microglobulin. The single carbohydrate moiety described earlier has been definitively shown to be attached to asparagine 19 in the DR beta chain at a typical carbohydrate acceptor site sequence ASN-GLY-THR. The DR beta chain also shows considerable homology to the beta chain of the I-E molecule of the mouse with about 70% sequence homology.

The second half of the DR beta chain displays extensive homology to immunoglobulin constant regions, in particular the third constant domain (CH3) of IgG. As mentioned above, the DR beta chain also has sequence homology to the third domain (alpha-3) of HLA-A and -B and murine H2-D and -K heavy chains, and to human beta-2 microglobulin. These extensive homologies are illustrated in Figure 9. Sequence gaps have been added to maximize homology. While the overall homology is only 25 to 30%, the impressive part of the data is that the amino acids which are preserved among different immunoglobulin domains seem to have been preserved in the histocompatibility antigens, beta-two microglobulin and both the alpha and beta chains of the DR antigens. For example, at 31 positions of the Ig constant domain sequence, one residue appears in at least 50% of all

sequences determined in all species. Eighteen of these conserved positions are identical in the DR beta chain sequence. In particular, the same residues corresponding to DR Cys 117, Pro 124, Trp 131, Cys 172, Val 174 and His 176 are identical in 80% of the Ig constant domains.

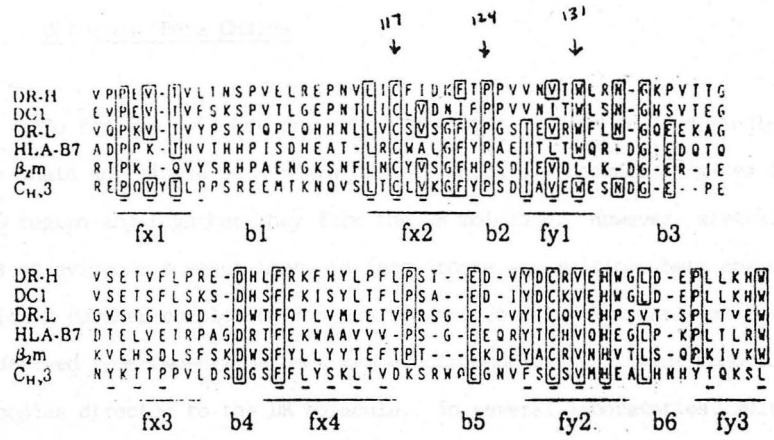

Figure 9. Comparison of the amino acid sequence of the alpha-2 domain of HLA-DR alpha chain to Ig-like domains in DC1 alpha chain, HLA-B7, HLA-DR beta chain, beta-2m and Ig C_H 3. The alignments are made to illustrate those features that are held in common with the structure of an immunoglobulin C region determined by x-ray crystallography. The underlined segments refer to stretches of beta pleated sheet, whereas underlined residues refer to those amino acids that reside in the internal portion of the folded Ig domain. (from reference 32)

In summary, the C-terminal half of DR beta chain (and of DR alpha chains), the third domain of HLA antigens, and human beta-two microglobulin all have significant sequence homology to immunoglobulin constant region domains especially at the conserved residues important in the secondary and

tertiary folding of the Ig domain. All five sequences have a disulfide loop of approximately the same size. In addition, HLA-A and -B antigens and beta-two microglobulins have beta sheet secondary structure. These data support the hypothesis that all of these domains arose by gene duplication and sequence divergence of a common ancestral gene.

V. The DR Antigen Probably Consists of a Single Alpha Chain but Several Different Beta Chains

So far the evidence presented indicates that there is a single alpha chain and single beta chain both of which are encoded by genes in the HLA-D region and together they form the DR molecule. However, several lines of evidence suggest that, in fact, there are multiple beta chain loci providing further complexity for the system (36-38). The earliest evidence was derived from two-dimensional gel electrophoresis utilizing monoclonal antibodies directed to the DR molecule. In several laboratories, multiple beta chain spots were noted. Later, these different beta chains were isolated from presumably homozygous cell lines and primary structural data was obtained on the separated chains. Each was different. Later complete amino acid sequence analysis was performed on pooled beta chains and multiple amino acids were noted in several positions. Finally, analysis at the DNA level utilizing beta chain probes suggests that there are multiple genes encoded in the germline which hybridize to the same beta chain probe. While it has not been definitively demonstrated that each of these genes detected by DNA probes is expressed, the clear indication from previous work suggests that at least some of them are.

We might then ask if each of the beta chains included in the DR complex, are encoded on the human sixth chromosome or, encoded elswhere? The definitive experiments to answer this question have not been done but based on the observed two-dimensional gel polymorphism, the limited sequence analysis available on proteins derived from different HLA-DR homozygous cell lines, preliminary evidence suggesting linkage of different beta chain genes by DNA analysis, as well as recent evidence at the DNA level in the mouse, strongly argues that each of the beta chains are separately encoded within the HLA-D region.

The number of different beta chain loci is still unclear but a minimum of three seems necessary to accomodate the available data. The model presented in Figure 10 which derives in part from the work of Dr. Carolyn Hurley in our laboratory, shows a hypothetical arrangement with a single DR alpha chain and three DR beta chains. Whether all normal individuals actually express the gene products of these three beta chain loci is not known. There is some evidence in the mouse, for example, to suggest that there may be null alleles. Finally, it should be apparent that the ordering of the genes is conjectural although the definitive gene order should be available in the near future.

There is a fair possibility that differences between DR serologic typing vs D region typing which is performed by the mixed lymphocyte reaction may be accounted for by these multiple beta chain loci.

It is fair to point out that there is some evidence, albeit not compelling, that there are additional DR alpha chain loci as well, but most investigators at this point have reinterpreted the evidence for multiple alpha chain loci in light of the SB and DS molecules that we will turn to at this point.

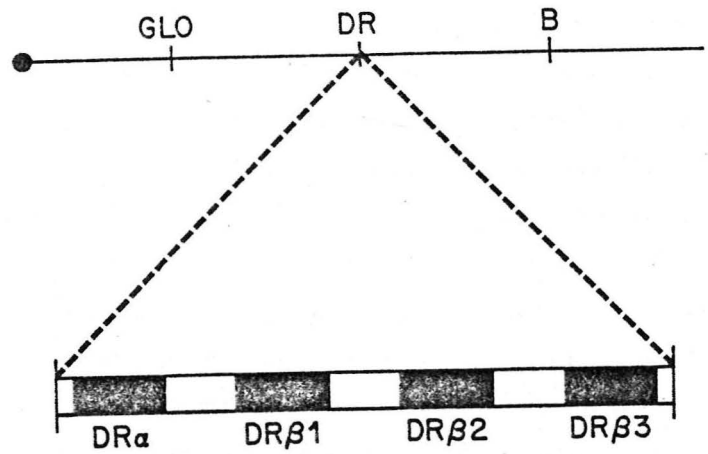

Figure 10. The HLA-D region is thought to consist of at least four loci. A single alpha locus along with three beta chain loci. Neither the order of these genes nor their presence and/or expression in all normal individuals has been definitely established.

THE SECOND D LOCUS (MB/MT/DC/DS)

I. Introduction

Evidence that a second locus existed within or near the HLA-D region in man has been available for several years. Serologists established certain disease associations that were difficult to map to either the HLA-D or the HLA-B regions. At about the same time, several enormously complex serologic systems were described from several laboratories that were called the DC, MB, MT, Te and BR supertypic specificities (see Table II). Each of these specificities had one thing in common - they were typically associated with some but not all DR specificities (39-42). Two schools of thought emerged early. One view was that particular DR antigens, for example, DR4 and DR5 molecules, had certain antigenic determinants in common and, as such, antisera could be raised that could recognize DR4 and DR5 but no other DR molecules. They were termed supertypic specificities because they clustered different DR antigens with single antiserum. A second interpretation of these specificities was that separate gene products -closely associated with DR but present on distinct molecules -were being detected. Since there is extensive linkage disequilibrium throughout the entire HLA complex and few if any crossovers have been described in family studies between, for example, MB and DR, the subject has awaited biochemical verification in order to distinguish the two possibilities (43-45).

TABLE II

Associations between defined HLA-DR antigens and additional reactivities

DR	1	2	3	4	5	6	7
MB_1	+	+				+	
MB_2			+				+
MB_3				+	+		
MT_1	+	+				+	
MT_2			+		+	+	
MT_3				+			+
DC_1	+	+				+	

It is fair to say that there is still no unifying hypothesis to explain the "second locus". However, some of the biochemical experiments suggest that these so-called supertypic specificities reside on separate molecules which are more closely related to the murine I-A antigen. Several authors have recently referred to this locus as DS for second D locus.

II. Biochemical Characterization of DS Gene Products

Radiolabeling so-called DR homozygous cell lines and immunoprecipitation either with alloantisera or monoclonal antibodies were done for "second locus" antigens and the result shown in Figure 11 was typically obtained. A two-chain molecule non-covalently associated on the cell surface with chains of approximate molecular weight 34,000 and 29,000 daltons was found. In immunodepletion experiments, it was shown that these molecules were different from molecules detected by either alloantisera or monoclonal antibodies directed against the DR molecules. These data provided the first evidence that "second locus" molecules were, in fact, distinct from DR molecules.

Amino acid sequence analysis of these molecules soon established their structural uniqueness and clearly defined them as distinct molecules from the DR molecules (46,47). Both their alpha and beta chain sequences differed from the DR alpha and beta chains isolated from the same cell line and, as such, provide a powerful argument that a second distinct locus exists.

Two-dimensional gel electrophoresis performed with these same alloantisera and monoclonal antibodies established that at least the beta chains are polymorphic and encoded by the human sixth chromosome.

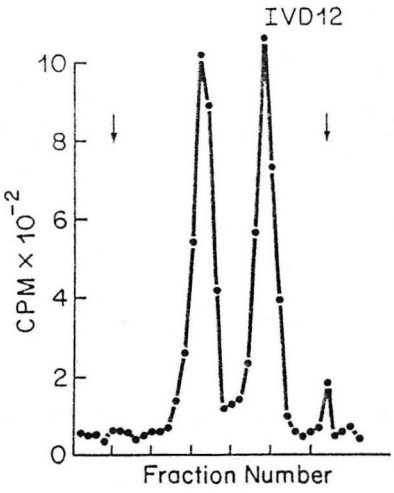

Figure 11. SDS polyacrylamide gel electrophoresis of immunoprecipitated radiolabeled MB antigens from a human EBV-transformed lymphocyte cell line. Molecular weight markers are indicated. The chains migrate with a molecular weight of approximately 34,000 and 29,000 (from Giles, R.C., Nunez, G., Hurley, C.K., Winchester, R., Stastny, P. and Capra, J.D. submitted for publication).

Recently DNA clones have been isolated and sequenced for the alpha chains of this molecule (48). Coupled with the available primary structural evidence obtained by radiolabeled as well as cold amino acid sequence analysis, we now have available considerable primary structural data for both of these chains. While they bear striking homology to the DR molecules, they are clearly distinct as illustrated in Figure 12. However, for the alpha chain, for example, which has been studied most extensively, the organization both at the gene level and the likely organization of the domain structure of the mature protein is remarkably similar to the HLA-DR antigens. A comparison of the structures of the DR alpha and DS alpha chains is shown in Figure 12.

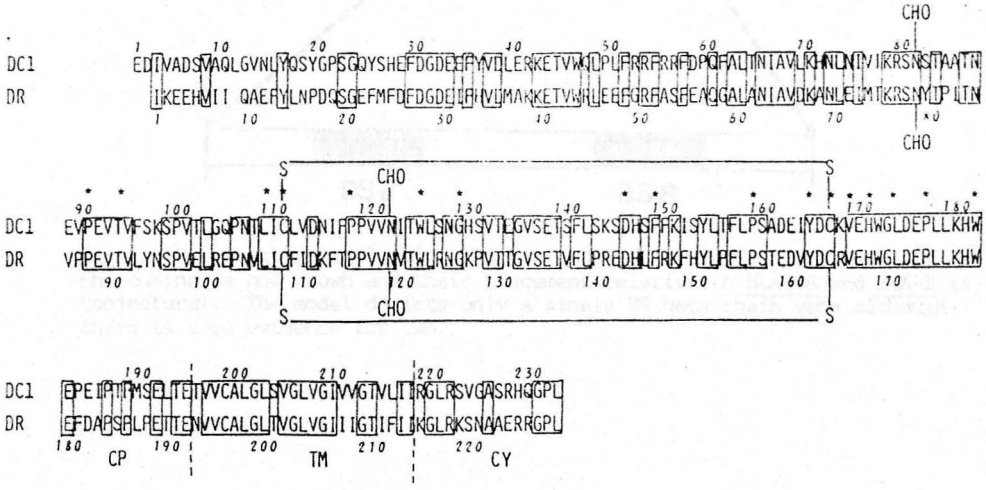

Figure 12. Comparison of DC-1 alpha and DR alpha chain sequences. Identical residues are boxed. The limit of the transmembrane region is indicated by dashed lines. Stars indicate positions in the alpha-2 domain conserved among Ig-like domains. (from reference 48).

These studies demonstrate conclusively that a second locus exists in the human HLA-D region. The available evidence suggests that there is but a single DS alpha and either one or possibly two DS beta chains although much more work is needed to establish this point. This increases the number of genes encoded by the previously defined HLA-D region from four (DR) to six or seven as shown in Figure 13. The actual location of the DS region be it centromeric or telomeric to HLA-D is not known.

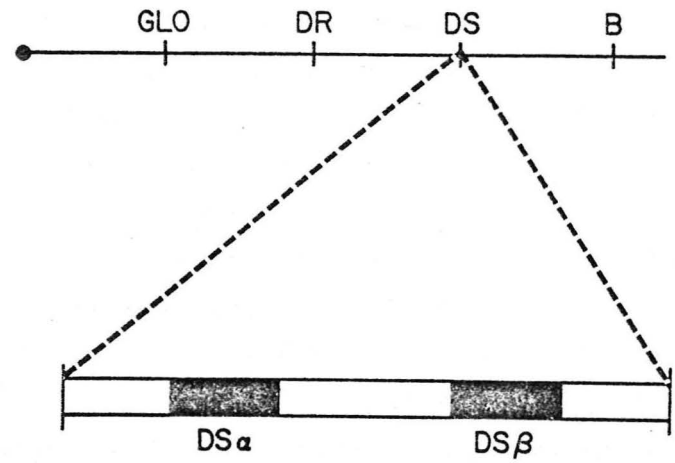

Figure 13. Working model of the Second Locus (DS/MB/DC). The order of the chains is not known and their placement relative to HLA-DR and HLA-B is conjectural. The model depicts only a single DS beta chain gene although there is some evidence for two.

As was the case shown in Figure 11, the order of the chains is not known, however, that information should become available in the near future. The extraordinary sequence homology between these molecules and the DR molecules and their homologies with immunoglobulins and beta-two microglobulins suggests that at least parts of all of these molecules will have similar three-dimensional structures.

The exact relationship between DS, MB, MT, Te and BR is not known at the present time. Table II illustrates a compilation of some of these "supertypic" serologic specificities among the various DR phenotypes. While it appears that from independent laboratories one can correlate DC1, MB1 and MT1, from that point on logic does not provide any particular insights into the genetic organization of this complex. Evidence provided from our own laboratory would suggest that MB antigens (at least in DR4 and DR5 cell lines) have amino acid sequences that are similar to if not identical to DS amd DC-1 molecules (Giles et al., submitted). Similar studies using additional monoclonal antibodies should settle this question in the near future as there are now monoclonal antibodies available that detect presumed MT specificities as well as DC specificities.

THE SB LOCUS

I. Introduction

Primary allogeneic proliferative responses to MLC's between normal individuals matched for all known HLA antigens are very weak. Secondary responses of lymphocytes primed in this fashion, however, can be as strong as those of cells primed to HLA-D/DR differences. Such tests are referred to as PLT (Primed Lymphocyte Typing) tests and PLT reagents generated between such HLA "compatible" priming combinations provided the first evidence for a new segregant series of highly polymorphic cell surface alloantigens (49-50). These antigens are encoded by HLA and map between HLA-DR and GLO. They are preferentially expressed on B lymphocytes rather than T lymphocytes and have, therefore, been designated HLA-"SB" ("secondary B cell") antigens. Their tissue distribution and role in vitro as strong stimulators of secondary lymphocyte proliferation and as targets in cell mediated lympholysis suggests that products of HLA-SB are functionally analagous to those of HLA-DR. Thus far, 7 alleles have been defined in the SB series and studies to analyze disease associations are in progress.

The mapping of the SB locus between GLO and DR (that is, centromeric to DR) was accomplished not only by linkage analysis but by deletion-mutation studies - an innovative new technique that has recently been applied to the HLA system. In this particular technique, gamma irradiated human cell lines of defined HLA specificities are selected with appropriate alloantisera or monoclonal antibodies and the resultant mutant lines

studied both serologically, cytogenetically and structurally (51-52). Point deletions have been extraordinarily important in providing cell lines with more clearly defined specificities. The production of hemizygous lines by this technique has also been important and large deletions in such hemizygous lines have provided important insights into the gene order of various MHC encoded antigens.

II. Structural Studies

Approximately a year ago, Nadler and Schlossman described a monoclonal antibody that recognized a bimolecular complex which was independent of HLA-DR (53). Later studies by Shaw demonstrated that this monoclonal antibody reacted with some of the gene products of the SB locus (54). Carolyn Hurley in our laboratory was able to demonstrate biochemically that this molecule while similar to DR was distinct from it, thereby providing biochemical evidence for a third locus within the human HLA-D region (55). Figure 14 shows the bimolecular complex precipitated by the antibody ILR-1 from a HLA-DR homozygous cell line. The 34,000 molecular weight alpha and 29,000 beta chains are obviously reminiscent of the structures of the DR and DS molecules described above. However, by amino acid sequence analysis, both chains were shown to be distinct from the DR molecules isolated from the same cell line. A portion of these data are shown in Table III where a few radiolabeled amino acids are compared between the alpha and beta chains of DR vs. SB molecules.

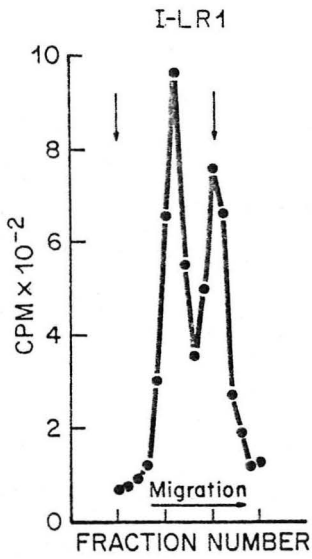

Figure 14. SDS polyacrylamide gel electrophoresis of immunoprecipitated radiolabeled SB antigens from a human EBV-transformed lymphocyte cell line. Molecular weight markers are indicated. The chains migrate with a molecular weight of approximately 34,000 and 29,000. (Kindly provided by Dr. Carolyn Hurley).

TABLE III

Amino Acid Sequence Comparison of SB and DR Antigens

Alpha chains

Position	9	12	13	22	24	26
DR	-*	F	Y	F	F	F
SB	Y	F	-	F	F	F

Beta chains

Position	7	9	16	17	18	24	26	28	30	32
DR	F	-	-	F	F	-	F	-	Y	Y
SB	Y	Y	Y	-	F	F	-	Y	Y	Y

* Indicates the absence of the assigned amino acid at that position.

The extent of biochemical polymorphism at this locus is being addressed in several laboratories including our own at this time. There is no information at the present time which allows us to define both chains as being encoded on the human sixth chromosome. The likelihood, however, if we can extrapolate from DR and DS would be that the HLA-SB region encodes a minimum of two polypeptide chains, an SB alpha and an SB beta chain and that both are adjacent and encoded between GLO and DR. Whether there are multiple SB alpha and/or beta chains is not known but there is no reason to presume so based on the available data.

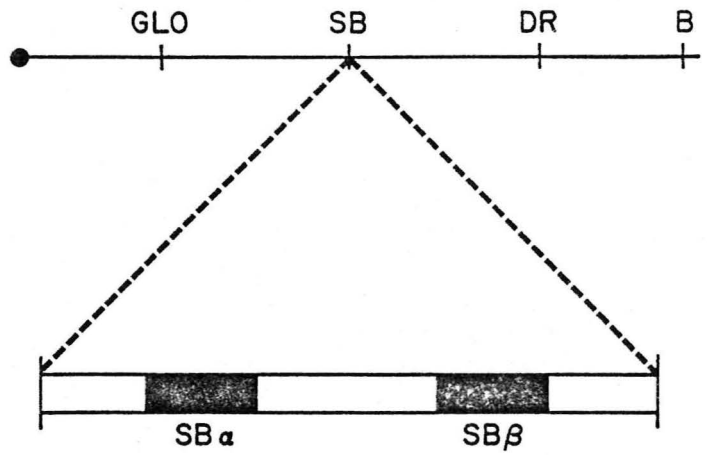

Figure 14A. Working model of the SB locus. The order of the chains is not known.

A WORKING MODEL OF THE HLA-D REGION

Figure 15 summarizes the information that is presently available with the appropriate reservations and extrapolations that have been described above and presents a working model of this complex genetic locus. As can be seen, three genetic regions are now defined between GLO and HLA-B: SB, DR, and DS. Within the SB and DS regions, two polypeptide chains have been defined, an SB alpha and SB beta within the SB region, and a DS alpha and DS beta within the DS region. The DR region, consists of a single alpha chain and a minimum of three beta chains. Thus, these

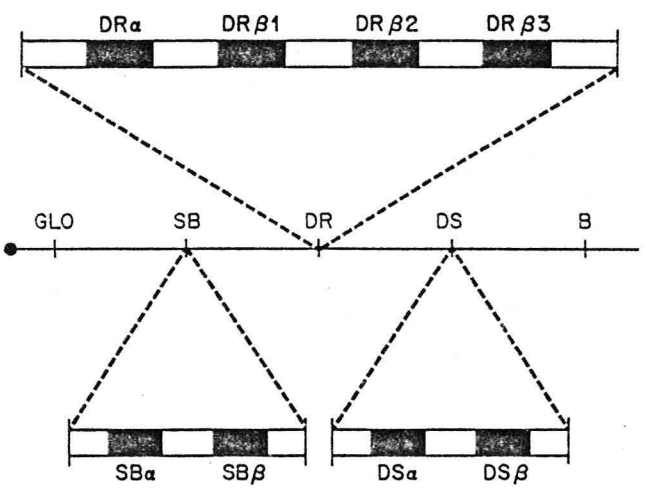

Figure 15. Working model of the genetic organization of the short arm of the human sixth chromosome. The HLA-D region has been expanded to include SB, DR and DS. The order of most of the genes is not known.

eight polypeptide chains (three alpha chains and five beta chains) are presently thought to be encoded where previously only a single specificity was defined between the markers previously defined as GLO and the major transplantation locus, HLA-B. The promise for the future is a more precise linkage of various disease associations within this expanding genetic complex.

A PRIMITIVE GENE?

Several years ago, a glycoprotein was identified on the surface of rodent thymocytes and neurons which became called the Thy-1 antigen. This is a major cell surface protein of these two cell types and homologues have been identified both in the human, rat, mouse and very recently in invertebrate neuronal tissue. In the mouse, this particular molecule has been extremely important as there are two allotypic forms which differ in their amino acid sequence by one amino acid and the allotypes have been used to identify different types of T cells in inbred strains of mice.

All of these Thy-1 molecules have molecular weights of approximately 18,000 and recently with the completion of the amino acid sequence of several of these molecules the strong evolutionary homology of these molecules with immunoglobulins has been appreciated. Indeed, the homology between Thy-1 and immunoglobulins is nearly as great as the homology between various domains of immunoglobulins or various classes of immunoglobulins from one species to the next. A disulfide loop of approximately the same size as in immunoglobulin domains has been identified and extensive computer analysis indicates that it is likely that the three-dimensional structure of the Thy-1 molecule is similar to immunoglobulin domains.

Williams has recently presented arguments that Thy-1 was the precursor gene which gave rise to a whole series of cell surface molecules that are crucial in recognition (56). In many cell surface proteins, the

domain that is closest to the plasma membrane is virtually always homologous with Thy-1. Figure 16 illustrates the molecules described in this Grand Rounds as members of a super gene family. They start on the left with Thy-1, proceed through HLA-DR which we have assumed to be very similar to DS and likely SB. Beta two microglobulin is associated with the major transplantation antigens and finally, the immunoglobulin M and not shown immunoglobulin D molecules, both of which are major cell surface glycoproteins crucial in recognition. Thus, a human lymphocyte can have on its surface molecules such as Thy-1, HLA-SB, HLA-DR, HLA-DS, HLA-A, HLA-B, HLA-C, beta two microgloblin, immunoglobulin M, and immunoglobulin D, all of which probably evolved from a common precursor gene.

Thy-1 seems to be the best candidate for a molecule which may approximate the primordial immunoglobulin domain. The recent ellucidation of the full sequence of a cell surface molecule isolated from the squid showing evidence of extraordinary homology to the mouse molecule is further evidence for this hypothesis. Since there is no known recognition role for the Thy-1 molecule, either our notions that all of these molecules have evolved for recognition needs to be altered or the function of Thy-1 is, in fact, in recognition. The fact that invertebrates which do not have classical lymphocytes do express Thy-1 suggests that the primordial immunoglobulin and histocompatibility antigen domain evolved from neuronal structures. It may well be that Thy-1 functions as a receptor for another molecule (for example, a hormone) with the combining site being the region homologous to the antigen recognition site in immunoglobulin domains.

Alternatively, one could argue that Thy-1 functions in a manner analogous to the constant domains of immunoglobulin and is in effect a ligand that is recognized by receptors on other cells. As such, it may be an important molecule in morphogenesis. In this hypothesis it can be considered as a stable "platform" for the display of determinants that could be protein as in the IgG C region and potentially protein or carbohydrate in the case of Thy-1 antigens. The idea of the Ig domain as a basic recognition unit for cell interaction would then be the functional common denominator of this entire super gene family rather than a function that is limited to immunity. Nonetheless, the notion that a whole host of molecules which have been of interest to immunologists for many different reasons should derive from a common ancestral building block seems far from fortuitous and an unraveling of the mechanisms which led to the evolution to each of these molecules from a presumed neuronal primitive ancestor should be a rewarding avenue of investigation for the future.

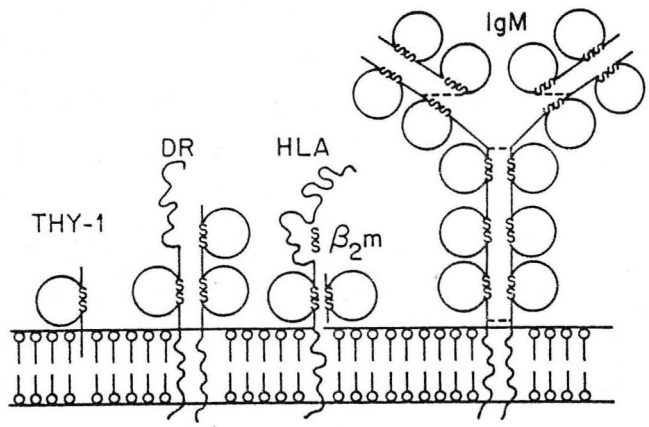

Figure 16. The molecules of the immunoglobulin super family. (Adapted from reference 56).

FUNCTION OF THE MAJOR HISTOCOMPATIBILITY COMPLEX

I. Class I Molecules

Class I molecules are expressed on essentially all cells of the body. With the exception of mature red blood cells in many species, as well as sperm, essentially all cells in mammals contain class I molecules. Thus, presumably the function of these molecules is related to this distribution on all cells.

An increasing body of evidence implicates Class I molecules in the immune response to viral infections. When viruses infect cells, those cells can be killed (lysed by a subset of T lymphocytes called T killer cells or cytotoxic T cells. Cytotoxic T cells can kill virally infected cells before synthesis of new virus is complete thus preventing the spread of the infection. But how do T killer cells distinguish normal cells from virally infected cells? It is thought that the class I molecules play a critical role in this distinction.

The T cell population consists of billions of circulating lymphoctyes, each one bearing receptors able to bind to one particular antigen. This results in a heterogeneous population which can recognize a wide variety of foreign molecules. T cells normally are quiescent and activation is required for them to be effective killer cells. This activation is not simple, but involves the presentation of antigen by a cell thought to be in the monocyte-macrophage series. Thus, viruses

which enter the body are picked up by these "antigen presenting cells" which process the viral antigens and present them to T killer cells which are constantly circulating. Those few killer T cells which have receptors specific for a viral structure will bind the presenting cell. Although there may be many resting cells that can bind to the virus alone, these are not activated. Only those T cells which recognize both the class I surface molecules and the viral structures on the presenting cell will differentiate into T killer cells which are specific for that virus. Such activated T killer cells are very precise in their specificity and will recognize only that specific virus in the context of that specific class I molecule and even closely related viruses and class I molecules will not suffice. This obligate recognition of foreign molecules in the context of class I proteins is called MHC restriction of T cell recognition.

The fact that viruses can infect virtually all tissues of the body might explain the widespread tissue distribution of class I molecules. The extent of polymorphism found on these molecules by this hypothesis might be explained by the notion that the more polymorphic the cell surface molecules are, the greater the potential is the repertoire for viral-MHC interaction. This becomes an important element in a drive for heterozygocity of the organism as it should be appreciated that a heterozygous individual would be able to form far more potential viral-class I molecule complexes than an "inbred" or homozygous individual. Thus, the possibilities on a population scale of a single extremely virulent virus being able to decimate an entire population is decreased. Possibly, the variations seen in HLA alleles that are expressed in different populations around the world result from geographically limited viral selection pressures. Presumably,

disease associations with various HLA A, B and C antigens would be explained on the basis of either underreactivity or overreactivity of T killer cells based on the particular virus-class I molecule interaction.

II. Class II Molecule Function

Class II molecules which have been the subject of this Grand Rounds are largely confined to cells that are characteristic of the immune system. While T killer cells cope with cell bound viruses, antibodies secreted by activated B cells deal with free virus particles. In order to be activated, resting B cells require signals from a subset of T cells called T helper cells. If a T helper cell specific for virus is to "help" a B cell make antibody against the virus, it must have the capacity to recognize not only the virus, but also the B cell. It would be futile for T helper cells to send signals to cells outside the immune system, so unlike T killer cells, T helper cells do not recognize virus in the context of the ubiquitously expressed class I molecules, instead they are limited to the recognition of virus in the context of class II molecules, which are expressed mainly by B cells and antigen presenting cells such as macrophages. The activation of T helper cells is, thus, similar to the MHC restricted activation of T killer cells. Circulating T helper cells encounter virus structures on the surface of a presenting cell. They are activated as a result of interacting with virus and class II molecules on the presenting surfaces and will subsequently assist virus specific B cells carrying the same class II molecules.

Historically, the class II molecules were initially described as IR (immune response) genes. The Nobel Prize in medicine was awarded to Benacerraf two years ago largely for this discovery. The antibody response of an inbred animal is highly dependent upon genes which map to what became known as the I region. It is now known that the molecules that mediate this immune responsiveness or non-responsiveness are, in fact, the Ia (mouse) or DR (human) antigens. These are collectively referred to as Class II molecules because it is still not clear in man, at least, which of these functions is mediated by SB, DR, DS or other still undiscovered Class II loci. It is thought that animals bearing class II alleles that are poorly recognized in association with the foreign molecule will not generate activated T helper cells and will consequently be unable to produce strong antibody responses.

III. How do class I and class II molecules activate T cells?

There is probably nothing in immunology today that is more widely debated than the mechanisms whereby MHC proteins activate T cells. Many feel that like hormones they might work by directly activating any T cell which binds them. But most people feel that the binding of MHC molecules by T cells triggers the antigen presenting cells to present activation signals to the bound T cell. T cells binding virus alone would, therefore, not trigger the antigen presenting cell and would not receive the activation signal. The fact that both class I and class II molecules are transmembrane proteins and the observation that not every cell expressing these molecules can act as T cell inducers supports this notion.

I need not remind you that the MHC genes being highly polymorphic are the major barriers to tissue transplantation. T cells distinguish between the products of self and foreign MHC alleles with great precision destroying any tissue bearing foreign MHC products.

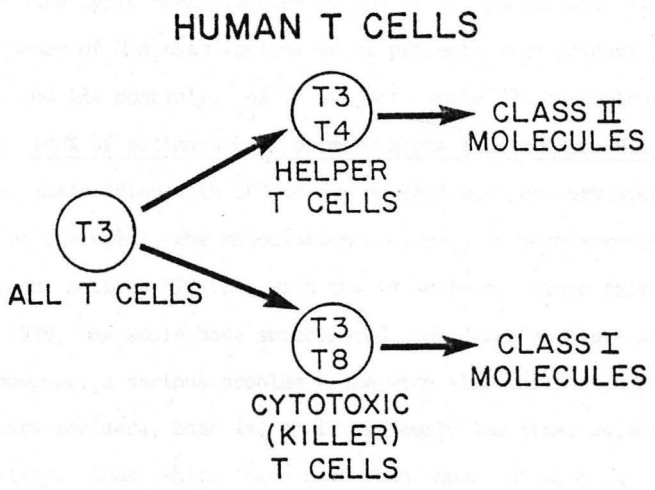

Figure 17. Human T cell subsets interact with different MHC encoded molecules.

THE EXPANDING HLA-D REGION IN HUMAN DISEASE

Just six months ago, Dr. Stastny presented a Grand Rounds describing the association of human disease with HLA-D/DR antigens. He stressed that many infectious, metabolic, neurologic, and particularly rheumatic diseases showed very strong associations with the HLA-D/DR complex. Many of these associations were originally described from this

institution and a review of that area is clearly superfluous. What I would like to stress here is the even stronger association of certain diseases with the additional loci that I have described for you today and, in particular, present evidence for the first association of human malignancy with major histocompatibility complex genes.

Perhaps the earliest associations of "second locus" antigens were with the supertypic specificities of the MT and MB series. Table IV shows the frequency of HLA associations in 22 patients with primary Sicca syndrome and 184 controls. As is evident, while 37% of controls are MT2 positive, 100% of patients with primary Sicca syndrome are MT2 positive. While the association with DR3 is strong (64% with primary Sicca syndrome vs. 31% of controls), the association obviously is much stronger with the second locus antigen MT2 than with the DR antigen. Since this study was done in 1979, one would have anticipated considerable progress in this area. However, a serious problem arose with almost all supertypic specificity antisera, that is, their extremely low titer as well as lack of availability. Thus, while there have been important associations with subsets of patients with systemic lupus erythematosus, and various forms of rheumatoid arthritis with MT, MB or DC specificities, this association of primary Sicca syndrome is prhaps the best example of an association of an "expanding D region" with human disease.

Another important association has been with successful intra-familial kidney transplantation. The MB system which has been worked on extensively on by Duquesnoy was assessed in renal transplantation in a retrospective study of 21 patients who had received a kidney transplant

from a related donor matched for a single HLA haplotype. One year transplant survival was closely correlated with the presence of a compatible MB antigen in the unshared haplotype of the donor: 8 of 8 rejected kidneys were obtained from MB-incompatible donors, and 12 of 13 successful transplants were from MB-compatible donors. The association between MB

TABLE IV

FREQUENCIES OF HLA ANTIGENS IN 22 PATIENTS WITH PRIMARY SICCA SYNDROME AND 184 CONTROLS*

Specificity	Controls	Primary Sicca Syndrome
B8	24%	59%
DR3	31%	64%
MT2	37%	100%

* From refererence 57.

compatibility and allograft acceptance was highly significant, but no significant relation could be demonstrated between the transplant survival and donor-recipient compatibility for the antigens of the HLA-A, -B or -DR loci. These findings suggest that matching for MB may be critical for the selection of donors in intrafamilial kidney transplantation (58).

TABLE V

Influence of Compatibility for Antigens Controlled by Different HLA Loci on One-Year Survival of Kidney Transplants from Related Donors Mismatched for a Single Haplotype

Locus	Number of Compatible Kidneys/Total Number of kidneys		Significance of Difference
	Survival <1 year	Survival >1 year	P
HLA-A	2/8	4/13	0.37
HLA-B	2/8	0/13	0.13
HLA-DR	0/8	4/13	0.12
MB	0/8	12/13	0.000044

Despite the association of murine viral leukemogenesis with particular major histocompatibility complex haplotypes, in man a considerable effort has not revealed tangible evidence of a similar association between HLA-A, B and DR locus alleles and susceptibility to common forms of malignancy. However, in certain families the occurrence of multiple incidents of chronic lymphatic leukemia or Hodgkin's disease has been correlated with the inheritance of particular HLA haplotypes (59,60). The findings of these two reports raised the possibility that previously

unstudied alleles of histocompatilibity might be associated with susceptibility.

Recently in collaboration with Dr. Robert Winchester in New York, we have found an association between a monoclonal antibody to a second D locus antigen MB3 and susceptibility to chronic lymphatic leukemia. This association is shown in Tables VI.

TABLE VI

CONTRASTING FREQUENCIES OF CERTAIN IA ALLOANTIGENIC DETERMINANTS BETWEEN INDIVIDUALS WITH CHRONIC LYMPHACYTIC LEUKEMIA AND A CONTROL POPULATION

Ia Alloantigen Specificity	Individuals with Leukemia n=29 (% positive)	Normal Controls n-28 (% positive)	Relative Risk
DR1	10.3	21.4	-2.4
DR2	6.9	35.7	-7.5
DR3	20.7	25.0	-1.3
DR4	31.0	25.0	+1.4
DR5	62.1	21.4	+6.0
DRw6	13.8	17.9	-1.4
DRw7	20.7	21.4	-1.0
IVD12	93.1	50.0	+13.5

As shown in Table VI, of 29 individuals with B cell type chronic lymphatic leukemia, 27 or 93.1% were reactive with monoclonal antibody IVD12 which defines an MB3-like specificity. Only approximately 50% of normal controls are positive for this MB3-like specificity providing a relative risk of approximately 13.5%.

Recently again in collaboration with Winchester's group, we have demonstrated the relative risk in Hodgkin's disease to be over 50 for a second locus antigen. Virtually every individual with Hodgkin's disease tested in our study bears cells which are reactive with the IVD12 monoclonal antibody.

From these initial studies, it is apparent that as reagents become available to study the expanding HLA-D region gene products, associations with human disease should be even more evident. Recall that in the mouse the vast majority of immune responses are associated with I-A-like molecules. Since the human counterpart of this locus has only recently been defined and since monoclonal reagents have been available for only 6-9 months work in this area should be an extremely exciting and productive area of clinical investigation.

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