ADVANCES IN
Immunology

EDITED BY

FRANK J. DIXON

VOLUME 37

1985

ADVANCES IN
Immunology

EDITED BY

FRANK J. DIXON

Scripps Clinic and Research Foundation
La Jolla, California

VOLUME 37

1985

ACADEMIC PRESS, INC.
(Harcourt Brace Jovanovich, Publishers)

Orlando San Diego New York London
Toronto Montreal Sydney Tokyo

ACADEMIC PRESS, INC.
Orlando, Florida 32887

United Kingdom Edition published by
ACADEMIC PRESS INC. (LONDON) LTD.
24–28 Oval Road, London NW1 7DX

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 61-17057

ISBN 0-12-022437-2

PRINTED IN THE UNITED STATES OF AMERICA

85 86 87 88 9 8 7 6 5 4 3 2 1

CONTENTS

ADVANCES IN IMMUNOLOGY, VOL. 37

Structure, Function, and Genetics of Human Class II Molecules

ROBERT C. GILES AND J. DONALD CAPRA

Department of Microbiology,
University of Texas Health Science Center at Dallas,
Dallas, Texas

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-Da heavy chain and a noncovalently associated 11,500-Da light chain known as β_2-microglobulin. The heavy chain is an intrinsic membrane glycoprotein which is *HLA* encoded and structurally polymorphic. β_2-Microglobulin is an extrin-

ISBN 0-12-022437-2

sic, 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 in the MHC. These subunits are noncovalently associated and consist of a heavy or alpha chain of molecular weight 34,000 and a light or β-chain of molecular weight 29,000 (see Fig. 1). The fact that both subunits of the class II molecule are encoded within the major histocompatibility complex is somewhat unusual. In most other cases in which multimeric proteins are made up of *different* subunits, the genes encoding the separate polypeptide chains are unlinked. Exceptions such as insulin and C4 (the fourth component of complement—see below) arise when a single large precursor polypeptide (polyprotein) 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. Class II molecules are involved in mediating mixed lymphocyte reactions (MLR) and communicating between lymphoid cells. 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 peripheral blood monocytes, macrophages, Langerhans' cells, and dendritic cells of the lymphoid organs.

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)

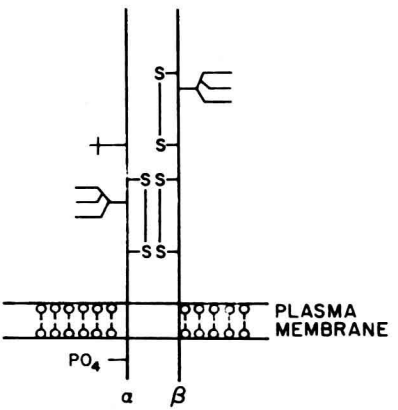

FIG. 1. General structure of human class II molecules. S–S indicates disulfide bridges. The carbohydrate side chains are depicted as well as a possible phosphorylation site. Adapted from Shackelford *et al.* (1982).

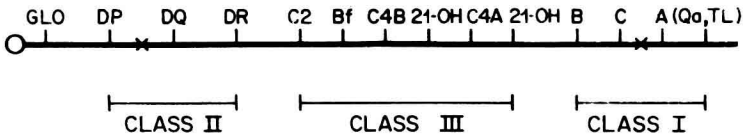

FIG. 2. Genetic map of the human major histocompatibility complex. The precise order of some of these loci is not known. The centromere is located to the left. See text for details.

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, 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 Fig. 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" and, 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 α and a β) are encoded within the subregion and in most instances multiple polypeptide chains are encoded within each subregion of *HLA-D*. The number of these genes that are actually expressed is still a subject of controversy. The two "x's" in the figure indicate areas which may be recombinational hotspots as many of the recombinations that have been observed both within populations and families occur near these two points (Bodmer, 1984).

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. This new nomenclature will be adopted and used throughout this review. While we will explain this in detail later, suffice it to

say that 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).

The first hint of what is now known as the human analogs of the murine Ia antigens came in the early 1970s when Yunis and Amos (1971) demonstrated that the antigens responsible for the proliferation of human lymphocytes in the mixed lymphocyte culture were encoded by a locus closely linked to *HLA-A*, *-B*, and *-C*. This mixed lymphocyte culture locus is now known as *HLA-D*. The serologic characterization of human Ia-like antigens was more difficult than in the mouse system. It was first described by van Rood and coworkers (1975) and depended on the use of selected antisera that inhibited the mixed lymphocyte reaction of cells from *HLA-A*, *-B*, and *C* identical persons.

Polymorphism is a hallmark of the molecules encoded by the MHC. Class I, class II, and class III molecules all exhibit a high degree of polymorphism. The nature and mechanisms of this diversity are likely to be comprehended rather quickly as our knowledge of their primary structures, both at the DNA and protein levels, is increased. A crucial question concerning this system is, "What was the evolutionary pressure that led to and maintained this polymorphism in the population?" In addition, the close association of some alleles of the *HLA-A*, *-B*, *-C*, and *-D* genes with human diseases has made this field an especially interesting one. However the precise role(s) of this polymorphism may be somewhat more ellusive until some of the questions regarding the exact immunologic (or other) functions of these MHC-encoded molecules have been answered.

As our understanding of major histocompatibility complex-controlled immune responsiveness broadens and hybridoma and gene cloning technology advances, specific enhancement of desired immume 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). This review will explore the complexity of the *HLA-D* region, with emphasis on the genetic organization, structure, and function of molecules encoded in this region.

Other reviews detailing more of the history, serology, distribution, and function of the DR and Ia antigens can be found in the literature (Ferrone *et al.*, 1978; Bodmer, 1981; Shackelford *et al.*, 1982; Gorzynski and David, 1983; Auffray *et al.*, 1983), and two recent reviews have appeared in *Advances in Immunology* (Winchester and Kunkel, 1979; Gonwa *et al.*, 1983).

II. HLA-DR Serology/HTC

This review was written immediately after the completion of the Ninth International Histocompatibility Workshop held in Munich, Germany in May 1984. At that meeting, none of the HLA-Dw specificities was upgraded to full status but many new workshop designations were developed. While our understanding of the genetics of class II molecules largely comes from the application of classical serology—especially alloantisera, a major development has been the discovery of monoclonal antibodies with polymorphic specificities for HLA-DR products (Hansen *et al.*, 1981; Pierres *et al.*, 1981; Accolla and Pierres, 1983) including some that appear specific for the products of a single allele (Radka *et al.*, 1983). Excellent reviews of the serology, particularly the historical notions of the serology of the *HLA-D* region and its relationship to DR are available (Winchester and Kunkel, 1979; Ferrone *et al.*, 1978). While some new techniques have improved and simplified HLA-DR typing (Grumet *et al.*, 1983) we will only briefly describe some of the new developments particulary as they relate to the structural aspects which will follow.

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 Fig. 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. Because it has not yet been unambiguously demonstrated which of the various subregions contributes to most MLR reactivities, the Ninth International Workshop Nomenclature Committee chose not to upgrade the Dw specificities to full status. In addition, (as will be detailed in the next section), "w" has been added to all DQ and DP specificities. The designation HLA-Dw with no further indication of subdivisions, is retained for the MLC defined specifities which have yet to be mapped to a subregion.

Tables I to IV summarize our present understanding of the various

TABLE I
GENE FREQUENCIES FOR HLA-DR[a]

HLA-	Caucasians	Orientals	Negroids
DR1	9.5	5.0	5.1
DR2	15.8	15.1	15.1
DR3	12.0	1.8	14.9
DR4	12.7	21.8	7.6
DR7	12.0	2.9	13.2
DRw8	3.0	7.3	0.8
DRw9	0.8	11.5	1.5
DRw10	0.8	0.5	2.3
DRw11	12.3	4.0	16.5
DRw12	2.0	7.2	3.4
DRw13	5.4	2.9	3.8
DRw14	5.8	6.8	10.7
DR X	7.9	13.2	5.3
Sample size[b]	1926	752	263

[a] From Baur, M. P., Neugebauer, M., Deppe, H., Sigmund, M., Mayr, W. R., Albert, E. D., *in* "Histocompatibility Testing 1984."
[b] Number of haplotypes counted.

serologic specificities extant in the *HLA-DR* region. Table I lists the Dw a gene frequencies for HLA-DR reported at the Ninth Workshop. Table indicates ne provisional designations for HLA-DR specificities. Note, p.

TABLE II
NEW PROVISIONAL DESIGNATIONS FOR HLA-DR SPECIFICITIES[a]

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

[a] These designations (like those in Tables III and IV) are derived from the Nomenclature Committee of the Ninth Workshop and will be published in "Histocompatibility Testing 1984" (E. D. Alpert, M. P. Baur, and W. R. Mayr, eds.), Springer-Verlag, Berlin and New York, 1984.

TABLE III
NEW PROVISIONAL DESIGNATIONS FOR HLA-Dw SPECIFICITIES

New	Previous equivalents
Dw13	DB3
Dw14	LD40
Dw15	DYT, YT
Dw16	DB8, B8
Dw17	7A, (Dw7A)
Dw18	6A, (Dw6A)
Dw19	6B, (Dw6B)

ticularly, that DR5 has been "split" into DRw11 and DRw12; and that DRw6 has been similarly split into DRw13 and DRw14. It will not always be possible in this review to use this newer nomenclature as often the typing which might have revealed the "split" was not done. Table III lists new provisional designations for HLA-Dw specificities and their equivalents and, finally, Table IV indicates our present understanding of Dw and DR relationships.

At the present time there are still approximately 8% *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.

The DR1 specificity exists on a DR or I-E-like molecule and there is no evidence that there are splits of this specificity.

DR2 is split into at least two and possibly a third serologic grouping (Kasahara *et al.*, 1983). There are two separate Dw specificities that correlate with DR2, Dw2, and Dw12 (see Table IV). There is an additional third

TABLE IV
Dw AND DR RELATIONSHIPS

Dw specificities	Associated DR specificities
Dw1	DR1
Dw2, Dw12	DR2
Dw3	DR3
Dw4, Dw10, Dw13, Dw14, Dw15	DR4
Dw5	DRw11 (5)
Dw6, Dw18, Dw19	DRw13 (w6)
Dw9, Dw16	DRw14 (w6)
Dw7, Dw11, Dw17	DR7
Dw8	DRw8

grouping that may exist. By restriction fragment length polymorphism (see below) there may be as many as three additional splits. At the present time, however, firm evidence exists for only two splits of DR2.

There is no evidence at the present time for splits of DR3 and essentially all DR3 specificities are associated with Dw3.

DR4 is extremely complicated and is conveniently divided serologically into a minimum of three separate groupings that have been referred to as 4.1, 4.2, and 4.3. 4.1 includes the LD40 and Dw4 groups. It may include DYT as well. 4.2 includes the DB3 group but includes other specificities as well. The 4.3 group includes the Dw10 group. By HTCs there is a minimum of five splits of DR4, Dw4, Dw10, Dw13, Dw14, Dw15 (see Table IV). There is biochemical evidence from several laboratories that different DR4 specificities can be explained by variations in DR (I-E-like) β-chains. The biochemical evidence for this will be presented later but suffice it to say that by both isoelectric focusing in one dimension, by two-dimensional gel electrophoresis and by restriction fragment length polymorphism several groups have demonstrated biochemical variation among DR4 genes and/or their products which correlate with one or more serologic or HTC splits.

As mentioned above, DR5 has been split into DRw11 and DRw12. DRw11 associates with Dw5 and DRw12 associates with DB6.

DRw6 is extremely complicated. It has been split serologically into a minimum of two groups previously referred to as 6.6 and 6.9 as shown in Table II. The 6.6 and 6.9 terminology has been changed to Dw13 and Dw14 (other equivalents are also noted in Table II). At the recent Ninth Workshop, evidence was presented utilizing two-dimensional gels that there were four separate biochemical patterns among DRw6 individuals. The alloantisera which define Dw13 and Dw14 correlated with two of these patterns.

At the present time, there are no obvious serologic splits of DR7, however by HTCs, DR7s can be divided into two separate groups so it is likely that a minimum of two separate groups of DR7's exist.

DRw8, like DRw6, still does not have full Workshop designation. However, no evidence exists at the present time for serologic splits, although by HTCs there is evidence for a minimum of three separate groups of DRw8 (Mickelson *et al.*, 1983).

Despite some attempts DRw9 has not been split serologically or with HTCs.

DRw10 seems homogeneous serologically with only a single group. However this has not been studied extensively and there may well be additional splits.

Thus DR1, 3, 7, w8, w9, w10, w11, w12, w13, and w14 presently exist as single serologic entities; DRw9 as two; and DR2 and 4 as three. This makes a

total of 15 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 (see below) 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 α-chain (nonpolymorphic) and probably three β-chains (see below). It has been exceedingly difficult to assign specific β-chain gene products to these polymorphisms. However it seems quite clear that I-E-like β-chains are the polymorphic component of class II antigens as no variation in α-chains has been observed to explain these specificities. Whether differences in reactivity are explicable based on different β-chain gene products is still a matter of controversy.

Finally, since approximately 10% of the population are officially listed as "DR blank" it is likely that many additional specificities will be discovered. Recently, Wallin *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 HLA-DR blank phenotypes will be dissected in the near future.

Thus, the situation in the human *HLA-DR* subregion is quite reminiscent of the mouse *I-E* subregion. While the bulk of evidence suggests that the human DR α-chain is nonpolymorphic, the murine *I-E* α-chain is modestly polymorphic. This has been seen both serologically, biochemically, and recently at the DNA level. However, all would agree that the bulk of the polymorphism in both the murine *I-E* subregion and the human *HLA-DR* subregion relates to the beta chain. In man, it is clear that a minimum of two and probably three β-chains is expressed. In the mouse, there is some evidence for more than one expressed β-chain. In the genome there are clearly additional I-E-like β-chain genes but evidence for their expression is not definitive (Steinmetz and Hood, 1983).

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 Seventh Histocompability 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

Eighth Workshop, additional clusters of cross-reactive alloantisera had beer described. Due to their ability to recognize several *HLA-DR* allelic prod ucts, these antisera were classified as recognizing "supertypic specificities." Those recognized to date include the MB, MT, DC, LB, BR and Te serie: (see Table V). Each of these specificities is found in linkage disequilibriun (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 serie: 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 betweer these independently defined supertypic series has not been clear. For exam ple, 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 V) 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 dif ferent series. With this in mind a simplified scheme relating the supertypic specificities to each other is presented in Table V (taken from Hurley *et al.* 1983a). In this formulation, specificities that have similar distributions are assumed to be identical; slight differences observed by different laboratories in the association of the supertypic specificities with DR haplotypes and the degree of linkage disequilibrium are assumed to be differences in alloan tisera used by these groups. 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.

One of the more controversial questions involving HLA-D region mole cules over the last 5 years has centered around the highly complex pattern of reactivity of these alloantisera recognizing the supertypic specificities. A issue has been the question of the molecular bases for these patterns o

TABLE V
SUPERTYPIC SERIES

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

"cross-reactivity" as seen by these alloantisera and more recently by monoclonal antibodies. 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 specificity was originally identified by radioimmunoassay using the cell line Daudi (DRw6, blank). By performing both quantitative and qualitative binding studies of a large number of alloantisera on an ^{125}I-labeled class II preparation from Daudi, they were able to demonstrate two well-defined class II subsets; one subset corresponded to the DRw6-bearing antigens, while the other subset defined the DC1-bearing antigens. This was the first real evidence suggesting the existence of a second *HLA-D* region locus, although the structural basis of the DCl-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—see HLA-DQ Biochemistry).

Shortly before the Eighth Histocompatibility Workshop, Duquesnoy *et al.* (1979) defined serologically the MB supertypic specificities. Their data demonstrated that anti-MB1 activity could be removed by absorption with either DR1 or DR2 positive cells, but it could not be lysostripped by anti-DR1 or anti-DR2 antisera. During the Eighth 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 V) 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—see below) 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 questions and some of the answers generated in this debate are dealt with in Section X of this review.

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. Its oldest nomenclature and 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