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Molecular Basis of Human Leukocyte Antigen Class II Disease Associations

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I. Introduction

Current knowledge of the structures and biological functions of the human leukocyte antigen (HLA) system provides a framework for unraveling the mechanisms of one of the most puzzling enigmas of modern biology and medicine, which consists of the definite associations among components of the system and genetic susceptibility or resistance to disease (Dausset, 1981). Although known for over 25 years, this phenomenon is only now beginning to be understood in molecular terms. Indeed, recent immunological, biochemical, and molecular biological developments have led to precise understanding of the structures and functions of the human major histocompatibility complex (MHC). This knowledge is prerequisite in evaluating the genetic and somatic aspects of the role of the HLA complex in the physiology of the immune response and their contribution to autoimmunity. The most significant initial observation has been an increase in the frequency of certain serologically defined alleles of the HLA complex in several autoimmune diseases.

During the past 15 years fastidious attempts have been undertaken to improve the identification of the most significant HLA components involved in genetic associations. However, since the HLA system displays the unusual feature of a strong linkage disequilibrium between loci and alleles, the genetic traits found to be associated with disease did not emerge at random. The pattern of genetic associations followed an almost constant trend. The associations gained strength each time an additional locus centromeric to the precedent was individualized.

The advances made in this respect almost paralleled the introduction of progressively more refined typing procedures, which allowed the division of former genetic entities (loci and alleles) into additional subtypes. This is best illustrated in the case of insulin-dependent diabetes mellitus (IDDM), which was initially found to be associated with alleles

within the A, then the B, locus and finally the HLA-D region. Among the HLA-associated diseases, or at least for those diseases in which an autoimmune process is suspected to be directly relevant to the pathogenesis, the associations are with genes and molecules of the HLA-D region (HLA class II genes and products). The most recent data assign the disease susceptibility to common amino acid sequences present on a HLA class II molecule within its "active" site.

It can be postulated that a specific antigen (or set of antigens), presumably in the form of short peptides, bind with exquisite affinity to the precise structure of the class II molecules associated with a particular disease. This would lead to inappropriate antigen presentation and T cell activation, resulting in an impaired immune response. However, the exact nature of the antigens (foreign or self) remains enigmatic as the potential influence of the T cell receptors (TcR) repertoires. Present knowledge is still insufficient to construct a definitive representation of the trimolecular structures presumably involved in controlling the immune response (MHC–antigen–TcR).

Moreover, with regard to disease susceptibility, one cannot discriminate between two possibilities. Is the disease susceptibility associated with one or a few contiguous specific linearly displayed amino acids, or with several amino acids (or sets of amino acids) distantly spaced in the molecule, creating a unique three-dimensional conformation? These two hypotheses have practical implications for selecting the most pertinent typing approach in studies of disease susceptibility. Indeed, in the first case identification of the crucial amino acids (or stretch of amino acids) should suffice, while in the second case a three-dimensional structural model ultimately would be required to identify the exact composition of the disease susceptibility elements.

This chapter focuses strictly on the HLA MHC class II genes and molecules with regard to how they contribute to better delineation of the genetic associations and how the current knowledge of their structure, expression, and functions can be used to speculate on their role in the pathogenesis of disease. Because of the strong linkage disequilibrium between loci and alleles, I feel justified in restricting the description of the genetic associations to only the most recent data (mainly generated by molecular means), since they supercede in precision and accuracy the previous data obtained by serological methods.

Furthermore, emerging studies of gene regulation and expression provide information concerning the function of these genes and molecules. Such approaches are intended to identify which HLA molecules confer the highest susceptibility or resistance to a disease and how they contribute to disease development. I discuss the central contribution of three-dimensional conformational structures of the HLA class II mole-

cules and emphasize in particular the possibilities generated by the formation of hybrid HLA class II molecules, the prominent role of which has been highlighted by the most recent epidemiological studies of disease susceptibility. In addition, such genetic and molecular approaches have practical implications in predictive medicine (i.e., identifying individuals at risk for a disease) and may provide new rationales for therapeutic intervention.

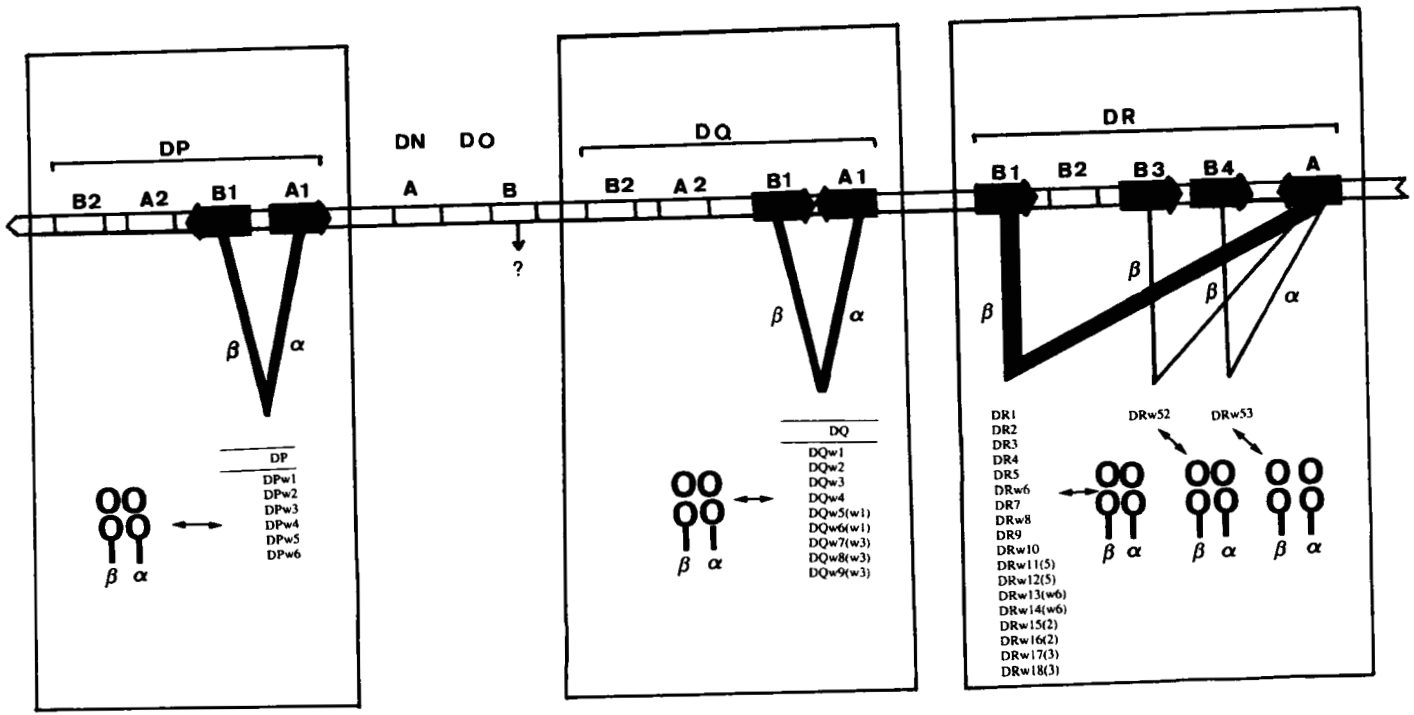
II. HLA Class II Structures: Genes and Molecules

Class II molecules have a central immunoregulatory role. They are cell surface glycoproteins expressed principally on macrophages, monocytes, and B lymphocytes, which present processed antigens (in the form of peptides) to antigen-specific T lymphocytes, leading to their specific activation and proliferation. Such T cell features are implicated in the development of autoimmunity. A brief description of the class II genes and molecules is required in order to discuss their role in disease susceptibility (Fig. 1).

The human MHC class II genes are located on a segment of chromosome 6 and consist of several sets of α–β species, for a total of 14–15 individual genes per haplotype. Three major subregions—namely, DR, DQ, and DP—are identified by their cell surface-expressed products, consisting of one α–β dimer each for DQ and DP and one or two concomitantly expressed α–β dimers for DR, depending on the haplotype (e.g., DRA–DRB1 and DRA–DRB3 or DRA–DRB4) (Korman *et al.*, 1985; Kappes and Strominger, 1988). In addition to the expressed α–β genes, MHC class II pseudogenes (e.g., DRB2, DPA2, and DPB2), nonexpressible genes (e.g., DQA2 and DQB2), and weakly expressed genes (e.g., DNA and DOB) are found (Trowsdale and Kelly, 1985; Tonnelle *et al.*, 1985).

Overall, MHC class II genes are characterized by two apparently opposing structural features, both of which are critical to our understanding of the system. First, the HLA class II genes and proteins are homologous to each other (as well as among species). Second, most of the class II genes are highly polymorphic at the population level. Therefore, gene duplication, gene conversion, and intralocus double crossings-over are the most likely mechanisms to have generated the present isotypic and allelic diversities (Auffray *et al.*, 1984; Gustafsson *et al.*, 1984; Gorski and Mach, 1986; Andersson *et al.*, 1987). Together, these facts explain several features of the observed polymorphism. Most of the class II polymorphism is located at the first domain of the molecule and appears to be clustered into two to four hypervariable regions.

These structural variations between the class II gene products are the


DP
B2
A2
B1
A1
β
α
DP
DPw1
DPw2
DPw3
DPw4
DPw5
DPw6
DN
DO
A
B
?
DQ
B2
A2
B1
A1
β
α
DQ
DQw1
DQw2
DQw3
DQw4
DQw5(w1)
DQw6(w1)
DQw7(w3)
DQw8(w3)
DQw9(w3)
DR
B1
B2
B3
B4
A
β
β
β
α
α
DR1
DR2
DR3
DR4
DR5
DRw6
DR7
DRw8
DR9
DRw10
DRw11(5)
DRw12(5)
DRw13(w6)
DRw14(w6)
DRw15(2)
DRw16(2)
DRw17(3)
DRw18(3)
DRw52
DRw53

fundamental differences permitting immune recognition and are therefore likely to be critical in disease susceptibility. The crystal structure of one HLA class I molecule has recently been obtained, and, because of the overall similarity in amino acid composition and general secondary conformation of classes I and II molecules, it was subsequently used to create a three-dimensional model for class II molecules (Bjorkman *et al.*, 1987; Brown *et al.*, 1988).

Although hypothetical, such a model provides a structural framework for understanding the function of an MHC class II molecule. An antigen recognition site can be proposed which is composed of an internal cavity lined by two α-helical structures and closed at the bottom by a platform of eight β-pleated sheet structures. When the model is compared with a bulk of published and unpublished serological, functional, and genetic data, residues which are monoclonal antibody epitopes (i.e., protruding into the solvent) or class II-restricted T cell recognition epitopes (i.e., facing into the site or upward) can be predicted. Thus, a model is provided for the binding of foreign or self-peptides and for the identification of the putative contact sites between the peptides and the class II residues.

The amino acid stretches of class II allelic variability previously identified can then be positioned in this three-dimensional model to delineate the components of the active sites of the molecules. For example, along the DR β1 chain the polymorphic residues are part of the β-pleated sheet

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FIG. 1. The class II genes of the HLA system are clustered on the short arm of chromosome 6 into three subregions: DR, DQ, and DP. Overall, the genes are organized into exons coding for domains of the protein and noncoding introns. A class II molecule is a glycoprotein dimer composed of a transmembrane α chain coded for by an A gene, noncovalently associated with a transmembrane β chain coded for by a B gene. The DR subregion contains a nonpolymorphic DR A gene and three or four DR B genes, depending on the haplotype. The DRB1 genes are highly polymorphic and correspond to the DR1–DRw18 specificities. DRB2 is a pseudogene. The former are associated with either a DRB3 gene (in haplotypes 3, 5, w6, and w8), which is moderately polymorphic and corresponds to the specificity DRw52, or a DRB4 gene (in haplotypes 4, 7, and w9), which is monomorphic and corresponds to the specificity DRw53. The DQ molecules are α–β dimers composed of a DQ α chain coded for by a DQA1 gene and a DQ β chain coded for by a DQB1 gene. Both are highly polymorphic. The DQA2 and DQB2 (formerly DX α and DX β) genes are homologous to the precedent, but are not expressed. The DQw1–DQw9 specificities reflect the DQ β chain. The DP subregion contains two expressed genes: DPA1 and DPB1. DPA1 has only two known alleles. DPB1 polymorphism is more extensive and corresponds to the DPw1–DPw6 cellularly defined subtypes. DP α and β chains associate in a DPα–β dimer. Additional genes are present which are not regularly expressed or are expressed at low levels.

platform for the two first hypervariable regions (amino acids 9–13 and 25–33; HVR1 and HVR2, respectively), while the major HVR region (HVR3) from amino acids 57 to 76 (extending to amino acid 85 and centered on amino acids 67–74) is located on turns of the α-helical structure. In the class II molecules both the $\alpha 1$ and $\beta 1$ domains participate in the putative antigen recognition site. Indeed, an intermolecular dimerization must be postulated in the case of the HLA class II model, in order to be consistent with the three-dimensional structural pattern of the class I molecule. This dimerization is likely to be formed by a disulfide bridge between the two spatially close amino acids at positions 78 and 15, respectively, on the α and β chains.

III. HLA Class II Polymorphism and Typing

This section evaluates the contribution and potential of the different typing procedures used to identify disease susceptibility factors.

A. Serology

Serologically defined HLA class II specificities consist of conformational motifs (or epitopes) present on at least one set of HLA class II molecules expressed at the surface of the B lymphocyte population and recognized by allospecific antibodies (monoclonal or polyclonal) (Fig. 2). Such discrete epitopes can be unique [monoclonal antibody (mAb) binding] or grouped (alloantibodies) and expressed on the same molecule; an epitope can eventually be found on different types of molecules (belonging to the same isotype or to several different isotypes). In the latter case the epitope represents a shared determinant between different alleles or different isotypes (i.e., interallelic or interisotypic determinant, respectively) and is termed "supertypic."

Serological typing can thus identify the presence of an epitope, but cannot directly assess the molecular species on which it is present. This can be deduced from immunoprecipitation or gene transfection studies which would, in addition, allow gene assignment of the epitope. Examples of supertypic epitopes are numerous; DR2 and DR4 specificities are found on several different HLA molecules (i.e., interallelic epitopes) (Wu *et al.*, 1986; G. T. Nepom *et al.*, 1983). DRw52 can be found on different alleles of the same gene (DRB3) but also on a different gene product (DRB1) (Haziot *et al.*, 1986). Similarly, DRw53 is found on either DRB1 or DRB2 (Matsuyama *et al.*, 1988).

If one excludes the rather unlikely case of an alloantibody recognizing by chance a genuine disease susceptibility epitope, serological typing reflects a complex situation of linkage disequilibrium. Indeed, the epi-

a

D	DR	DQ	DP
Dw1	DR1	DQw1	DPw1
Dw2	DR2	DQw2	DPw2
Dw3	DR3	DQw3	DPw3
Dw4	DR4	DQw4	DPw4
Dw5	DR5	DQw5(w1)	DPw5
Dw6	DRw6	DQw6(w1)	DPw6
Dw7	DR7	DQw7(w3)	
Dw8	DRw8	DQw8(w3)	
Dw9	DR9	DQw9(w3)	
Dw10	DRw10		
Dw11(w7)	DRw11(5)		
Dw12	DRw12(5)		
Dw13	DRw13(w6)		
Dw14	DRw14(w6)		
Dw15	DRw15(2)		
Dw16	DRw16(2)		
Dw17(w7)	DRw17(3)		
Dw18(w6)	DRw18(3)		
Dw19(w6)			
Dw20	DRw52		
Dw21			
Dw22	DRw53		
Dw23			
Dw24			
Dw25			
Dw26			

b

DR2	DRw15, DRw16
DR3	DRw17, DRw18
DR5	DRw11, DRw12
DRw6	DRw13, DRw14
DQw1	DQw5, DQw6
DQw3	DQw7, DQw8, DQw9
Dw6	Dw18, Dw19
Dw7	Dw11, Dw17

c

HLA-D Specificities	Associated DR Specificities
Dw1, Dw20	DR1
Dw2, Dw12	DRw15(2)
Dw21, Dw22	DRw16(2)
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
Dw23	DR9
Dw24, Dw25, Dw26	DRw52

FIG. 2. (a) Listing of the HLA-D region specificities, (b) listing of the new subdivisions (splits), and (c) correlation between HLA DR specificities and HLA D typing. (Data obtained from the HLA Nomenclature Committee.)

tope recognized by an antibody can be present on the class II molecule involved in the susceptibility, while also present on a distinct molecule irrelevant to the disease. Assignment of the antibody specificity to disease susceptibility could only be allowed in the case of recombination between two genes. Such recombination is rarely found within a homogeneous population and is more frequently found between different ethnic groups. This explains the interest in studying HLA and disease associations in these groups.

Since there are only 20 officially recognized HLA-DR typing serological specificities (HLA nomenclature) and the structural variation accounts for over 50 alleles, most of the serologically defined specificities are likely to be supertypic. Moreover, since serological reagents were selected through allorecognition, they are not necessarily suitable for detecting disease susceptibility epitopes. HLA serological typing thus may presently represent a rather incomplete approach to typing for HLA disease associations. New serological reagents are being developed to circumvent these difficulties. HLA class II transfectants or transgenics can be used to produce specific antiisotype and/or antiallele antibodies.

A series of even more refined approaches will be undertaken when a precise sequence of the class II molecule is identified as the disease susceptibility element. Site-directed mutagenesis of the class II molecule or immunization with synthetic peptides should generate antibodies uniquely directed toward discrete sequences of the class II product (i.e., disease-specific epitope) (Atar *et al.*, 1989). This approach would not only be invaluable for epidemiological studies, but should be ultimately considered for epitope-specific therapy. This approach is particularly pertinent in the case of hybrid determinants (i.e., determinants created by the formation of particular α–β heterodimers, as discussed below) for which no serological reagent is available.

Apart from serology for the detection of cell surface-expressed (thus, conformational) epitopes, several molecular methods have been developed and have been widely used in HLA typing over the past 10 years. They include analysis of the polymorphism at either the protein or DNA level and therefore largely reflect linear amino acid or nucleic acid sequences. My purpose is not to review these approaches in detail, but to highlight some of their characteristics and the implications for HLA and disease association.

B. Biochemistry

Biochemical typing, which includes one-dimensional isoelectrofocusing (IEF) and two-dimensional polyacrylamide gel electrophoresis (PAGE), usually requires radioactive cell labeling and immunoprecipita-

tion with specific mAb's), steps which are both time consuming and expensive. Two-dimensional PAGE at least has proven to be highly efficient in detecting new HLA class II variants (Fig. 3). Furthermore, it represents the best technique for definition of the reactivity of mAb's in association, eventually, with immunodetection by Western blotting of their individual chains or complex reactivity. The first dimension (of a two-dimensional PAGE) can be nonequilibrium pH gradient electrophoresis or IEF, which provides better resolution of the class II β and α chains, respectively. Since our initial description of the DR β chain polymorphism, two-dimensional PAGE has been widely used to identify molecular variants belonging to DR2, DR4, DR5, DRw6, DR7, and DRw52.

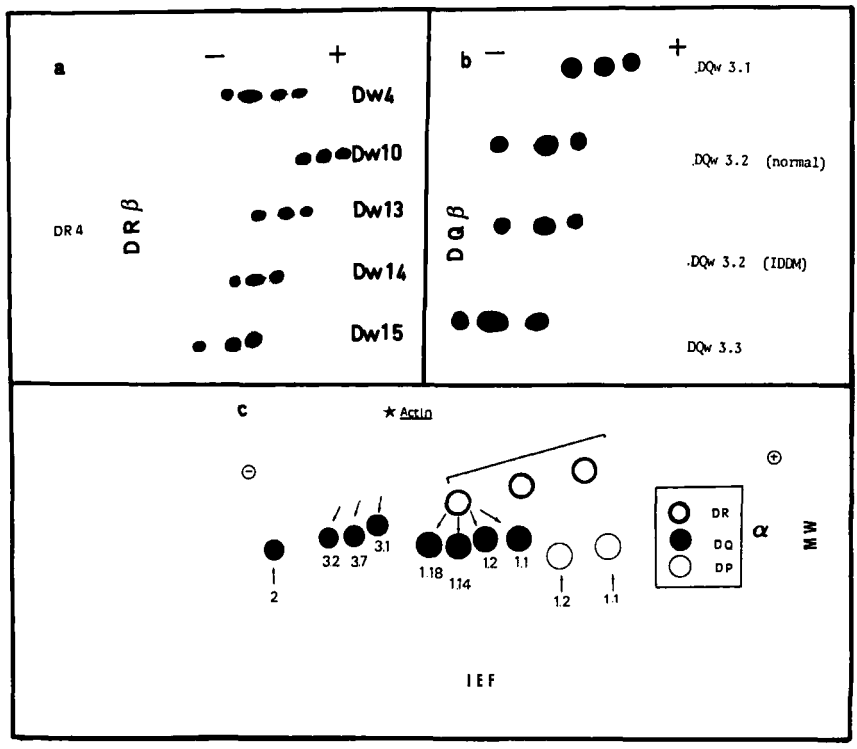


FIG. 3. Two-dimensional gel electrophoresis analysis of HLA class II molecules. Schematic representation of (a) DR β1 chain variants in DR4 individuals, (b) DQ β chain variants in DQw3 individuals, and (c) DR, DQ, and DP α chains of the different haplotypes. IDDM, Insulin-dependent diabetes mellitus; IEF, isoelectrofocusing.

So far, this is one of the best typing procedures for the identification of DQ α and DP α variants, with eight and two alleles, respectively (Charron and McDevitt, 1979, 1980; Knowles, 1989; Charron, 1990; Charron and Fernandez, 1990).

The theoretical limitation is that in some instances conservative amino acid substitution will not be detected electrophoretically and thus will not be resolved by two-dimensional PAGE. In practice, this appears to be very rare. Further, in the case in which the initial (i.e., most basic) location of two different alleles is electrophoretically indistinguishable, the more processed forms (more acidic locations) are distinct. This reflects the fact that, although the net charges of two molecules may be similar, the type and the variety of amino acids which compose the molecule do, in fact, slightly affect their electrophoretic migration. This is clearly the case when the DR β1 chains from DR1 and DRw9 cells are analyzed (R. Fauchet and D. Charron, unpublished observations). In contrast, limited substitution (e.g., point mutation) at the genomic level may induce drastic changes in the net charge and variants, which are silent by genomic analysis [restriction fragment-length polymorphism (RFLP)] but are readily distinct by two-dimensional PAGE as in the case of DR–BON versus DRB1 gene products (Coppin *et al.*, 1987).

Biochemical typing has a unique potential of importance in disease association, since it is at present the only typing technique which can detect hybrid molecules, such as intraisotypic hybrid molecules, obtained by *trans*-association and interisotypic association from *cis*-and/or *trans*-complementation. (Charron *et al.*, 1984; Lotteau *et al.*, 1987b). Although the detection of such hybrid molecules requires rare reagents (such as chain-haplotype-, or allele-specific mAb's), it is a critical step in studying HLA and disease association, since several epidemiological studies have documented the high incidence of such hybrid molecules in certain diseases (Nepom *et al.*, 1984; Ronningen *et al.*, 1989).

The use of silver staining instead of radioactivity in two-dimensional PAGE and the development of Western blotting and IEF may provide more simple ways to perform biochemical typing for HLA class II in the near future (Hermans *et al.*, 1989a; Rodriquez de Cordoba *et al.*, 1989). Apart from the genetic polymorphism, biochemical studies of HLA class II molecules are also of interest in the study of the diversity of expression of class II molecules in cells of the diseased organs in which they may appear in an aberrant manner. Protein analyses are thus complementary to studies at the transcription level performed by Northern blot, run-on/run-off, and S1 nuclease protection assays.

In brief, the biochemical approach encompasses the genetic and somatic aspects of HLA class II, both of which are pertinent to the question of HLA and disease association.

C. DNA (RFLP/ALLELE-SPECIFIC OLIGONUCLEOTIDES)

1. RFLP

The combination of restriction endonucleases and specific probes enables one to study allelic polymorphism arising from genomic neucleotide sequence variations in both coding and noncoding regions of a gene. RFLP analysis is based on length variation of fragments, resulting from the cleavage of specific genomic DNA at polymorphic restriction sites, as revealed by Southern blotting. The use of RFLP became popular a few years ago, in the HLA field in particular, for the study of HLA class II and disease association (Cohen *et al.*, 1985; Bell *et al.*, 1985). The potential value of RFLP analysis in genotyping class II alleles was tested as soon as specific probes became available, and the method has since been developed and extensively refined, in particular during the Tenth International Histocompatibility Workshop (Dupont, 1989).

A great contribution was made when it became possible to perform the typing of cells not expressing HLA (e.g., leukemic cells and cells from Bare lymphocyte syndrome) (Marcadet *et al.*, 1985a). However, it was hoped that this genomic approach would identify new genetic markers uniquely associated with and specific for a disease. Indeed, had these markers existed, this approach may have localized in the HLA region non-HLA disease susceptibility genes. In such a case the previous association with certain HLA alleles detected serologically would have had to be interpreted as part of the strong linkage disequilibrium which is a constant feature of this gene segment.

A few pitfalls emerged in using RFLP analysis, because of the presence of several HLA class II pseudogenes and inter- and intraloci nucleotide sequence homology. Cross-hybridization rendered problematic interpretation of the early RFLP studies using full-length probes. RFLP patterns reflected multiple hybridization signals, some being cross-hybridization. In general, it is the strong linkage disequilibrium among polymorphic restriction sites (a large number are present in noncoding region) and coding sequence variations that are highly informative in RFLP studies. Improvement came from the use of shorter and fewer cross-hybridizing probes "exon specific," which can be considered operationally as locus or gene specific (DR β, DP α, and DP β). For DQ α–β, although high sequence homology with DX α–β results in cross-hybridization, the low polymorphism of the DX α–β genes enables identification of the relevant fragments and interpretation of the polymorphism as specific for the DQ genes.

Integrated DNA RFLP typing strategies and procedures have been developed (Bidwell *et al.*, 1988; Cohen, 1989) which are convenient tools for the investigation of HLA polymorphism in large population samples.

However, these procedures do not provide any advantage with regard to definition and have some drawbacks. Since both haplotypes may share one or more allelic patterns, the identification of heterozygosity can be complicated. Moreover, some alleles may not be adequately resolved. This is illustrated in the cases of DR–BON versus DR1 (Coppin *et al.*, 1987) and the splits of DR4 β chains (i.e., Dw4, -10, -13, -14, and -15), which are of great importance in rheumatoid arthritis (RA) studies (Gregersen *et al.*, 1986).

The development of nonradioactive detection may provide a means of introducing this technique into additional typing laboratories (Erlich *et al.*, 1986; Saiki *et al.*, 1985). Moreover, interest is increasing in studying the regulation of individual HLA class II gene expression which may affect disease states. It is at present unclear whether regulatory sequences (5′ upstream promoters and enhancers) have any genetic variability. If this is the case, RFLP analysis may provide a means to identify and characterize these regulatory polymorphisms and to study their epidemiology in diseased and control populations.

2. *Allele-Specific Oligonucleotides (ASO)*

ASO is an additional molecular technique which has been introduced to circumvent the inability of cDNA probes to identify some HLA class II splits, particularly the different DR4 Dw haplotypes. This technique was rapidly combined with the polymerase chain reaction procedure, which greatly facilitates the obtaining of specific DNA sequences suitable for further analysis (by RFLP or ASO) (Saiki *et al.*, 1985, 1986). From the known nucleotide sequences of the individual DR, DQ, and DP α and β genes of the different haplotypes, it is feasible to select short sequences specific for one (or for a group of) alleles. These allele-specific oligonucleotides are synthesized and used in combination with a radioactive (or nonradioactive) detection system.

Hybridization of ASO following specific amplification by thermal cycles (polymerase chain reaction) of the hypervariable exons represents an elegant technique for the identification of a given short linear class II sequence within the genome. Ultimately, the comparison of sequences of every class II gene (α–β) from normal versus diseased cells will be attained. This will undoubtedly focus interest on relevant amino acids and stretches of amino acids which are important for conferring high susceptibility to a particular disease. However, considering the three-dimensional structure and recent functional data, it is likely that additional information will be revealed by the study of conformational parameters relevant to the identification of disease susceptibility factors.

Since HLA and disease association may finally reflect specific immune

recognition of an antigen presented to a T cell by a class II molecule, the use of T cell clones would permit identification of the disease-susceptible versus non-susceptible individuals. The suggested conformational nature of the disease susceptibility factor predicts that it is likely to be composed of one or several amino acids or a series of amino acids localized on one class II chain (α or β) or on the two chains (α and β) of a particular class II dimer. This arouses interest in the role of the determinants created when hybrid HLA class II molecules are formed. Hybrid epitopes are consistent with the overwhelming epidemiological data showing a heterozygous (or haplotypic) effect in several diseases (Nepom *et al.*, 1984; Ronningen *et al.*, 1989), as initially suggested by Svejgaard *et al.* (1983) in IDDM.

IV. HLA Class II Hybrid (Intra- or Interisotypic) Molecules

Cell surface expression of the class II molecule as a stable heterodimer appears to be a logical requirement for immunological function and is likely to play a major role in disease association. The expressed repertoire of the class II molecules is the combined result of allelic and isotypic variations. Thus, particularly for disease associations, an appreciation of the full repertoire of class II dimers has to be evaluated. In addition to the number of class II α–β loci combined two by two within a haplotype to form isotypes, a powerful way to increase class II antigen diversity would be the association of the α and β chains of the two haplotypes by *trans*-complementation within an isotype. These molecules are found only in heterozygous individuals. An even more efficient way of generating additional diversity would be to pair α and β chains from different isotypes either in *cis* and/or *trans*. Here, I summarize the data on hybrid molecules and explore their potential for the understanding of associations between HLA and disease.

A. HLA-DQ Hybrid Molecules

The HLA-DQ products have the potential of forming hybrid molecules, which are dimers created by α–β chain pairing resulting from gene *trans*-complementation (Fig. 4). Such molecules include chains belonging to both the paternal and maternal haplotypes. Indeed, the DQ α and β chains are both highly polymorphic (Goyert and Silver, 1983; Charron *et al.*, 1984; Giles *et al.*, 1985). The use of allele-specific mAb's directed uniquely at the DQ α or β chain resulted in the isolation of hybrid (DQ) molecules. The use of allospecific sera and recombinant strains led to the initial description of such molecules in F_1 mice (Jones *et al.*, 1978; Silver *et al.*, 1980).

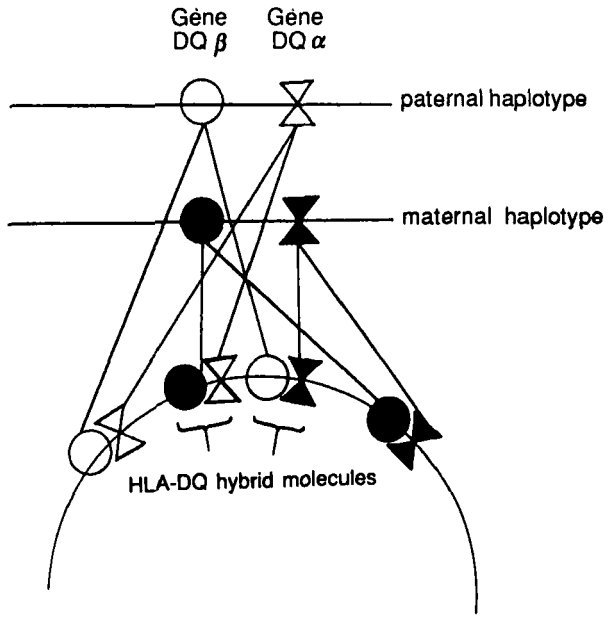


FIG. 4. *Trans*-Complementation leading to the formation of HLA-DQ hybrid molecules.

Our own approach included the use of two-dimensional PAGE to detect and identify electrophoretic variants of DQ molecules. A DQw2 (DR7) β–DQw1 (DR1) α hybrid molecule was the first to be demonstrated in humans in a DR1–DR7 heterozygous cell line (Charron *et al.*, 1984). Experiments with ^{125}I surface-labeled antigens demonstrated that these hybrid molecules were also expressed at the cell surface. The lack of adequate reagents explains the limited work that has been published since our first demonstration of hybrid DQ α–DQ β molecules in heterozygous individuals.

Giles *et al.* (1985) found the presence of a DQw3 β–DQw2 α dimer in a DR5–DR7 cell. Using the same anti-DQw3 mAb, Nepom *et al.* (1987) were able to identify, by two-dimensional PAGE and peptide mapping, a DQw3 β–DQw2 α chain complex in DR3–DR4 normal individuals. Moreover, the same DQ α (DQw2)–DQ β (DQw3) dimer was present at a comparable level in DR3–DR4 individuals, whether or not they had IDDM.

Recent results using transfection suggest that all four DQ heterodimers are present in a DQw2–DQw3 cell (G. T. Nepom, personal communication). The ability to form hybrid DQ molecules by gene *trans*-complementation should not be surprising (Fig. 4). Indeed, when DQ α and β

alleles from distinct haplotypes are compared, a series of "naturally occurring" hybrid DQ molecules are found which are identical in their α and β chain composition to the hybrid molecules found in heterozygotes, but differ only in that their α and β genes are coded on the same haplotype (*cis*-configuration). Recent data at the nucleotide level described DQ dimers in DR7–DQw2 and DR7–DQw3 haplotypes sharing a DQ α chain, but differing in their DQ β chains (Song *et al.*, 1987). Although the sequences provided are restricted to the first domain of the α and β chains, the data suggest that the DR7–DQw3 haplotypes encode in *cis* a natural hybrid molecule formed by a DQw3 α chain originating from a DR4 haplotype and the typical DQw2 β chain present in every DR7–DQw2 haplotype.

Additional data were recently obtained on a larger panel of homozygous typing cells in which DQ α and β polymorphism was studied at the protein level by IEF analysis (Bontrop *et al.*, 1987). Many, but not all, of the possible combinations of α and β chains were found. Thirty hypothetical dimers which could arise as a result of the various combinations between five DQ α and six DQ β chains were considered, but only 20 dimers were observed experimentally. Whether the absence of a given α–β combination is due to the inability to associate (i.e., forbidden α–β pairing) or whether the population studies are, as yet, insufficiently extensive to include all possible combinations is unresolved.

Indeed, because of strong linkage disequilibrium between alleles of the DR and DQ loci, certain combinations of alleles predominate in a given population. Different combinations of genes which would generate *cis*-derived hybrid molecules are most likely to be found in different populations. Natural hybrid molecules thus represent minor variant haplotypes. It is likely that these "natural" hybrid DQ molecules evolved by recombination between two distinct (heterozygous) haplotypes and were then fixed. This type of data argues in favor of a recombination "hot spot" between DQ α and β chains (Song *et al.*, 1987) and justifies a search for sequences facilitating recombination events, as have been described in the mouse I region (Steinmetz *et al.*, 1986; Smith *et al.*, 1981).

The demonstration in heterozygous individuals of the cell surface expression of hybrid HLA-DQ molecules raises several questions. Although there is no convincing report of alloantisera behaving as if they could recognize hybrid molecules, it may well be that such sera have been overlooked by serologists. Indeed, such sera would only recognize heterozygous individuals and would not segregate with a given HLA haplotype in family studies. Alternatively, it may be that these determinants are less immunogenic in terms of alloantibody response.

Transfectants containing hybrid molecules will provide an exquisite tool for the production and screening of hybrid-specific antibodies which

can be subsequently used in epidemiological studies. While in the mouse the Ir gene *trans*-complementation phenomenon is unequivocally explained on the basis of hybrid Ia molecules, among the mixed-lymphocyte reaction-stimulating determinants there appear to be some that represent conformational epitopes created by the *trans*-association of α and β chains (Fathman, 1980). Few functional data are available from humans. T cell clones have been found to be restricted by such hybrid molecules (Hansen *et al.*, 1982). However, modification of the cloning procedure has recently demonstrated several clones which may reveal hybrid epitopes involved in celiac disease (CD) (Lundin *et al.*, 1989).

The case for a role for hybrid HLA-DQ determinants in disease susceptibility requires particular attention. Indeed, in numerous epidemiological studies, first in IDDM (Svejgaard *et al.*, 1980) and later in juvenile RA (Nepom *et al.*, 1984) and in CD (Betuel *et al.*, 1980), an unexpectedly high incidence of the disease has been observed in particular combinations of DR haplotypes (i.e., heterozygous effect) (Charron, 1986). As a consequence, the relative risk is dramatically higher in some heterozygous situations than for any unique allele, even when present in a homozygous state. Such data cannot be explained by the model of monoallelic association.

If HLA class II molecules are involved, it becomes logical to propose that the particular determinant derives from a combination of products, one from the first haplotype, the other from the second haplotype. Because of the lack of polymorphism in the DR α chain, it is unlikely that HLA-DR molecules themselves would be the structural basis of this heterozygous effect. The DQ loci fulfill the two requirements of being in strong linkage disequilibrium with DR alleles and having structurally polymorphic subunits. *trans*-association of DQ α–β chains creates hybrid molecules which may be consistent with the observed heterozygous effect, since only these HLA-DQ hybrid molecules will bear conformational epitopes unique to the combination of paternal and maternal haplotypes. These possibilities are emphasized in the discussion concerning IDDM and CD. The expression of hybrid HLA-DQ molecules thus becomes an important parameter to consider.

Recent work in the mouse using appropriate combinations of transfected genes has shown that, while haplotype-matched A α–β genes (haplotypes *k*, *b*, *d*) resulted in the optimum cell surface expression of the dimers (as one would expect from the biochemical studies previously conducted in normal cells), the level of expression of haplotype-mismatched A α–β dimers was extremely variable and depended on the combination of haplotypes used. As an example, A α^k–β^b transfectants had poor surface Ia expression, while A α^k–β^d transfectants had no detectable Ia at their

surface. Furthermore, it was demonstrated that appropriate combinations of polymorphic sequences in the NH_2-terminal half of the A α and β chains could control the pairing. This allele-specific control of Ia molecule surface expression was rather unexpected. It is, however, consistent with most functional and evolutionary features of class II genes (Braunstein and Germain, 1987; Sant *et al.*, 1987).

The inability of certain DQ α and β chains to form functional dimers has yet to be demonstrated in humans. Although some DQ α and β genes have never been found to occur in *cis* in the population, this may not preclude the possibility that they occur in rare cases or even in *trans* (Bontrop *et al.*, 1987). It is still not known whether the pairing efficiency is the same for DQ α–β gene products coded in *cis* versus in *trans*. Rules underlying the formation of DQ α–β pairs are still enigmatic, and data are not yet available on which part(s) of the α–β chain is important in order for the dimer to be correctly assembled and transported to the cell surface.

Mechanisms similar to those described in the mouse system are expected to be found in humans. However, several other factors have not yet been investigated which could interfere with hybrid molecule formation. These include numerous posttranslational processing events and quantitative regulatory mechanisms, some of which are noticeably different in mice and humans (Neel *et al.*, 1987). These phenomena could affect gene repertoire and, subsequently, disease association.

Finally, the possibility for other homologous hybrid molecules should be envisaged. While the total lack of structural polymorphism in the DR α gene and protein means that there is no difference in whether the constitutive α chain of a DR α–β dimer is coded for by either parental chromosome, other class II subsets are potential candidates for generating intraisotypic hybrid molecules. This is clearly a possibility for the DP subset, both subunits of which are structurally polymorphic (Ando *et al.*, 1986; Lotteau *et al.*, 1987a). Since several structurally distinct DP β chains can pair with the same DP α chain, this provides another example of naturally occurring hybrid molecules. Whether the two DP α chains can pair indiscriminately in heterozygous cells with any DP β chain is unknown.

B. Interisotypic Hybrid HLA Class II Molecules

It has been suggested that, due to a presumed higher affinity between the α and β chains when they are coded for by loci of the same isotype, the expression of class II dimers will occur only within one isotype. Isotype-mismatched molecules were therefore considered forbidden pairs and were not expected to be found at the surface of normal cells (Travers *et al.*, 1984). A series of laboratory experiments using normal Epstein–Barr

virus-transformed human B cell lines provides direct evidence for the assembly and the cell surface expression of mixed isotypes consisting of DR α and DQ β chains. Quantitation of the isotype-mismatched dimers versus the conventional class II dimers using immunoprecipitation of labeled cell lysates is not precise. It appears, nonetheless, that DR α is associated with DQ β than with DR β and, similarly, less DQ β is associated with DR α than with DQ α.

In the mouse transfection of *I-E* and *I-A* genes into L cells has recently demonstrated the unexpected formation of similar isotype pairs consisting of I-E α and I-A β chains (Germain and Quill, 1986; Malissen *et al.*, 1986; Germain and Malissen, 1986). This unorthodox pairing appears to be predominantly influenced by the allelic polymorphism of the I-A β chains, since the I-A β–I-E α complex could be detected as an I-E α–I-A β^d dimer, but not as an I-E α–I-A β^k or I-A β^b dimer. Furthermore, this work emphasized the unexpected influence of the polymorphic NH_2-terminal domain of the I-A β molecule in permitting pairing, since the I-E α chain is virtually nonpolymorphic.

It should be remembered that, in the case of transfected genes, the α and β chains have no alternative partner with which to pair. In normal cells the situation is more complex, since each α or β chain has the opportunity to pair with its homologous chain (in *cis* or *trans*) and eventually with the chains of other isotypes (in *cis* or *trans*). When we used the three-dimensional structural model of an HLA class II molecule proposed by Brown to test the different DR α–DQ β dimer formations, we identified three distinct areas in the α1 and β1 domains which are the presumed to be interchains contact sites. Interestingly, while most of the bands between the DR α and DQ β residues are found in every DR α–DQ β combination tested, some are nevertheless restricted to particular dimers.

Altogether, the data predict that DR α–DQw1 β pairing is favored over DR α–DQw3 β and over DR α–DQw2 β (Hermans *et al.*, 1989a; Charron *et al.*, 1990a). This was verified at the product level, since we detected DR α–DQw1 β molecules, while DR α–DQw2 β molecules were not detectable in the appropriate haplotypes. Besides this qualitative regulation, the quantitative aspects of DR α–DQ β dimer expression were analyzed. We have observed that a permissive haplotype does not automatically result in the appearance of an interisotypic heterodimer. We therefore investigated the amount of individual α–β chain mRNA present in Epstein–Barr virus B cell lines in which biochemical analysis was unable to detect the presence or absence of isotype-mismatched α–β pairs. We have noted a correlation between the ratio of α–β transcripts of the different isotypes (i.e., DR and DQ) and the presence or absence of the

DR α–DQ β product. DR α–DQw1 β molecules could only be biochemically detected in cells in which we had observed a large excess of DR α transcripts over DR β and DQ β over DQ α (Lotteau *et al.*, 1989b).

In order to address some of the mechanisms underlying the formation of mixed-isotype dimers in a more direct manner, we undertook experiments designed to specifically modulate the expression of the DR α gene in Epstein–Barr virus-transformed human B cell lines. From the latter experiments we can conclude that transfection of a DR α gene into a cell line not expressing DR α–DQ β molecules induces the expression of DR α–DQ β dimers. In a second series of experiments, cells naturally expressing DR α–DQ β dimers were transfected with a DR α antisense cDNA. This, in turn, results in the extinction of the DR α–DQ β isotype-mismatched pair. In conclusion, the absolute amount of each chain, the relative amount of each chain within an isotype, and the reciprocal affinity of chains for each other are potential regulatory parameters of DR α–DQ β hybrid formation (Lotteau *et al.*, 1989a; Charron *et al.*, 1990b).

Whether isotype-mismatched molecules are important in the physiology of the immune response is an open question. The fact that few data are available may be due to the presumption that they were nonexistent. The reactivity of several published T cell clones has suggested that they could be restricted by such isotype-mismatched class II molecules (Gomard *et al.*, 1986; Eckels *et al.*, 1986). In serological terms allele-"specific" human alloantisera are likely to have broad reactivity within a haplotype, rather than be strictly allele specific. These alloantisera may contain populations of antibodies recognizing interisotypic specificities (e.g., combination of DR α–DQ β). In this case the interisotypic hybrids would not be distinguished from the dominant allelic specificity, since DR and DQ are in strong linkage disequilibrium and DR α is monomorphic.

New tools can be designed to search for such sera, and mutant cells (deleted of genes for one or several chains) should be very useful. Similarly, transfectants containing various combinations of mixed α–β isotype pairs should be suitable targets. This approach will be of great importance in the future, especially if one or several of these combinatorial determinants appear to be directly implicated in the pathophysiology of an HLA-associated disease.

Interisotypic class II dimers are indeed suitable to provide a structural basis for any haplotypic effect within the HLA-D region which might be found in epidemiological studies. They could account for the contribution of both DR and DQ or DP subregions to susceptibility to a particular disease, as was recently suggested in CD and multiple sclerosis (MS).

The suggestions that specific conformation within HLA class II dimers may represent a critical element for susceptibility and that hybrid class II

molecules occur either by *cis* or *trans*-complementation within an isotype(s) are discussed below in the context of well-characterized diseases. For this purpose I review the data on RA, IDDM, CD, and MS which are most relevant to the structural and functional framework of the HLA class II molecules provided.

V. Diseases

A. Rheumatoid Arthritis

Although likely to be multifactorial, the search for an etiology of RA has focused mostly on a few immunological aspects: rheumatoid factors and HLA studies. The latter were initiated by the first reports over 10 years ago of the association of RA with the cellular subtype Dw4 (Stasny, 1976). Thus, the DR4 haplotypes became the most-studied HLA class II factors. Approximately 70% of RA patients are DR4, versus 28% in controls (Stasny, 1978). Shortly after its serological definition, the DR4 haplotypes were subdivided into five or six cellular specificities defined by homozygous typing cells: w4, w10, w13, w14, w15, and KT2 (Reinsmoen and Bach, 1982).

A general characteristic of the HLA system is that the different subtypes are unevenly distributed among normal populations in different parts of the world, Dw10 being frequent in Jews and Asian Indians, Dw15 or KT2 occurring in Orientals. Among these subtypes only two—Dw4 and Dw14—are prevalent in RA and account for the found association with RA in Caucasian studies, while Dw10 appears neutral. Great effort was then devoted to biochemical dissection of the molecular basis of the alloreactive splits of the DR4 haplotypes, which in turn helped to define the RA-associated haplotypes.

Two-dimensional PAGE studies revealed large heterogeneity of DR β and DQ α–β electrophoretic patterns in DR4 (B. S. Nepom *et al.*, 1983; G. T. Nepom *et al.*, 1983) (Fig. 2). The different DRB1 genes associated with a DRB4 gene (homogeneous in the DR4 haplotypes and corresponding to the DRw53 specificity) were subsequently sequenced. Only limited nucleotide differences were observed in circumscribed areas of the most external exon of the DR β1 gene. This HVR (i.e., HVR3) encompasses a stretch of ten amino acids between positions 65 and 75 and, by extension, a residue at position 86 (Gregersen *et al.*, 1986). Dw4–, Dw10–, and Dw14–DR β1 molecules have identical nucleotide sequences, except for seven nucleotides between Dw4 and Dw10 and three between Dw4 and Dw14. These nucleotide differences result in only four amino acid differences for Dw10 and two for Dw14 (Gregersen *et al.*, 1986; Seyfried *et al.*, 1987).

It is of interest to note that previous Southern blot RFLP studies, although usually efficient in revealing allelic diversity, were not very informative in the case of the DR4 haplotypes, notwithstanding their extensive diversity. This can be explained by the fact that the nucleotide variation affected a limited area of the gene, therefore probably corresponding to recent evolutionary processes with conservation of the restriction sites.

Although several DR4 haplotypes are found to be positively associated with RA in different ethnic groups (e.g., Dw4 and Dw14 in Caucasians and Dw15 in the Japanese), DR4 is not associated with RA in other ethnic groups, such as Jews or Asian Indians. While Dw10 is the most common DR4 haplotype in Jews, it is almost completely absent among RA patients, suggesting that DW10 in this population is probably "protective," or at least neutral. The position of the molecules which differ in amino acid composition between Dw10 (not associated or protective) and Dw4–Dw14 (both associated with disease susceptibility in RA) can be considered the most likely to be critical in determining the impaired immune response which underlies susceptibility to RA. These include primarily amino acids 67, 70, 71, and 86.

Moreover, DR1 is overrepresented in the group of $DR4^-$ RA patients, irrespective of the ethnic group. This parallels the fact that DR1 is one of the least variable haplotypes among ethnic groups. Furthermore, the relationships between DR1 or DR4 haplotypes and RA include the supertypic specificity, MC1 (Duquesnoy *et al.*, 1984; Lepage *et al.*, 1985). MC1 is a class II determinant defined by both serological (i.e., with alloantibodies or mAb's and cellular (i.e., with cloned and primed lymphocytes cells) typing. DR1, DR4, and MC1 are strongly associated, since DR1 and DR4 are entirely included within MC1 (at least in Caucasians), although MC1 is found in rare DR2, DRw9, and DRw10 haplotypes.

Interestingly, MC1 represents highest single determinant associated with RA, since in a recent study of 80 patients MC1 is present in 83% of the patients and 43% of the controls (Carpenter *et al.*, 1988). This illustrates that, although supertypic (i.e., present on several distinct molecules), some "epitope" once identified may be of better value in predicting susceptibility haplotypes to a disease than a precise class II molecular subtype.

Similar data were reported concerning the reactivity of 109 D6 mAb's, which recognizes an epitope on DRw53 molecules (Lee *et al.*, 1984). The risk for RA appears to be higher for 109 D6 than for DR4, since 109 D6 was found in a greater number of individuals with RA than those who are either DR4 or DRw53. However, DR7 individuals are also 109 $D6^+$ (at least the one expressing the DRw53), but do not have a higher incidence

of RA. Thus, 109 D6 positivity does not itself confer full susceptibility, but could contribute to an increase of susceptibility in the context of other RA-associated alleles.

Indeed, non-DR4 non-DRw53 109 $D6^+$ RA individuals were found to possess DRw10 molecules by assessed serology. Moreover, the individuals analyzed so far were reported to be also DR1 on the other haplotype. The 109 D6 serological epitope may therefore reflect a common class II structure present on both DRw53 β and DRw10 β chains (Winchester and Gregersen, 1988). $MC1^+$ and 109 $D6^+$ individuals may be more closely related than was previously thought. Although MC1 is not present on DRw53 (since DR7 individuals are $MC1^-$), the DRw53 sequence (amino acids 65–73) is identical to the one found in the DRw10 β1 chains, while some DRw10 individuals belong to the $MC1^+$ group (Merryman *et al.*, 1988; Carpenter *et al.*, 1988). This suggests that among DRw10 individuals there is some heterogeneity interconverting 109 D6 and MC1. The 65–75 region of the β1 area appears to be critical when the positively associated haplotypes (i.e., DR1, DR4w4, DR4w14, and DR4w15) are analyzed.

Positions 67, 70, and 71 have conserved residues, except for a relatively conservative lysine-to-arginine substitution at position 71 in Dw4. This contrasts with DR4–Dw10 (a neutral or protective haplotype), in which positions 67, 70, and 71 are consistently substituted similarly to those which are found in most of the DR2 β chain and which have been claimed to be protective for RA, at least in Caucasians (Dw2) and in the Japanese (Dw12) (Maeda *et al.*, 1981; Ohta *et al.*, 1982). Interestingly, the serological and functional epitope MC1, which is also an RA "allele," could be contributed to by a conformational structure shared by DR1, DR4w4, and DR4w14 and thus could be potentially encoded by the same 65–75 region of the DR β1 gene. However, not every amino acid may be important, even in this 65–75 short linear sequence. Only a few, perhaps the one exposed at the surface of the molecule (toward the TcR) or part of the peptide recognition structure will be involved in eliciting an immune response.

T cell clone reactivities are informative in this respect. Indeed, alloreactive T cell clones have been generated against HLA-Dw14 which have revealed a "cellular epitope" present in all seropositive RA patients tested, irrespective of their being DR4 (Goronzy *et al.*, 1986). A similar reactivity pattern is found using an ASO whose sequence was derived from the DR4–Dw14 β chain 68–74 sequence. Furthermore, one can notice that a rare DRw6 (Dw16) subtype which possesses the DRw14 epitope is also associated with RA (Seyfried *et al.*, 1988). In other studies T cell clones have been found to recognize shared determinants between DR1 and DR4 RA haplotypes (Weyand *et al.*, 1986). Overall, the data

localize the functional RA-associated (i.e., shared) epitope to an area of the DR β1 molecule common to DR1, DR4w4, and DR4w14 (also Dw15 and Dw16). However, it was impossible to strictly correlate the recognized epitope with this sequence.

This suggests that the disease susceptibility factor is not a common continuous linear sequence between all of the recognized epitopes, but is likely to be a three-dimensional structure of a conformational epitope, which may be of importance independently of the overall structure of the molecule on which it is found. Interestingly, in the mouse a mutant, Bm12, shows that a structural epitope can be fully exchanged between an I-E and an I-A β chain and remain fully functional (Mengle-Gaw *et al.*, 1984). Considering the above concepts, it could be that T cell clones would be the most specific tools to be used in disease susceptibility analysis.

If the molecular substratum of the disease-associated immune process resides in an antigen presentation phenomenon to trigger (auto)reactive T cells, it is conceivable that some variability is allowed for the linear structure of the class II molecule, so long as the three-dimensional conformation of several important residues is conserved: residues directly involved in association with the antigen and/or with the TcR, for which no variation would be allowed. The concept of molecular mimicry was logically proposed based on the assumption that an environmentally provided antigen could exist which would induce T cell recognition of the previously defined disease susceptibility epitope. This would result in the initiation of a self-T cell activation and further autoimmunity. A search for molecular sequences shared between a foreign antigen (e.g., a pathogen) and the "self" molecule was initiated. In this quest the gp110 glycoprotein from Epstein–Barr virus revealed a QKRAA–QRAA sequence from residues 808–816.

Since an arginine-to-lysine substitution is considered conservative, this sequence is reminiscent of the QRRAA or QKRAA sequences present at residues 70–74 in the hypervariable region of several RA susceptibility DR β alleles. These include DR1, DR4w4, DR4w14, and DR4w15 (Roudier *et al.*, 1988). The nonassociated haplotypes DR4–Dw13 and DR4–Dw10 differ noticeably (QRRAE and DERAA, respectively). Moreover, a hydrophobicity plot of the Epstein–Barr virus gp110 reveals that the QKRAA–QRAA is located on the α-helical structure similarly to the class II HVR3 and is thus exposed. T cells recognizing and proliferating to the QKRAA determinant could be initiated and expanded by the initial pathogen sharing the QKRAA sequence. Perpetuation of the anti-QKRAA response would then occur even after the initiating antigen (i.e., the Epstein–Barr virus) had disappeared.

It was recently shown that Epstein–Barr virus induces T cells that

recognize QKRAA containing a gp110 peptide as well as a Dw4 peptide. However, no evidence was provided that the T cells involved were recognizing identical epitopes, since they could also be overlapping epitopes (Roudier *et al.,* 1989). Thus, the self-antigen–mimicry hypothesis requires further dissection of the T cell responses, the TcR repertoire, and the peptides involved. It also remains questionable why these "self"-specific T cell clones have not been deleted during thymic selection, since their specificity is self (class II peptide) in nature.

Although T cells reactive with self-histocompatibility molecules are found in the synovial fluid and are increased, whether these autoreactive T cells recognizing DR1–Dw4, –Dw14, and –Dw15 would trigger RA and whether these T cells are expanded by recognition of Epstein–Barr virus gp110 are still unknown. Besides self-mimicking antigens, more conventional antigens have also been considered as triggering agents in RA. These include *Mycobacterium tuberculosis,* for which T cell hyperresponsiveness has been described in DR4 individuals (Palacios-Boix *et al.,* 1988). Moreover, *Mycobacterium tuberculosis*-specific T cells have been characterized from synovial T cells in patients with RA as well as anticollagen type II T cells.

Although the anticollagen type II response is under MHC control in several animal models, the data from humans cannot conclusively validate the hypothesis of a central role for this latter molecule in RA. The isolation of heat-shock protein-specific T cell clone from an RA patient (Holoshitz *et al.,* 1989) is puzzling, and the isolated clone's being γ–δ is even more enigmatic.

Apart from the overwhelming evidence for a role for the DR β1 gene in RA susceptibility, the involvement of other locus products remains unestablished. Indeed, DR4 haplotypes include polymorphic DQ β genes. However, individuals with DR4-associated RA carry either the DQw3.1 or the DQw3.2 species, and the DQw3.1 and DQw3.2 specificities are equally distributed in RA patients and in normal controls. Thus, polymorphism of DQ does not appear to greatly contribute to the disease susceptibility, and any DQ β association in RA is likely to be secondary to DR4. However, DQ may influence the phenotypic expression of RA and superimpose some feature on the main genetic susceptibility, due to the DR β1 gene association. In this respect an increase of the DQw3.1 (DQw7) specificity has been found in severe (i.e., seropositive) RA and in Felty's syndrome or RA associated with nodules and/or erosions (Sansom *et al.,* 1987, 1988).

Patients with Juvenile Rheumatoid Arthritis (JRA) constitute a group of clinically and serologically heterogeneous individuals, accounting for the diversity of the HLA data generated in this disease. Weak associations

have been reported with DR4, DR5, and DRw8. Some particular aspects were revealed in the group of seropositive JRA patients associated with DR4. Although DR4 homozygosity was increased in serological studies, this in fact reflects an even higher relative risk conferred by a heterozygous state Dw4–Dw14 (relative risk, 116) (Nepom *et al.*, 1984), contrasting with a relative risk of 7.2 for DR4.

Since the epidemiological data suggest a contribution of both haplotypes to the disease susceptibility element in JRA, the conformational epitope responsible is likely to be located on a hybrid HLA class II molecule, the α and β chains of which have not yet been identified. It may be of interest to note that a recent association was reported between JRA and the DPw2 specificity (Odum *et al.*, 1988). Determination of whether the DR and DP associations are independent, additive, or synergistic should help to identify the JRA susceptibility conformation. In the latter case an interisotypic DR–DP or DP–DQ molecule is a likely candidate. The latest data suggest that the DPBw2.1 allele found in JRA patients is independent of the DR5 and DRw8 specificities (H. Erlich, personal communication). However, one should wait until we know the DPA allele present in these patients, since the best disease linkage may well be subsequent to an isotype–mismatched DR β–DP α molecule.

In addition to the genetic aspects, there are several somatic aspects of the disease association which are important. Cell surface expression of the HLA class II molecules is likely to elicit, at least in part, the pathogenic effect of the susceptibility gene(s). Indeed, numerous abnormalities of HLA expression have been observed in RA patients. The most relevant finding is an aberrantly high expression on the synovial tissue, in particular, on the adherent synovial lining cells of the HLA class II species. All three subsets—DR, DQ, and DP—are concomitantly expressed at levels comparable to those found on B lymphocytes of the same individual (Teyton *et al.*, 1987; Charron and Teyton, 1987). This absence of differential isotype expression does not favor the prominent role of any class II subset in mediating the susceptibility. The capacity of synovial tissue to express HLA class II antigens may be interpreted as an argument in favor of an ability to initiate and perpetuate a local immune response processing and presenting an antigen (auto- or foreign) to T cells. This has yet to be rigorously demonstrated in the model.

It is of hypothetical interest that the Ii chain [a proteoglycan associated with α–β class II dimers (Charron *et al.*, 1983) and thought to be involved in antigen processing and/or presentation] is strongly expressed and hypersialated in adherent synovial lining cells (Teyton *et al.*, 1987). Alternatively, HLA class II expression may provide a beneficial effect, allowing HLA class II-restricted cytotoxic T cells to eliminate altered or in-

fected synovial cells. Class II expression on synoviocytes correlates with their activation and proliferative capacity. Furthermore, we have recently shown that synovial cell proliferation could be modulated by anti-HLA class II antibodies (Teyton *et al.*, 1990). The class II molecules could therefore provide to the synovial cells an intracellular signal which affects proliferation and/or activation.

In addition to an immune function, the presence of HLA class II molecules of synovial cells may be important in down-regulation of their proliferation. Although γ-interferon is a likely candidate (alone or in conjunction with other cytokines) for induction of the *in vivo* expression of HLA class II in synovial tissue, the exact mechanisms of such expression are unknown. *In vitro* inducibility of HLA class II expression by γ-interferon in adherent synovial lining cells does not differ between cells from normal and RA individuals (Teyton *et al.*, 1987).

Thus, considering the hypothesis that the disease could correlate with hyperinducibility of class II molecules on synoviocytes, several key pieces are missing from the puzzle. Among them, the most important are probably identification of the antigen(s) involved in triggering of the autoimmune process and the composition of the T cell repertoire capable of responding to the above aggression.

B. Insulin-Dependent Diabetes Mellitus

IDDM has been the most investigated pathology with regard to the HLA class II system association and autoimmunity (Table I). This is remarkable for a disease which was not considered to involve the immune system less than two decades ago. Study of the HLA has been highly rewarding over the last years. The existence of an IDDM susceptibility gene within the MHC derives from two basic epidemiological observations: (1) the presence of particular HLA specificities, with a higher frequency in affected individuals than in the normal population, and (2) HLA haplotype sharing within a family, which confers a higher risk (Thomson, 1984; Svejgaard *et al.*, 1983). However, the early data obtained in twins suggested that the disease is polygenic and that the HLA system contributes approximately 50% of the inheritability.

Most of the efforts have since been concentrated toward the identification of the best HLA linkage from IDDM. Progress almost parelleled the improvement in HLA typing and reflected successive significant technological advances. The first association was described in 1973, when the specificity of B15 was reported, followed in 1974 by the report of a B8 association (Nerup *et al.*, 1974; Singal and Blajchmann, 1973). The most exquisite recent association is with the absence of aspartic acid at position 57 in the DQ β chain (Todd *et al.*, 1987). This illustrates the development

of HLA typing procedures from serology to molecular biology and from sera to sequences and the subsequent subdivisions of loci and alleles.

It is feasible that, during this period, improvements in HLA typing (mainly identification of variants within supertypic specificities) led to an increase in the relative risk for the disease. Thus, IDDM is an exemplary case for further dissection of the polymorphism of the HLA system in order to identify HLA factors related to the highest susceptibilities and the molecular basis for the fine specificities of the different alleles implicated. An extensive review of the individual steps which have improved the definition of IDDM and HLA association is not required, since they reflect intermediate approximations of the most accurate genetic linkage which is presently known.

Briefly, the early B15 and B8 associations were due to high linkage disequilibrium of these haplotypes with DR4 and DR3 associations, respectively, found years later. Indeed, over 90% of Caucasian IDDM

TABLE I
SUMMARY OF HLA FACTORS CONTRIBUTING TO IDDM SUSCEPTIBILITY OR RESISTANCE[a]

Susceptibility		Resistance	
Dr4		DRw15	(DR2)
DR3		DRw15	(DR2)
DR1	(DQw1.1)	DR4	
DRw16		DR5	
DRw13		DRw13	(DRw6)
DQw8	(DQw3.2)	DQw6	(DQ1.2)
DQw2		DQw6	(DQ1.12)
DQw5	(DQw1.1)	DQw7	(DQw3.1)
DQw5	(DQw1.AZH)	DQw7	(DQw3.1)
Dw19	(DQw1.19)	Dw19	(DQw1.18)
			DQw6
DQβ57: Ala-Val-Ser		DQβ57:Asp	

[a] Heterozygous effect: hybrid HLA class II molecules. DR4 DQw8/DR3 DQw2 and DR4 DQw8/DRw8 DQw4: synergistic effect. DR7 DQw2 in blacks (DQw2β + DR4 DQα); DR9 DQw2 in blacks (DQw2β + DR4 DQα). The new HLA nomenclature is used whenever possible. The previous corresponding designation is provided in parenthesis. Data are taken from several sources (Todd *et al.*, 1987; Todd *et al.*, 1988a,b; Ronningen *et al.*, 1989; Horn *et al.*, 1988).

patients are positive for DR3 and/or DR4, as compared to 45% of normal controls (Svejgaard *et al.*, 1980). Subdivision (mainly by cellular and molecular means) of the DR4 haplotypes contributed to better definition of the disease susceptibility markers. However, the cellular splits of DR4 into five or six subtypes gave a rather confusing picture, with an increase in DR4–Dw4, but also in DR4–Dw14 (and Dw10 in Jews), while the situation for DR4–Dw13 and DR4–Dw15 was uncertain (Bach *et al.*, 1985).

These uncertainties are now explained by the molecular composition of the DR and DQ α and β chains present in these cellularly defined haplotypes. Indeed, when the three segregant series—DR, DQ, and DP—are considered, the association with IDDM is the strongest with HLA-DQ (Owerbach *et al.*, 1983; Cohen-Haguenauer *et al.*, 1985; Bohme *et al.*, 1986). This includes DQw3 and DQw2 subtypes. Among DQw3, at least three molecular subtypes have been found using serological, cellular, and molecular approaches (DQw3.1, DQw3.2, and DQw3.3, or DQw7, DQw8, and DQw9, respectively). A higher relative risk is associated with one of the DQ β subtypes (this subtype being shared with the different DQw subtypes of the DR4 haplotypes, which were found earlier to be susceptible). This linkage was first suspected by RFLP studies.

In 1983 Owerbach *et al.* reported a 3.7-kDa *Bam*HI DQ β gene fragment which was decreased in frequency in $DR4^+$ IDDM patients compared to $DR4^+$ controls. This fragment turned out to be allelic to the specificity DQw3.2 or DQw8, which was later shown (using several RFLP DQ β fragments[1]) to be highly associated with IDDM (Owerbach *et al.*, 1984; Cohen-Haguenauer *et al.*, 1985).

The importance of the RFLP data was strengthened by a perfect correlation of these fragments with a DQ β chain profile, as assessed by two-dimensional PAGE (Kim *et al.*, 1985). Moreover, it linked the putative susceptibility epitope on the DQ β molecule of a given type (DQw8 or DQw3.2). The molecular subtype DQw3.2 (i.e., DQw8) is thus the most prevalent accurate disease susceptibility linkage specificity found in IDDM in Caucasians, while in Orientals DR4, DRw8, and DRw9 are associated with IDDM. In contrast, DR2 appears to be highly protective in all studies (Bertrams and Baur, 1984).

Further refinement of the disease susceptibility factor has been attempted. Indeed, since IDDM is positively associated not only with DR4, but also with DR3 and DR1, in different distinct populations, a search for a structure common to these positively predisposing haplotypes was conducted based on the available nucleotide and amino acid sequences of

[1] For example, 1.9-kb *Taq*I and 12-kb *Bam*HI.

HLA class II genes. When inspecting the individual class II amino acid substitutions, Todd *et al.* (1987) noticed that all class II haplotypes which were not positively associated but neutral or negatively associated with IDDM possessed in common an aspartic acid at position 57 in the DQ β chain. In contrast, there is no common polymorphic determinant (or structural stretch of amino acids) in the IDDM positively associated haplotypes (noticeably DR4, DR3, and DR1) and amino acid 57 of DQ β is an alanine, a valine, or a serine.

Interestingly, Asp^{57} is also found in every mouse *I-A* β gene sequenced so far, with the exception of the NOD mouse, which represents the best mouse model for spontaneously developing IDDM, in which it is serine (Acha-Orbea and McDevitt, 1987). This finding of specific DQ β amino acids associated with $DR4^+$ IDDM has allowed the construction of oligonucleotide probes, which are helpful in population studies, since they can be used in dot blot analysis, a method adapted for large-scale studies. For example, when an oligonucleotide probe detecting DQw3.2 versus DQw3.1 was used in conjunction with an oligonucleotide probe detecting DQw2 and DQw1.1 in a population of 39 Caucasian IDDM patients, only 10% of these patients were heterozygous for DQ β Asp^{57} (Todd *et al.*, 1987). DQ β Asp^{57}-negative homozygozity was thus correlated with disease in 90% of the patients.

The results are therefore in full agreement with previous RFLP and two dimensional PAGE studies, showing a large increase of the DQw3.2 β chain over DQw3.1 and DQw3.3 (Monos *et al.*, 1987). DQw3.1 and DQw3.2 can also be distinguished by their reactivity with the mAb TA10 (Schreuder *et al.*, 1986). Similarly, two-dimensional PAGE would have detected specifically the DQw2, DQw1.1, and DQw1 AZH β chains in the non-DR3–non-DR4 IDDM.

Since the initial reports of the importance of position 57 of DQ β in IDDM susceptibility, the data have been both largely confirmed and extended. The analysis of haplotypes rarely associated with IDDM has been rewarding: DRw6, the DQ β sequence derived from $DRw6^+$ IDDM, contained a Val^{57} of the DQ β chain, which corresponds to the DRw6–Dw19 subgroup of DRw6 individuals. This haplotype was present in all seven DRw6 IDDM patients observed, while it was present in only three of 13 DRw6 controls (most of the controls were Dw18 and possess an Asp^{57}-positive DQ β chain, DQβ1.6) (Horn *et al.*, 1988; Todd *et al.*, 1988a).

Also, rare DR2 IDDM has been reported, since DR2 confers a dominant resistance to IDDM, as discussed later. However, in the DR2 IDDM examples (which are of a specific DR2 cellular subtype named AZH) the DQ β chain possesses a Ser^{57} DQ β chain, contrasting with the Asp^{57}

present in the two resistance-conferring haplotypes (DR2–Dw2 and DR2–Dw12) in their respective DQ β chains. Similarly, the positive association reported with DR1 and the negative association with DR5 are consistent with the codon 57 pattern, since in DR1 the DQ β chain has a positive Val^{57}, while the DQw3.1 β chain of DR5 contains Asp^{57}. In the frequent case of DR3 association, the DQw β2 chain contains an Ala^{57}. This is commonly found in Caucasians, while in blacks the DR3 is mostly associated with a specific DQw4 β chain. This chain contrasts to the regular DQw2 β chain, as it possesses an Asp^{57}. However IDDM DR3 blacks appear to be $DQw2^+$, and, given that $DQw2^+$ serology reflects the DQ β chain, they are likely to be Asp^{57} DQ β.

Since associations of certain DR and DQ antigens are very different among ethnic groups, one should only consider the individual DR β and DQ α and β chains present (and their sequences) and not rely on the "classical," but overestimated, linkage disequilibrium phenomenon. The emerging picture suggests that it is the charge of the polymorphic residue at position 57 of DQ β that is associated with IDDM susceptibility. Thus, the presence at position 57 of the DQ β chain of amino acids with a nonpolar hydrophobic R group (e.g., alanine or valine) or amino acids with a polar but uncharged R group (e.g., serine) is preferentially associated with an autoimmune response to an as yet unknown diabetes-related antigen, and this contrasts with the positively charged R of aspartic acid found in the IDDM negatively associated with haplotypes.

Several important exceptions exist to the absence of Asp^{57} DQ β as a requirement for IDDM susceptibility. The DRw9 haplotypes associated with IDDM in the Japanese have Asp^{57} DQ β. This is also the case for two other susceptible haplotypes found in Japanese DR4 and DQw9. Moreover, the presence of a non-aspartic acid residue at this position may not be sufficient to confer the highest susceptibility to IDDM. In fact, a heterozygous effect was reported early in IDDM studies, which forces the consideration of the contribution of both HLA haplotypes in order to delineate the best disease susceptibility element (Svejgaard *et al.*, 1983).

It was already apparent in the early studies in which B8 and B15 were found to be associated with IDDM that both haplotypes could contribute to the susceptibility of IDDM. A dramatically increased relative risk was further demonstrated for individuals possessing a particular heterozygous combination of HLA class II antigens, namely, DR3–DR4. This prompted a search for the class II molecule inducing the most susceptibility. Since the DR α chain is monomorphic, it cannot therefore contribute to the heterozygous effect. The discovery that both α and β chains of the DQ molecules were polymorphic led to the ideas that the formation of hybrid class II molecules obtained by *trans*-complementations could oc-

cur and that those molecules present only in heterozygous individuals will fulfill the requirement imposed to explain the heterozygous effect (similar to an F_1 effect in animal genetics) (Svejgaard *et al.*, 1983; Charron *et al.*, 1984). Ultimately, these ideas support the concepts that it is more the structural three-dimensional conformation than an individual sequence which is the likely genuine disease susceptibility element and that several distinct amino acids can contribute to its formation. Indeed, this would agree with our present understanding of the structural model for antigen presentation, which implies a series of dynamic interactions among the antigenic peptide, the MHC, and the TcR.

Hybrid class II molecules provide the best model to explain the heterozygous effect observed for IDDM. The demonstration of the existence of homologous hybrid HLA-DQ molecules lends weight to this hypothesis (cf. Section III). It was subsequently shown that these hybrid molecules are in fact expressed in DR3–DR4 individuals. This occurs in both normal and IDDM individuals, and no obvious quantitative difference was observed on the cells studied (EBV B cell lines) (Nepom *et al.*, 1987). Nevertheless, these results support the possibility that hybrid conformational epitopes are created *de novo*, which may affect the immune response and lead to the autoimmune process.

Indeed, in the mouse such hybrid determinants would be only and specifically recognized by alloreactive T cells and T cell clones (Fathman, 1980). These hybrid molecules may bear a fixed three-dimensional structure, which contains a unique conformational entity itself bordered by a set of interacting residues (on the same, as well as opposing, chains). Some degree of degeneracy in the amino acid composition could be allowed by the model, leading to a gradient of "susceptibility" molecules. The data are consistent, the highest susceptibility being mediated by a conformational structure present in an hybrid molecule formed by the DQw3.2 (i.e., DQw8) β chain of the DR4 haplotype and the DQw2 α chain of the DR3 haplotype. The "auto"-antigen, a foreign or autologous peptide, will be better accommodated by the molecule of highest susceptibility and less along the gradient of molecules.

In this hybrid, or conformational, model similar conformations could be obtained in class II dimers resulting from *trans-*, but also from *cis-*, complementation. The minimal requirement at the DQ β chain level would be the absence of Asp^{57}. Indeed, the heterozygous effect was observed not only in DR3–DR4 individuals, but also in DR4–DR1 individuals. This could be interpreted in that the conformation of the DQ hybrid molecule formed in DR4–DR1 is very close to that formed in DR4–DR3 (functionally and/or for peptide binding), and thus confers a similar level of susceptibility.

It is of interest to note that contrasting with DR4 is the case of DR3 association with IDDM. No distortion of allele frequency variation was found between patients and normal controls at either the DR β or DQ β locus. This was recently extended to the DQ α locus, for which the same heterogeneity was found in both patients and controls, thus not allowing identification of the residue(s) of the DQw2 α chain, which contribute(s) to disease susceptibility. When other DQ α–β combinations are present, the level of disease susceptibility appears to be lower, which may reflect less functional efficiency of the hybrid molecule created in these cases. There are even the possible combinations of DQ haplotypes in which the α–β associations are forbidden, as has been reported in the mouse for some I-A α–β. In this case only the homologous *cis*-encoded α–β DQ dimer would be expressed and could be the least active in terms of peptide recognition.

Overall, this may mean that the structural requirement may be less stringent for DQ α than for DQ β. This would explain how the heterogenous effect can still be accounted for by a capacity of association of the DQw3.2 β chain identical to that of any type of DQ α chain found in DR3 haplotypes. Alternatively, the heterozygous effect may not concern a DQ α–β dimer, but some other type of class II molecules. In this respect, it is noteworthy that in Japanese IDDM-susceptible haplotypes DR4–DQw4 and DRw9–DQw9, the DQ β chain has Asp^{57}, but that it is the DR β chains which may contribute to disease susceptibility (i.e., the absence of aspartic acid or the presence of serine in IDDM DR β chains from Japanese patients). In this population a characteristic DR β–DQ α dimer could be equivalent to the DQ β–DQ α dimer found in Caucasians.

In the IDDM-prone NOD mice which are I-E^-, the expression of I-E (i.e., DR-like) molecules prevents the development of diabetes, while the I-A β (i.e., DQ-like) chain possesses a predisposing Ser^{57} residue (Nishimoto *et al.*, 1987). While the authors suggest that protection may be due to an I-E-controlled suppression or to cross-tolerance to the self-antigen, I propose an alternative explanation. Introduction of an I-E α gene may impair the balance of the I-A and I-E α and β chains to the point that an excess of I-E α could associate with I-A β (interisotypic molecule) and thus prevent A α–β diabetogenic dimer expression at sufficient levels.

The possible contribution of molecules other than DQ was raised earlier, when a group reported the existence of a DX α polymorphism (detected as a *Taq*I fragment in RFLP) associated with IDDM (Festenstein *et al.*, 1986). However, the DX α gene has no known product, and several other groups did not find the DX α association. Again, the DX α polymorphism reflected a selection bias due to linkage disequilibrium. There is no reason to believe, therefore, that DX α alleles themselves add to IDDM

susceptibility. Additional sequences should thus be present in the DQw2 α chain with which the sequence identified on the DQw3.2 β chain coded in *trans* would combine in heterozygotes to form a DQα–β dimer with full susceptibility capacity.

The Brown model of a class II molecule predicts that the DQ β chain amino acid 57 is located at an extremity of an α helix and is pointing into the antigenic groove. Thus, the negatively charged non-Asp^{57} in DQ β is likely to form a salt bridge with the positively charged Arg^{79} of the DQ α chain. However, the fact that the IDDM-associated DR3 haplotypes and the IDDM-neutral DR7 haplotypes, although having different DQ α chains, display the same two opposing amino acids does not favor any particular role for the Asp^{57} DQ β–Arg^{79} DQ α interaction. Additional polymorphic adjacent sequences are evidently needed to explain the latter.

Interestingly, the study of populations (and cases) in which IDDM susceptibility does not strictly follow the DQβ57 pattern have provided support for the hypothesis that certain combinations of DQ α and DQ β obtained by *trans*- or *cis*-complementation are the crucial structures involved in IDDM susceptibility. In a series of 92 IDDM patients from Norway, an increased risk was found among DR4–DRw8 heterozygotic individuals, similar to that seen for DR3–DR4 heterozygotes. By RFLP and ASO typing, the DR4–DRw8 patients appear to bear the DR4–DQw8/DRw8–DQw4 haplotypes in eight of nine. This suggests that the two distinct combinations of haplotypes DR3–DQw2/DR4–DQw8 and DR4–DQw8/DRw8–DQw4 share an equivalent DQ α–β conformation encoded in *trans*. Interestingly, DQw4 has an Asp^{57} of its DQ β chain, and thus the DQw4–DQw8 heterozygotic individuals do not have two Asp^{57}-negative DQ β1 alleles. In these cases Asp^{57} DQ β is not protective as in DR2–Dw2.

DR7 (and DRw9) haplotypes appear to confer disease susceptibility in blacks that contrasts with a neutral effect of DR7 found in Caucasians. Todd *et al.* (1988b) analyzed the DQ α and β chains found in these black haplotypes. While the sequences of the amino-terminal domain of the DR β chains (β1 and β4) and DQ β were identical in black and Caucasian DR7–DQw2 haplotypes, a difference was found in the DQ α chain. The black DQA1 allele was identical to the DQA1 allele found on DR4 Caucasian haplotypes. Thus, replacement of the DR7–DQA1 allele (present in DR7 Caucasians) by a DR4–DQA1 allele (present in DR7 blacks) appears to be sufficient to switch from a neutral to a susceptible haplotype for IDDM.

Similar results were obtained in DRw9 black haplotypes in which DQ β was identical to the DQ β of black DR7 while the DRw9–DQ β from

Caucasians is different (DQw9 Asp^{57} positive). This is consistent with DRw9's also being a susceptible haplotype in blacks, although neutral in Caucasians. It should be pointed out that the DQA1 of the black DRw9 haplotypes was found to be identical to Caucasian DR9 and black DR7 and to correspond to the regular DQA1 of DR4. The DQα–β dimer composition found in DRw9–DRw9 blacks with IDDM is thus similar to that obtained in *trans* in the DR3–DR4 heterozygous Caucasians.

Overall, the genetic data argue that position 57 of DQ β, although prominent, is not the only contributary residue to IDDM susceptibility. An unknown sequence(s) presumably on DQ α is usually required to obtain full disease susceptibility. In some instances this DQ α factor may even overrule the causative effect of the specific DQ β sequence. Moreover, one cannot exclude a role for additional sequences of the DQ β chain. In some populations contribution to disease susceptibility by amino acids usually found on the DQ β chain could be absent and replaced by equivalent residues on DR β.

The exact mechanisms by which islet β cells which provide insulin are progressively destroyed in IDDM are presently unknown. The way in which the genetic susceptibility is phenotypically translated remains largely speculative, although of critical importance. This central question has prompted many laboratories to investigate the somatic expression of HLA class II molecules in the pancreatic endocrine tissue and its regulation and its role in the physiopathology of the disease. Following the thyroid model, in which the aberrant expression of class II molecules on the epithelial cell population was proposed as the central event leading to the autoimmune phenomenon observed in thyroiditis, a similar hypothesis was proposed for IDDM (Hanafusa *et al.*, 1983; Bottazo *et al.*, 1986).

Indeed, HLA class II expression was documented on β islet cells from a few IDDM patients, while other islet endocrine cells and the exocrine cells of the pancreas remained class II negative (Bottazo *et al.*, 1985). Moreover, among human β cells only a small proportion of cultured cells are induced to express class II by γ-interferon. Only when tumor necrosis factor α (or β), although not efficient alone, is combined with γ-interferon do human β islet cells become strongly positive in culture (Pujol-Borrell *et al.*, 1987). Recent data from mice have shown that class II expression alone is not sufficient to endow complete antigen-presenting cell function of β islet cells (Markmann *et al.*, 1988). This casts doubt on the possibility that class II expression could be solely responsible for the initiation of autoimmunity. Moreover, the β cell specificity of HLA class II expression observed *in vivo* remains unexplained (Timsit *et al.*, 1989).

It could be that islet β cells are uniquely sensitive to an as yet unknown specific combination of lymphokines or, alternatively, that a β islet cell

specific virus determines the class II induction via a mechanism similar to that which has been reported in rats, in which a noninfective neurotropic coronavinus induces class II expression in astrocytes (Massa *et al.*, 1987). This may also be related to the class II expression of thyrocytes in culture which is observed following the introduction of simian virus 40 DNA (Belfiore *et al.*, 1986).

A series of elegant experiments was designed to precisely address the question of the role of aberrant HLA class II expression in the pathogenesis of IDDM. These experiments provided some unexpected results. Mice were made transgenic using constructs containing the insulin promoter gene and class II α and β chains. It was anticipated that these animals would only differ from nontransgenic animals by the expression of class II in pancreatic islet β cells. This was indeed the case (Lo *et al.*, 1988; Sarvetnick *et al.*, 1988). Moreover, diabetes results in almost all of the animals. However, one of the most unexpected findings was the absence of an inflammatory infiltrate surrounding the islet cells (noticeably T cells). This may be consistent with the incapacity of these cells to actually function as antigen-presenting cells. Thus, class II expression does not appear to be capable, by itself, of inducing autoimmune destruction of the islet β cells. However, the islet cells were altered and progressively disappeared. The decrease in insulin secretion and subsequent cell death observed in the β cells of these transgenic animals in the absence of T cell infiltration are puzzling, as is the observation that similar transgenic animals with class I genes, as well as with class II genes, became diabetic (Allison *et al.*, 1986).

Although several highly speculative explanations were proposed, none has yet been validated experimentally. This is the case for competition for transcriptional factors between the transgenic MHC and the endogenous insulin gene, interaction between the intracellular pathway of insulin secretion and MHC class II expression leading to degradation of the insulin or direct binding between insulin and the MHC proteins (Parham, 1988).

Besides their immunological role in restricting the immune response, class II molecules are capable of transducing signals into the cell bearing them, which can result in activation of the cell (or proliferation, or differentiation). Using Sepharose-conjugated anti-HLA class II antibodies, we were able to directly activate human B cells via a secondary messenger pathway involving calcium flux, phospholipase C metabolism, and protein kinase C activation (Mooney and Charron, 1988; Mooney *et al.*, 1989). Stimulation of interleukin 1 synthesis and release by the B cell were also observed. I suggest that such a role for class II molecule may well be relevant to the observation made in the transgenic mice and

subsequently to the pathophysiology of IDDM. Indeed, hyperexpression of HLA class II on pancreatic islet β cells, once established, may alter the cellular biology of the islet cells and transduce activation signals contributing directly or indirectly to cellular destruction (via interleukin 1 or via the release of other cytotoxic agents). Such a hypothesis accommodates the different possibilities of HLA class II induction on the β islet cells (e.g., direct infection of the β cells, release of the cytokines, and gene transfer) and does not require any primary immunological event and effector.

It is important to consider that immune mechanisms (e.g., antigen presentation and T cell cytotoxicity) and nonimmune mechanisms are not mutually exclusive possibilities toward the destruction of the β islet cells. Both reflect the somatic expression of the class II genotype. Furthermore, recent data suggest that in mice the signaling capacity of a HLA class II molecule may well be allele specific (Bishop and Frelinger, 1989). Fascinating questions remain to be answered in order to understand the role of the HLA class II expression in IDDM susceptibility. As the genetics have become more precise, the expression leading to the pathology has become more enigmatic.

Identification of the self-peptide (or foreign peptide), which binds efficiently to the class II molecules involved in the genetic susceptibility, will obviously be an important breakthrough in our understanding and may also provide new therapeutical approaches. Alternatively, the demonstration that class II expression may alter in some way the cell biology of the pancreatic β cell would direct our thinking of pathophysiology and therapy onto different tracks.

The strong negative association of IDDM with DR2 has been a constant observation which warrants discussion (Bertrams and Baur, 1984). The supertypic DR2 specificity is protective, but only in some subtypes, as the subdivision of DR2 into DRw15 and DRw16 correlates exactly with disease susceptibility, DRw15 being dramatically decreased in IDDM, while DRw16 (a rare haplotype in Caucasians) is associated with the disease (Tiwari and Terasaki, 1985). Taking into account the molecular composition of the DR2–DRw15 and DR2–DRw16 haplotypes and comparing them with the sequences of the other positively and negatively associated haplotypes, Todd *et al.* (1987) concluded that only residue 57 of the DQ β chain correlates strongly with resistance and susceptibility to IDDM. The presence of an aspartic acid residue at this position would therefore explain the protective effect of most DR2 haplotypes. Indeed, it perfectly fit the earlier epidemiological data, DR2–Dw2 and DR2–Dw12 being protective and possessing an Asp^{57} DQ β, while DR2 AZH is susceptible and has a serine at the same position.

In contrast, the reason for the dominant effect of Asp^{57} DQ β in IDDM resistance is much debated. Several alternative explanations are proposed. Cross-tolerance between an antigen and the precise class II epitope could explain the dominant effect. The possibility that molecular mimicry is responsible for immunological tolerance has also been investigated (Todd *et al.*, 1988a). The envelope protein I-E2 of cytomegalovirus (CMV), a virus which may be involved in IDDM, possesses a stretch of six amino acids (81–88), five of these being present in the DR β1 and DQw2 β chains at residues 52–57. Anti-CMV T cell responses specific for this I-E2 epitope could only occur in individuals in which the I-E2-specific repertoire would not have been deleted as self during the thymic education process (non-DR2 individuals thus lacking the homology I-E2 are self).

Such speculation justifies the study of the antigen- and MHC-specific T cell repertoire expressed in the patients. Alternatively, the nonresponder status could be an active phenomenon (Boitard *et al.*, 1988). Suppression via T cells has been documented and found to be under MHC class II control. Moreover, specific suppression toward two distinct antigens (streptococcal cell wall antigen and schistosomal antigen extract) were found to be restricted to the DQ isotype (Sasazuki *et al.*, 1983). In these two models anti-HLA-DQ mAb's would abrogate the *in vitro* observed suppression (Matsushita *et al.*, 1987). However, it is not the DQ β position 57 residue which is involved, since $DW2^+$ individuals develop streptococcal cell wall-specific suppression, while Dw12 individuals do not, although they possess the same Asp residue.

The dominant resistance to IDDM cannot be unequivocally and solely explained by this suppression phenomenon. Several haplotypes, although possessing an Asp^{57}, are only weakly negatively associated or neutral, and some Asp^{57}-positive DQ β individuals are positively associated with IDDM. For example, in the Japanese DRw9, one could argue that a DQ α chain could abrogate the role of the Asp^{57} DQ β. Additional class II sequences of the DQ β chain or of another chain may be required to confer full dominant suppression.

It may be relevant to note that NOD transgenic mice possessing the DQw1 molecule from a DR2–Dw12 haplotype (an Asp^{57}-positive molecule) are not protected from becoming diabetic (Fukui *et al.*, 1989). These data do not support a unique role for Asp^{57} in suppression.

C. Celiac Disease

Among autoimmune diseases CD, or gluten enteropathy, is unusual in two ways. It has one of the strongest associations with HLA class II of the known HLA-linked diseases, and its triggering factor (wheat gluten) is

known. Although the genetic susceptibility appears to be predominantly associated with the DR3 specificity, other alleles are involved, including DR7 and DR5 (Betuel *et al.*, 1980). In serological studies of a large population of patients, the DR3–DQw2 haplotype was found in over 80% of the CD patients, in contrast with less than 30% in healthy controls (Tosi *et al.*, 1983). CD is also characterized by the heterozygous effect observed concerning DR3 and DR7, which in considerable in some populations (Betuel *et al.*, 1980). This highest relative risk found in the heterozygous DR3–DR7 individual suggests that possibility of a *trans*-complementation within the DQ system (Charron, 1986).

Rare are the non-DR3 non-DR7 CD patients. In a study of such a selected population all 16 were DR4. This suggests either the presence of a common and unknown epitope pattern shared between DR3–DR7 and the DR4 in these CD patients or the possibility that two distinct types of CD exist, one associated with DQw2 haplotypes and the other with DR4 (Tosi *et al.*, 1986).

Studies were recently conducted at both the DNA and protein levels. A DQ β chain cDNA probe was reported to identify a polymorphic fragment which was a better marker of the CD than the DQw2 serological specificity (Howell *et al.*, 1986). However, this was not confirmed in a group of homozygous DR3 CD patients in which the DR β and DQ β RFLP patterns were identical to those found in the control group (Sacks *et al.*, 1987).

In another study a 4-kDa *RSa*I class II fragment was found to discriminate the CD haplotype from matched controls (90% versus 18%). It was subsequently shown to be encoded by a DP β gene (Howell *et al.*, 1988). This was unexpected, given the degree of recombination between the DQ and DP loci. A possible explanation could be that DQ–DP recombination is lower in the DR3–DQw2 haplotypes. Alternatively, although independent in normal DQ haplotypes, DP may be more strictly linked in the CD haplotypes. Moreover, since a DP α polymorphism (84% versus 36%) was also increased in the same patients, along with the DP β polymorphism, this study may indicate a role for interisotypic hybrid molecules and determinants formed by DP α and DQ β and/or DPβ–DQα.

An increased frequency of two specific DP alleles was found in a study of 23 CD patients from Italy (Bugawan *et al.*, 1989). This study was conducted using a panel of DP-specific oligonucleotide probes on polymerase chain reaction-amplified material, which allowed the detection of 21 DP β and two DP α alleles. Two specific DP β alleles were increased in the patient population (DPB4.2 and DPB3), conferring relative risks of 93 and 2.8, respectively.

The present data could either reflect a linkage disequilibrium or an

independent contribution of DP β alleles, in addition to the well-established role of DQw2. Interestingly, in the two studies the DP β polymorphism (Bugawan *et al.*, 1989; Howell *et al.*, 1988) associated with CD susceptibility demonstrated differences among ethnic groups (Italy versus the United States). The U.S. patients had an increase in DPB1 and DPB3 variants. Altogether, the data focus on position 69 of the DP β chain, at which both DPB4.2 and DPB3 share a lysine residue (as in DPB1).

When the DQ polymorphism was further investigated at the DNA and protein levels in 30 white patients a 4-kDa *Bgl*II RFLP fragment was found in 97% of the CD patients, compared to 56% in the controls. Moreover, all of the CD patients tested possessed a biochemically detected DQ α chain variant, which was associated with the 4-kDa *Bgl*II fragment (Roep *et al.*, 1988). The identification at the DNA and protein levels of a CD-specific DQ α (DQw2.3) chain can be further considered. In DR3 and DR7 individuals structural analyses have revealed that the DQw2 molecules have almost identical β chains (they differ by only one amino acid in the second domain), while they have distinct α chains, as shown by two-dimensional PAGE and confirmed by nucleotide sequence data (Song *et al.*, 1987).

The constant presence in CD patients (including a non-DR3 patient) of a specific DQw2 α chain (usually associated with the regular DR3 haplotype) would not have been detected without the use of molecular probes, since the serological DQw2 specificity reflects the DQw2 β chain, and since this chain is structurally almost totally identical in DR3, DR7, and some DR5 and DRw8 haplotypes. The molecule of most importance in CD appears to be a DQw2.3 α–DQw2 β dimer.

Interestingly, this combination can be obtained by α–β chain complementation either in *cis* (same chromosome) or in *trans* (from both haplotypes). In the latter case the DQ molecule would belong to the hybrid type created *de novo* in heterozygous individuals (Fig. 5). Thus, in DR3 non-DR7 individuals this molecule would be encoded in *cis*, while in DR5–DR7 it would be encoded in *trans*. This would also be the case in DRw8–DR7 patients.

The hypothesis of a hybrid HLA-DQ molecule has received further support from a recent Norwegian study of 94 CD children, who were typed using two specific oligonucleotide probes (Sollid *et al.*, 1989). The first ASO detects the sequence 72–78 of DQ α present in the DR3–DQw2 and DR5–DQw7 haplotypes, and the second ASO detects the sequence 26–33 of DQ β present in DR3–DQw2 and DR7–DQw2. All but one patient are positive with ASO, which encodes a specific DQ α–β dimer. The same DQ α–β heterodimer (CD specific) could be coded either in *cis*

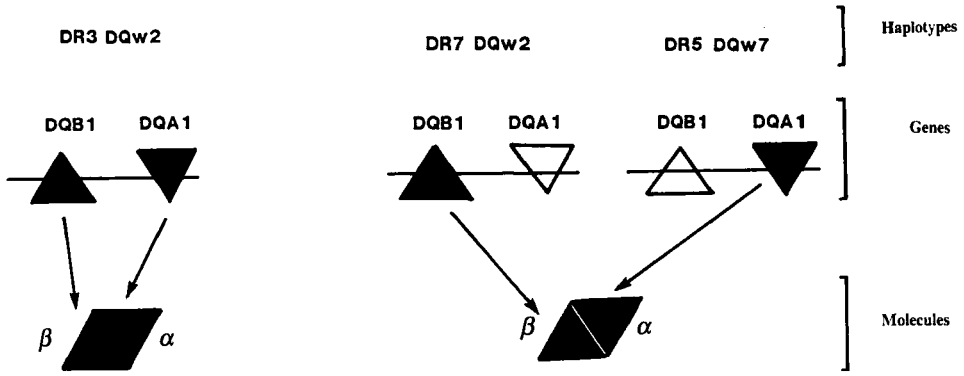


FIG. 5. HLA class II DQ haplotypes, genes, and molecules in celiac disease. In DR3–DQw2 individuals the genes which provide the DQ α–β dimer are encoded on the same haplotype (*cis*-complementation), while in DR7–DQw2/DR5–DQw7 individuals the same DQ α–β dimer is encoded by genes on both haplotypes (*trans*-complementation).

or in *trans* (the haplotype carrying the *cis* combination is likely to have evolved by crossing over during evolution between the two haplotypes carrying the *trans* combination).

These results would explain why DR3 appears to be a susceptibility haplotype, together with any other, while DR7 is associated in CD almost only with DR5 or DRw8 (and DR3). Since the DQw4 α chain (present in DRw8) differs in sequence from other chains in the 69–75 region, it may be relevant that there is a Ser^{75} present in DQ α of both DR3–DQw2 and DR5–DQw7 which is not found in any other DQ α allele. The rare non-DR3 non-DR7 patients are usually DR4. In the study cited above the only patient who was negative with the two ASO probes was DR4. Unless a common epitope(s) is found between the DR4 and non-DR4 groups, this result would again argue for two distinct forms of the disease (Tosi *et al.*, 1986).

It is conceivable that a gluten-derived peptide (acting as an antigen) binds similarly to the previously defined DQα–DQβ dimer in classical CD and to a different class II molecule (with similar or different conformation of the binding site) in the DR4 patients. Alternatively, the antigen may be different. This possibility is raised by the observation that in experimental allergic encephalomyelitis, which is under MHC control in the mouse, the triggering antigen, myelin basic protein, possesses two distinct epitopes, both of which are encephalitogenic.

CD is also concerned with the phenotypic expression of HLA class II antigens. It is of interest to note that while crypts and epithelium are DR^+ in CD, only the epithelial cells appear to bear the DQ^+ molecule, which are

also increased in the lamina propria. The direct role of these cells in CD is supported by the recent observation that Ia^+ gut epithelial cells can function as accessory cells in allogeneic reactions and process and present soluble antigen to primed T cells in an MHC-restricted manner (Mayer and Salien, 1987). Thus, the genotypic association with HLA class II in CD may result in an impairing of the local gut immune response through the expression of the susceptibility HLA molecules.

Although it has been not defined chemically, the triggering antigen is likely to be a gluten-derived peptide which could associate with some specific affinity to the MHC class II molecule involved in CD. It should be pointed out that, when the antigen is polysaccharidic, the HLA class II immune response appears to be preferentially restricted by DQ molecules (Durandy *et al.*, 1986).

D. Multiple Sclerosis

The first suggestion, dating from the early 1970s, that the HLA system contributes to the genetic susceptibility to MS is now well established. The association was initially found with HLA-A3 and -B7 (Jersild *et al.*, 1975; Compston, 1982; Tiwari and Terasaki, 1985), then more closely with DR2 and, in particular, the Dw2 subtype. This cluster of associations reflects the strong linkage disequilibrium affecting the A3–B7 and DR2–Dw2 haplotypes in Caucasians. In fact, a DR2–Dw2 association is found only in Northern Europeans and in North Americans. The association is with DRw6 in the Japanese and DR4 in Jordanian Arabs and Italians. This diversity along, with the fact that experimental allergic encephalomyelitis (a murine experimental model for MS) is linked to I-A (the mouse equivalent of DQ) (Fritz *et al.*, 1984), provides a rationale to search for a DQ polymorphism which would be shared by the different DR-susceptible haplotypes and would represent the genuine susceptibility factor.

Several groups have reported from both population and family studies an increased frequency of a particular allele of DQw1. Fauchet used a DQ β probe and four enzymes (i.e., *Bam*HI, *Bgl*II, *Eco*RI, and *Eco*RV) to identify two RFLP variants—DQw1a and DQw1b—the latter corresponding to the cluster DQR2–6 previously defined (Cohen *et al.*, 1984). The DQw1a subtype is associated with DR1–DR2 "short" (DRw16) and DR–BON, while the DQw1b subtype is associated with DR2 "long" (DRw15) and some DRw6 (DRw18). In 100% of the cases (14 DR2 and one DRw6), the patients were found to be DQw1b.[2] A segregation analysis demonstrated that the association was stronger with the DQw1b allele than with the DR2 allele (Semama *et al.*, 1988).

[2] DQw1a is the same as DQw5; DQw1b is the same as DQw6.

An earlier population study had provided evidence for an association of the DQR2–6 cluster with MS (Marcadet *et al.*, 1985a). This was confirmed and extended in a study of 61 patients from a Norwegian population (Vartdal *et al.*, 1989). Two oligonucleotides were used: one recognizing a common DQ β1 sequence of DQw6 (DR2), DQw8, and DQw9, the second recognizing a sequence common to DQw6 (DRw18) and DQw6 (DRw19). Among the 59 patients which were DR2, DR4, or DRw6 by serology, 59 carried shared DQ β1 polymorphic sequences, which can be identified as amino acids 10–29, 31–52, 58–69, and 71–83 of the respective DQ β1 chains.

The two patients who were negative with two oligonucleotide probes were $DR3^-$. These may be related to previous work identifying that the A1–B8–Dw3 haplotype was found in severe progressive MS, while the A3–B7–DR2 haplotype was associated with the more common milder disease (Madigand *et al.*, 1982).

Biochemical identification of the class II molecules involved in MS susceptibility has been attempted (Sriram *et al.*, 1985), but did not provide any definitive conclusion at the molecular level with regard to the disease susceptibility element. An ongoing study in this laboratory has provided some new interesting possibilities. Two-dimensional PAGE of the class II products from DR2–DQw1b haplotypes reveals that a common set of class II dimers is found. This includes a DQα–β dimer corresponding to the DQw1 α–DQw1 β subset and two additional dimers: DR α–DQw1 β and DP α–DQw1 β (Fig. 6). Thus, in the DR2–DQw1 haplotypes of controls and of MS-affected patients, we describe interisotypic hybrid molecules (Hermans *et al.*, 1990).

In view of the previous data on DQw1b polymorphism, this implies that the MS susceptibility could be borne by DQ α–DQ β, but also by a DR α–DQ β, a DP α–DQ β molecule, or combinations of the three. Thus, even if the epidemiological linkage data stress only polymorphic residues on one chain (e.g., DQw1 β), determinants which may be monomorphic on an α chain (of the same or a different isotype) could be involved in constructing the three-dimensional structure which is involved and functional in disease susceptibility.

An RFLP study of patients with MS from Northern Ireland and Scotland mentions a polymorphism of the DQ α gene (DQα–MSp1 3.25-kb fragment) which was found in increased frequency in DR2 MS patients (Heard *et al.*, 1989). Since this polymorphism appears to be allelic to the DQw1.2 α usually found in DR2–Dw2 haplotypes, it is likely that in the cells of MS patients who are DR2–Dw2 DQw1.2, the new DQ α fragment is contributed by the other haplotype. This *trans*-complementation raises the possibility that in these patients the determi-

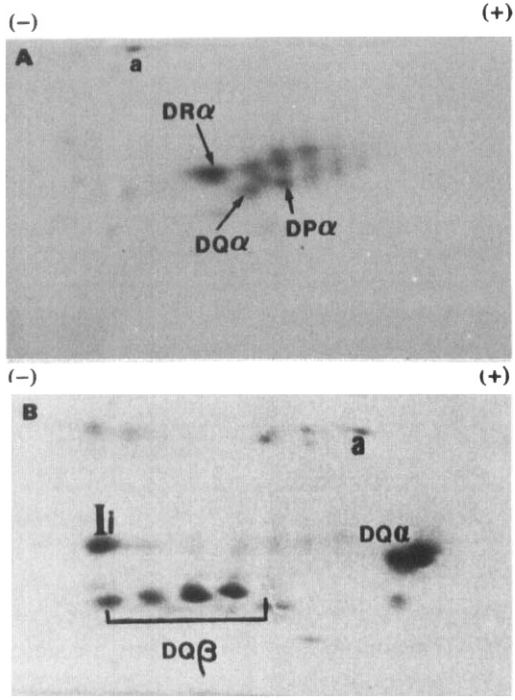


FIG. 6. Interisotypic hybrid HLA class II molecules DRα-DQw1β and DPα-DQw1β. [^{35}S]methionine-labeled extract from a DR2–DW2–DQw6 Epstein–Barr virus-transformed B cell line immunoprecipitated with G25a, an anti-DQw1 β chain monoclonal antibody (A) The α chain area of the gel (isoelectrofocusing, two-dimensional polyacrylamide gel electrophoresis) reveals DR α, DQ α, and DP α chains. (B) The β chain area of the gel (nonequilibrium pH gradient electrophoresis, two-dimensional polyacrylamide gel electrophoresis) reveals a DQw1 β chain.

nant responsible for the highest susceptibility to MS is present on an intraisotypic HLA-DQ hybrid molecule. The participation of DP in the disease susceptibility element is still debated.

Two Scandinavian studies reported an association with DPw4 (Moen *et al.*, 1984; Odum *et al.*, 1988), which was confirmed in a Japanese population (N. Odum, personal communication). The absence of a linkage between DPw4 and DR2 would argue that the two disease susceptibility elements have independent contributions. However, a determinant could be created by a dimer consisting of a DQ α and a DP β chain, both genetically linked to MS, although independent, which would represent the key element.

Alternatively, a disease susceptibility element could be formed by the

DQ β chain (DQw1 β) present in MS and a specific DP α chain. We await studies of DP α polymorphism in MS in order to conclude this point.

The way in which the HLA disease susceptibility factor participates in the mechanisms leading to the disease is still largely unknown. It seems that inducibility of Ia expression on astrocytes is under MHC control, at least in a rat model of infection with a coronavirus, giving a syndrome similar to experimental allergic encephalomyelitis (Massa *et al.*, 1987). It is thus of the utmost interest to explore in human brain cells their capacity for class II expression and the genetic (i.e., regulatory polymorphism) and somatic (i.e., virus or lymphokine) factors involved.

The T cell response is also a critical parameter in inducing and/or controlling the disease. Several reports mention modification of the TcR β repertoire in the MS population (Seboun *et al.*, 1989; Beall *et al.*, 1989). Whether the TcR β anomaly is in itself a disease susceptibility gene or a gene in linkage disequilibrium with another predisposing gene is still debated. It is also possible that other TcR genes contribute to the genetic background which interact with unknown environmental agents to create the disease.

VI. Concluding Remarks and Perspectives

In the past 15 years an enigmatic and almost impressionistic view of HLA and disease association has been clarified by structural definition of the class II molecules involved in disease susceptibility. The First International Symposium on HLA and Disease was held in Paris in 1976 (Dausset and Svejgaard, 1976) and was an essential contribution, drawing the attention of the medical and scientific community to the importance of the HLA system in disease and stimulating further research. At the time the genetics and statistics provided the hard data, while the immunology was speculative. The situation was confused, but McDevitt (1976) stated that the "future prospects seemed bright" in his concluding remarks and requested more precise typing techniques, while foreseeing mapping of the Ir genes and an unraveling of their structure: "Availability of such typing techniques will probably result in the detection of very strong associations between particular alleles of genes in the HLA-D region and particular diseases."

Indeed, the prediction is now superbly fulfilled. Clearly, the HLA class II molecules involved in disease susceptibility have a normal structure and may be found in the normal population, although at a lower frequency. The data reviewed here support the concept that the disease susceptibility element is composed of amino acids (contiguous and/or distantly spaced on the same chain, or on different chains of the same or a distinct class II isotype) which delineate a specific three-dimensional

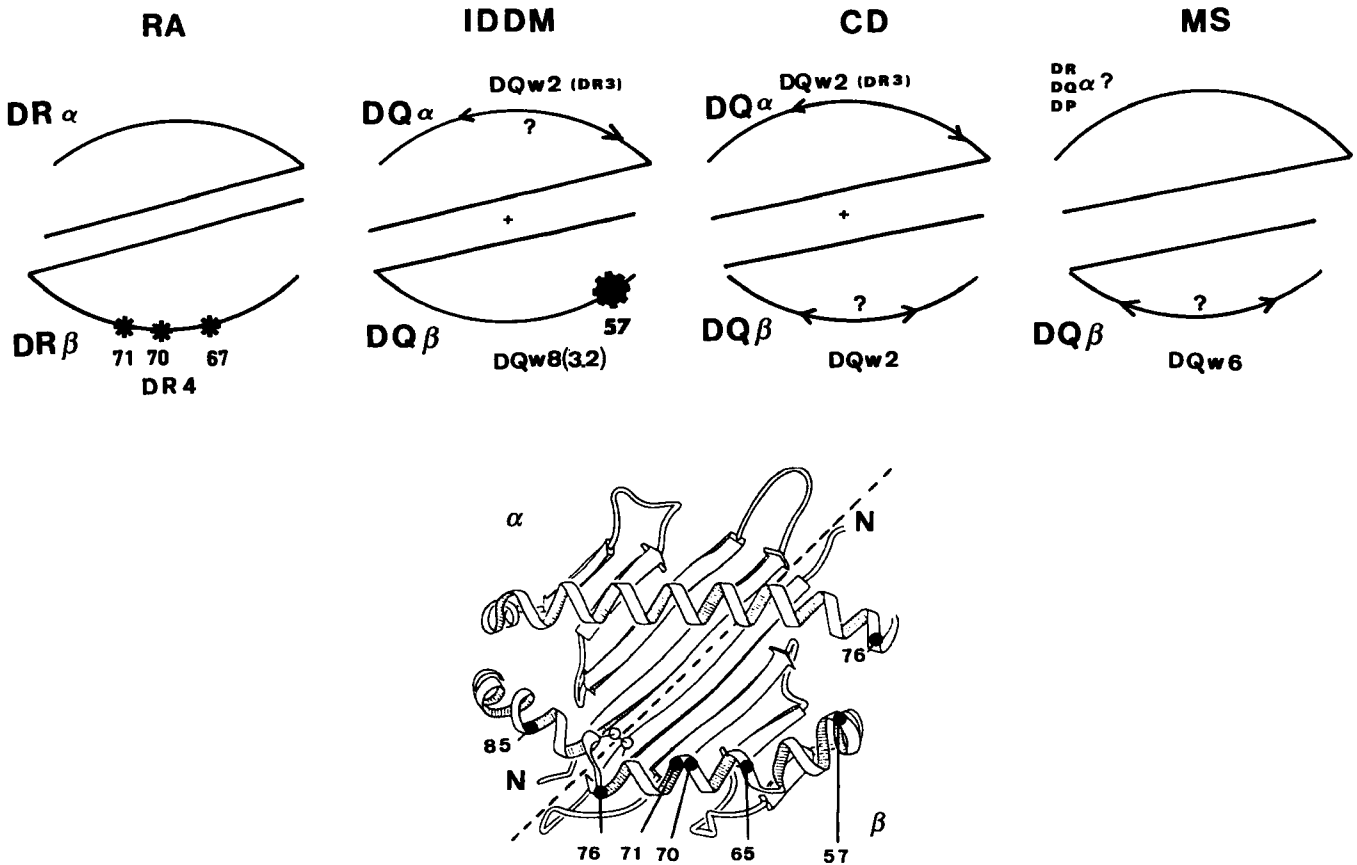
conformation. It is suggested that it is more the conformational epitope created than the linear stretch of amino acids which is important (Fig. 7).

Clearly, even when a portion of one class II chain appears to be predominant in disease susceptibility, additive and often synergistic contributions are found for a second chain. In conjunction with the fact that HLA class II molecules are α–β dimers capable of forming hybrid molecules (intra- or interisotypic) by *cis*- and/or *trans*-complementation, this provides an ideal structural framework for the localization of the conformational disease susceptibility epitope(s). The capacity of the conformational epitope to bind within a high-affinity range set of defined peptides varies, presumably according to the polymorphism of the epitope (Buus *et al.*, 1987). Whether it is always the same conformation of the class II molecule or a slightly different, but equivalent, structure which is involved in one disease is unknown.

Moreover, as a peptide receptor, an MHC class II molecule could be flexible, allowing for some variation in the amino acid composition of the antigenic peptide(s). Many peptides (but not all) are likely to bind to the disease susceptibility element. This questions the nature of the antigen involved in disease susceptiblity in autoimmunity. There is no obvious reason to exclude that the antigen itself has some degree of polymorphism, as it binds to the appropriate disease-specific conformational epitope with sufficient affinity and is able to induce T cell activation.

Future key studies include the definition of antigenic peptides, whether they are environmental (foreign) or autologous (self). The MHC molecule in itself may provide a molecular tool in this search, since the antigen can be defined through its complementary conformational interactions with the class II molecule. Candidate peptides are presently being tested for their ability to bind class II disease susceptibility elements. The TcR represents the third partner of the functional trimolecular complex involved in the control of the immune response. Already in animal models of autoimmunity (e.g., experimental allergic encephalomyelitis), experimental evidence for a selection of the T cell diversity gives support to the idea that the T cell repertoire is important (Acha-Orbea *et al.*, 1988). It is, however, unknown whether this reflects a secondary state or precedes and induces the disease. The T cell repertoire is the result of germ-line diversity, followed by thymic selection.

The expression of class II molecules on the thymus is set to eliminate MHC self-reactivity. However, this process may require specific amounts of any class II molecule and sufficient duration of expression of a particular class II molecule in order to efficiently result in a full depletion of autoreactive T cells (self-MHC). Hybrid molecules may be expressed at a lower level than regular class II species and their expression may not be as equally constant as the typical α–β isotypes.


RA
DR α
DR β
71 70 67
DR 4
IDDM
DQw2 (DR3)
DQ α
?
+
DQ β
57
DQw8(3.2)
CD
DQw2 (DR3)
DQ α
+
?
DQ β
DQw2
MS
DR
DQα ?
DP
?
DQ β
DQw6
α
N
76
85
N
76 71 70 65 57
β

Recent data from mice and preliminary results from this laboratory in humans suggest that there are favored α–β associations, while some are almost totally forbidden. This is determined by the affinity of different β chains for a given α chain. The end result may be that autoreactive T cells specific for class II hybrid molecules may not be totally depleted in all individuals. This may also depend on the local presence of lymphokines in the thymus during the selection process. The TcR repertoire would thus vary in different individuals, more susceptible individuals possessing a small amount of autoreactive T cells specific for the HLA class II hybrid molecule. This offers a tentative explanation for the concordance rate in twins, which is, at most, 50% in the HLA-associated diseases.

Ultimately, the HLA class II molecules are the potential target of therapeutic strategies. This possibility was suggested when anti-Ia mAb's were shown to prevent or reverse induced, as well as spontaneous, autoimmune diseases in animal models. This concept permits the perspective that either blocking or competing antibodies or peptides may be tailored toward the disease susceptibility element and will therefore display high specificity and predictably high efficacy against the disease-associated immune response.

Alternatively, antilymphokine reagents (e.g., antibodies, antagonists, and drugs) which could down-regulate HLA class II expression may also be considered. However, the exact mechanisms of such treatments are not known. They may block immune responses at the effector level, but may also solicit the induction of tolerance and/or the stimulation of suppression. Adverse potential effects mediated by immunological or nonimmunological pathways should not be ignored.

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FIG. 7. Molecular localization of the HLA class II epitopes involves in disease susceptibility. ?, Amino acids not precisely mapped; +, heterozygous effect. (*trans*-complementation). RA, Rheumatoid arthritis; IDDM, insulin-dependent diabetes mellitus; CD, celiac disease; MS, multiple sclerosis. Adapted from Bjorkman *et al.* (1987) and Brown *et al.* (1988).

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