Perspectives in Diabetes

HLA and Genetics of IDDM

Holism vs. Reductionism?

MIRIAM SEGALL

Analysis of HLA-associated susceptibility to insulin-dependent diabetes mellitus (IDDM) has largely focused on identifying *the* susceptibility gene. Adherents of a countertrend have long suggested the importance of analysis of HLA haplotypes (combinations of alleles on 1 chromosome) rather than individual genes. Accumulating data suggest that the relationship between IDDM susceptibility and HLA is much more complex than a single susceptibility gene. Consideration of this question should include the possibilities that *1*) more than one HLA gene is involved in determining susceptibility or resistance; *2*) different alleles of the same gene may be associated with different pathogenetic mechanisms; and *3*) different susceptibility-associated haplotypes, even if they share an allele at an IDDM-relevant locus, may behave differently in IDDM. A better understanding of the genetics, and perhaps the pathogenesis, of IDDM may be obtained by following up the clues offered by analysis of the association of HLA haplotypes (rather than individual alleles) with one another, with clinical features of IDDM, and with possible non-HLA-linked susceptibility factors. *Diabetes* 37:1005–1008, 1988

The HLA complex is the only well-documented genetic marker for susceptibility that has been defined in human insulin-dependent diabetes mellitus (IDDM). HLA molecules play a role in the recognition of antigen by T-lymphocytes and therefore in immunoregulatory processes. The hypothesis that HLA-associated susceptibility to IDDM involves the HLA antigens and their immunoregulatory functions is thus an appealing one, and the associations of IDDM with HLA have been extensively studied.

From the Immunobiology Research Center, Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, Minnesota.

Address correspondence and reprint requests to Dr. Miriam Segall, Immunobiology Research Center, Box 724 UMHC, University of Minnesota Hospital and Clinic, Harvard Street at East River Road, Minneapolis, MN 55455.

Received for publication 11 April 1988 and accepted 15 April 1988.

The extent to which these studies have contributed to the understanding of the etiology and pathogenesis of IDDM is, however, limited. The study of immunologic processes in IDDM has been restricted by lack of knowledge about the antigens recognized by T-lymphocytes, which are generally believed to be an essential part of the autoimmune process in IDDM. I argue, however, that the focus on *the* HLA-associated susceptibility gene has resulted in loss of potentially important information and that studying associations of HLA haplotypes with susceptibility will add significantly to the understanding of both the genetics and the etiology of the disease.

Data discussed herein suggest that more than one HLA gene may be involved in susceptibility to IDDM, and in this case, significant susceptibility may be associated with particular haplotypes. Interactions between susceptibility factors on different haplotypes may be important. If different haplotypes are associated with susceptibility through different pathogenetic mechanisms, haplotype analysis may also be useful in defining heterogeneity of the disease.

I do not intend to provide a complete review of HLA and IDDM, and the literature cited is selective. I use the HLA terminology that has become somewhat familiar in the broader scientific community rather than the recent changes based on the results of the 10th International Histocompatibility Workshop (World Health Org.). In Fig. 1, however, the newly assigned terminology is used for the individual HLA class II genes because of the lack of uniformity of the terminology currently used by different authors.

HLA COMPLEX

The HLA complex has been reviewed repeatedly and will not be dealt with in detail here; a simplified view is given in Fig. 1. There is potential for additive and synergistic interactions of many different genes as well as room for unidentified genes. In addition, the system is highly polymorphic (each locus has many different forms).

For this discussion, certain genetic terms are important. A *gene* is a locus or portion of genetic material that codes

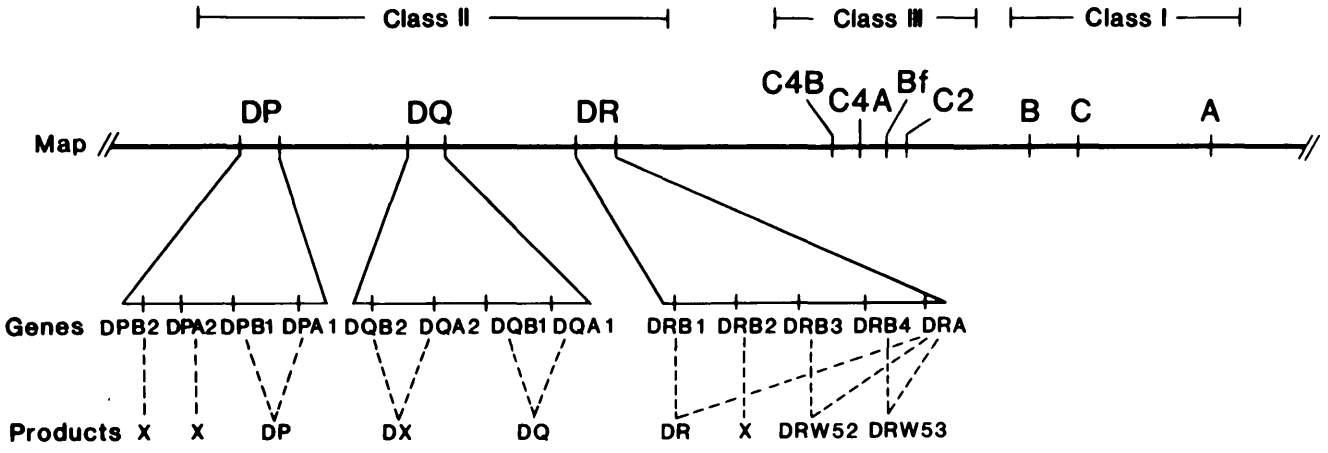

FIG. 1. Schema of HLA complex showing various regions (class I, class II, and class III). Distances are not to genetic scale. Individual class II genes are indicated according to recommended nomenclature of 10th International Histocompatibility Workshop; molecules or gene products are indicated according to common names. x, Pseudogene with no expressed product. Although DQB2 and DQA2 genes are normal according to genomic DNA sequencing, no expressed product of these genes has been identified. Number of DRB genes varies in different haplotypes; either DRw52 or DRw53 (or neither) is found in given haplotype. Additional genes (not indicated) are present in region between B and DR, and DOB and DZA genes are present in region between DQ and DP. DP alleles do not appear to be significantly associated with insulin-dependent diabetes mellitus.

for a particular product (polypeptide chain). An *allele* is one of the variant forms of a polymorphic locus; e.g., DR1, DR2, and DR3 are alleles of DR. An HLA *haplotype* is a particular combination of two or more alleles on one chromosome (e.g., A1-B8-DR3).

ASSOCIATION OF IDDM SUSCEPTIBILITY AND HLA

In family studies, a susceptibility factor for IDDM is closely linked to HLA; in population studies, there are strong associations between susceptibility and specific alleles of HLA genes. Certain HLA class II alleles appear to be more strongly associated with IDDM than any class I alleles, suggesting that the former are either susceptibility genes themselves or that the susceptibility genes are more closely linked to the class II than the class I genes (as is also indicated by study of intra-HLA recombination and IDDM susceptibility in families). It is unknown whether the class II genes or other unknown genes are the "real" HLA-associated susceptibility genes for IDDM. I therefore refer to the IDDM-associated HLA alleles as susceptibility or nonsusceptibility *markers*, using this term to cover both possibilities.

Association of IDDM susceptibility with single HLA genes. The associations of HLA and IDDM are defined by comparing the frequencies of various alleles in nondiabetic control and diabetic individuals; positively associated alleles are more frequent in diabetic patients, negatively associated alleles less frequent. The first identified associations of HLA with IDDM susceptibility were positive with the class I alleles B8 and B15 and negative with B7. With the definition of the class II specificities, stronger positive associations with DR3 and DR4 and negative associations with DR2, DR5, and DR7 were demonstrated in numerous studies; weaker positive associations with DR1 and possibly with DRw8 have been suggested (1).

Recent data suggest that the primary association may be with DQ rather than DR. DR4 is usually associated with the DQw3 specificity; DQw3 has been subdivided, and the DR4-DQw3.2 (DR4-DQR4) subset may be associated with susceptibility to IDDM, whereas the DR4-DQw3.1 (DR4-DQR5) subset is associated with nonsusceptibility (2,3). The DR1 and DR2-MN2(AZH) haplotypes, which appear to be secondary-susceptibility haplotypes for IDDM, both carry the DQw1.1 subtype of the DQw1 specificity, which is different from the DQw1.2 subtype found on the strong resistance haplotype DR2-Dw2 (4,5). A recent analysis of sequence data (6) has suggested that position 57 of the DQβ-chain may be an important factor in determining resistance to IDDM, because haplotypes that are "neutral" or "negative" with regard to IDDM susceptibility have aspartic acid at this position; i.e., the DQβ-gene may itself be a susceptibility gene. Association of IDDM with a particular allele of DX, the apparently nonexpressed second set of genes in the DQ region, has also been suggested (7).

Association of IDDM susceptibility with more than one HLA gene. Several examples that may represent such associations can be adduced. In DR4$^+$ IDDM patients, DQw3.2 appears to be associated with susceptibility and DQw3.1 with nonsusceptibility, as mentioned above. DR4 can be divided into several subtypes; in the Minnesota diabetic population, one of these subtypes, DR4-Dw4, is postively associated with IDDM in DR4$^+$ patients, whereas another subtype, DR4-Dw14, is not (8,9). Because both subtypes are associated with DQw3.2 in normal control and diabetic individuals, two factors, one associated with DQw3.2 and one with DR4-Dw4, may be involved in susceptibility. Similarly, in the case of position 57 on the DQβ-chain (6), the alleles that have aspartic acid at this position range from the DQβ of DR2-Dw12, classified as neutral, to the DQβ of the strongly negatively associated DR2-Dw2. Other elements, either within DQβ or elsewhere in the haplotype, must be involved in determining the behavior of a particular DQβ allele with respect to IDDM. In the case of DX, there appear to be associations with certain DQ specificities, particularly with the subsets of DQw3 (7).

Association of IDDM susceptibility with HLA haplotypes. In any discussion of HLA haplotypes, it is important to consider the phenomenon of linkage disequilibrium. This means that particular alleles at two or more linked loci are found together on a single chromosome more frequently than expected based on their frequency in the population; i.e., the distribution of alleles of these loci on the chromosomes of the population is nonrandom. Alleles of several loci over a relatively large stretch of chromosome may be in linkage disequilibrium; e.g., linkage disequilibrium is observed throughout the HLA complex. Especially important for this discussion is the linkage disequilibrium for particular sets of alleles in the region from HLA-B to HLA-DR (Fig. 1).

Whereas an analysis of the associations of HLA with IDDM is most conveniently conducted at the level of individual specificities, associations of particular HLA haplotypes with IDDM are well documented. Analysis of haplotypes may prove to be crucial to an understanding of the genetics of IDDM, in both its HLA and non-HLA aspects, and to be useful in the study of IDDM immunology.

Many of the associations of IDDM with HLA involve haplotypes. For example, although DR4 is in linkage disequilibrium with different HLA-B alleles, only certain haplotypes are positively associated with IDDM (i.e., are more frequent in IDDM patients than in control subjects.). B15-DR4 is positively associated with IDDM in Caucasian populations, whereas B44-DR4 is not. Both B8-DR3 and B18-DR3 are positively associated with IDDM in Caucasians, whereas B7-DR2 has a low relative risk.

The finding of haplotype associations with IDDM may mean that a positively associated haplotype contains a particular allele of another class I or class II gene that is the important one for susceptibility and is not found in the non-susceptibility haplotypes; that it contains a particular allele of some other gene (not class I or class II) that is the important one for susceptibility; or that alleles of several HLA genes together determine susceptibility, and all of the requisite alleles are only present on certain haplotypes. Obviously, with the current state of knowledge, no choice can be made among these alternatives, and different haplotypes may belong in different categories.

The haplotypes that are strongly associated with susceptibility or resistance to IDDM in Caucasian populations are also in strong linkage disequilibrium in nondiabetic individuals in these populations. Alleles of two loci may be in linkage disequilibrium for various reasons, and it is extremely difficult to distinguish among these possibilities in human populations. With respect to HLA, several common haplotypes that include a B and a DR allele in significant linkage disequilibrium also include particular complotypes or sets of class III alleles (Fig. 1; 10). These findings indicate the maintenance in the population of "units" of chromosome including at least the entire region between B and DR ("extended" HLA haplotypes). Significant associations of these extended haplotypes with IDDM have been described (11).

Possible association of alleles at same locus with different pathogenetic processes. DR3 and DR4 are important susceptibility markers in IDDM; among Caucasian patients, ≥90% are DR3, DR4, or both. Analyses of relative risk indicate that the DR3/4 heterozygote has a higher relative risk for IDDM than either the DR3/X or the DR4/X phenotypes (12). These two markers may represent different pathogenetic processes in IDDM (perhaps susceptibility to different initial triggering mechanisms). DR3 and DR4 may also have different associations with other HLA alleles in IDDM. For example, DR1 appears to be a secondary-susceptibility haplotype in $DR4^+$ but not in $DR3^+$ IDDM patients (1,9). In the Minnesota study, DR7 (generally considered a resistance haplotype) is reduced in frequency in $DR4^+$ $DR3^-$ IDDM patients but not in the small group of $DR3^+$ $DR4^-$ patients. The differences in behavior of DR3 and DR4 with respect to other alleles may reflect differences in the pathogenetic processes with which they are associated.

Possible associations of same allele at given locus with different pathogenetic processes, depending on rest of haplotype. A particular allele at a given locus may be found in more than one haplotype showing linkage disequilibrium. Data relating to certain such haplotypes in IDDM suggest they may indeed be significantly different.

Both the B8-DR3 and B18-DR3 haplotypes are associated with susceptibility to IDDM, but only B8-DR3 is associated with susceptibility to Graves' and celiac diseases, suggesting that either the B8-DR3–associated susceptibility to IDDM and to these other presumed autoimmume diseases are different or the B8-DR3– and B18-DR3–associated susceptibilities to IDDM are different. Data from Minnesota IDDM patients support the latter possibility; of the two DR4-carrying haplotypes positively associated with IDDM in this population, B40-BfS-DR4 tends to be preferentially associated with B8-BfS-DR3 in DR3/4 heterozygotes, whereas B15-BfS-DR4 tends to be preferentially associated with B18-BfF1-DR3 (13). Whether B15-BfS-DR4 and B40-BfS-DR4 represent different "forms" of DR4-associated susceptibility is unknown.

The B44-DR4 haplotype is not significantly associated with susceptibility to IDDM (11,13). However, our recent preliminary data suggest that the IDDM children in families with a non-insulin-dependent diabetic (NIDDM) parent have an increased frequency of the B44 specificity and that in $B44^+$ IDDM children with an NIDDM parent, the B44-DR4 haplotype is common, whereas in $B44^+$ IDDM children with an IDDM parent, this haplotype is infrequent. In the Minnesota IDDM study, patients carrying B44-DR4 tend to have DRw6 on the second haplotype more frequently than patients carrying other $DR4^+$ haplotypes. Further investigations should determine whether the B44-DR4 haplotype is indeed a marker for a distinct subset of IDDM and in what respect it is different from B15-DR4 and B40-DR4.

Haplotypes and heterogeneity in IDDM. Data on IDDM in monozygotic twins and in HLA-identical siblings demonstrate that environmental and possibly non-HLA-linked genetic factors play a significant role in the development of the disease. If the various associations of HLA with IDDM reflect two (or more) pathogenetic mechanisms, different environmental or non-HLA-linked genetic factors might be relevant to each mechanism, and/or each mechanism might have specific clinical features. Attempts to demonstrate associations of HLA specificities with parameters such as age of onset, seasonality, disease severity, or islet cell or insulin antibodies have yielded contradictory results, as have attempts to identify specific non-HLA-linked genetic factors.

Haplotype analysis in such studies may help to define associations and give additional clues to pathogenetic mechanisms.

CONCLUSIONS

I have tried to show that the associations of IDDM with HLA are much more complex than those encompassed by the search for *the* susceptibility factor. More than one locus may be involved, or as yet unimplicated loci may be important. Interactions between different susceptibility genes or between different sets of susceptibility genes associated with different haplotypes may be specific and related to different pathogenetic mechanisms associated with the genes or haplotypes. Analysis of the association of HLA haplotypes and IDDM susceptibility should yield new information on both the genetics and pathogenesis of IDDM.

ACKNOWLEDGMENTS

I take pleasure in acknowledging my debt to my colleagues Jose J. Barbosa, Stephen S. Rich, and Fritz H. Bach for collaboration and support.

This work was supported in part by Grant 186175 from the Juvenile Diabetes Foundation, Grant 6-496 from the March of Dimes Birth Defects Foundation, and a grant from the Arthritis Foundation, Minnesota Chapter.

This is paper no. 485 from the Immunobiology Research Center.

REFERENCES

1. Thomson G: HLA DR antigens and susceptibility to insulin-dependent diabetes mellitus. *Am J Hum Genet* 36:1309–17, 1984
2. Cohen-Haguenauer O, Robbins E, Massart C, Busson M, Deschamps I, Hors J, Lalouel J-M, Dausset J, Cohen D: A systematic study of HLA class II-beta DNA restriction fragments in insulin-dependent diabetes mellitus. *Proc Natl Acad Sci USA* 82:3335–39, 1985
3. Schreuder GMTh, Tilanus MGJ, Bontrop RE, Bruining GJ, Giphart MH, van Rood JJ, de Vries RRP: HLA-DQ polymorphism associated with resistance to type I diabetes detected with monoclonal antibodies, isoelectric point differences, and restriction fragment length polymorphism. *J Exp Med* 164:938–45, 1986
4. Segall M, Noreen H, Schluender L, Swenson M, Barbosa J, Bach FH: $DR2^+$ haplotypes in insulin-dependent diabetes: analysis of DNA restriction fragment length polymorphisms. *Hum Immunol* 17:61–68, 1986
5. Cohen D, Cohen O, Marcadet A, Massart C, Lathrop M, Deschamps I, Hors J, Schuller E, Dausset J: Class II HLA-DC beta-chain DNA restriction fragments differentiate among HLA-DR2 individuals in insulin-dependent diabetes and multiple sclerosis. *Proc Natl Acad Sci USA* 81:1774–78, 1984
6. Todd JA, Bell JI, McDevitt HO: HLA-DQ beta gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. *Nature* (Lond) 329:599–604, 1987
7. Hitman GA, Sachs J, Cassell P, Awad J, Bottazzo GF, Tarn AC, Schwartz G, Monson JP, Festenstein H: A DR3-related DX alpha gene polymorphism strongly associates with insulin-dependent diabetes mellitus. *Immunogenetics* 23:47–51, 1986
8. Bach FH, Barbosa J, Rich S, Segall M: Insulin dependent diabetes-associated HLA-D region encoded determinants. *Hum Immunol* 12:59–64, 1985
9. Segall M, Bach FH: HLA and diabetes from a T-cell perspective. *Diabetes Metab Rev* 3:803–17, 1987
10. Awdeh ZL, Raum D, Yunis EJ, Alper CA: Extended HLA/complement allele haplotypes: evidence for T/t-like complex in man. *Proc Natl Acad Sci USA* 80:259–63, 1983
11. Raum D, Awdeh Z, Yunis EJ, Alper CA, Gabbay KH: Extended major histocompatibility complex haplotypes in type I diabetes mellitus. *J Clin Invest* 74:449–54, 1984
12. Bertrams J, Baur MP: Disease reports: insulin-dependent diabetes mellitus. In *Histocompatibility Testing*. Albert ED, Baur MP, Mayr WR, Eds. Berlin, Springer-Verlag, 1984, p. 348–58
13. Rich SS, Weitkamp LR, Barbosa J: Genetic heterogeneity of insulin-dependent (type I) diabetes mellitus: evidence from a study of extended haplotypes. *Am J Hum Genet* 36:1015–23, 1984