MEDICAL GRAND ROUNDS

THE GENETICS OF IDDM: CLUES TO ETIOLOGY, PATHOGENESIS & TREATMENT

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I. INTRODUCTION

Diabetes mellitus has long been a problem child of medical genetics. It runs in families, yet its inheritance unlike that of most genetic diseases almost never follows any simple Mendelian pattern. Indeed, in many cases we cannot say it is the disease that is inherited, but rather a susceptibility to it. The quest to make sense of this puzzle takes on added urgency from the high prevalence of diabetes in Western Societies - much greater than that of other genetic pathologies. For example, Hemophilia-A affects 1 in 20,000 people, DMD 1 in 10,000; Cystic Fibrosis 1 in 2,000 and Sickle Cell Anemia 1 in 400 blacks. Diabetes Mellitus affects 1 in 50 people.

That diabetes is an inherited disease is unquestioned. Both Type I and Type II diabetes are known to have quite distinct inheritance patterns. And while a great deal is known about the genetic predisposition to both forms of diabetes mellitus, considerable confusion still exists and the precise type of genetics involved in the two diseases remains an enigma.

Since I am neither a geneticist nor a diabetologist, I hope I can be excused certain generalizations. I will attempt to convey to you some of the excitement that has been generated recently concerning the genetics of these two diseases particularly in the genetics of insulin dependent diabetes mellitus, with which I am a bit more familiar. If we have time, I hope to discuss how these new insights into the genetics of IDDM may provide important clues as to etiology, pathogenesis, prevention, and/or treatment of this devastating disease.

II. SIMPLE GENETICS

Geneticists learn an enormous amount by taking family histories and developing pedigrees. Additionally, geneticists gain information by looking at the pattern of disease in identical twins. For example, in most common genetic diseases both identical twins are affected with the disease. Thus, for example, in Sickle Cell Anemia, identical twins will both be affected or both be unaffected. We know sickle cell disease to be a single gene disorder and therefore the disease is said to have one hundred percent penetrance as individuals who are genetically identical have identical disease patterns. Indeed, in Sickle Cell Anemia individuals who inherit two doses of the Hemoglobin S gene are essentially all diseased. However, identical twins can both be affected with a particular disease and yet the disease can be multigenic. Thus, for example, if in order to develop a particular disease an individual has to inherit two genes independently from two different chromosomes, then identical twins would be one hundred percent concordant for disease and yet at the same time the genetics would be more difficult to sort out.

These two examples parallel the situation in diabetes. It is well known that insulin dependent diabetes mellitus (Type I diabetes) is inherited and yet in identical twins only approximately fifty percent of twins are concordant for disease. However, since we have a good idea of at least one of the genetic elements involved in IDDM (the HLA Complex) most

physicians view IDDM as a clear cut example of an inherited disease. In non-IDDM or Type II diabetes, we have very little knowledge of what the genetic elements are that predispose to disease so most physicians view Type II Diabetes as less inherited despite the fact that nearly one hundred percent of identical twins are concordant for Type II diabetes. Clearly we need some definition of terms and some understanding of the general modes of inheritance of clinical genetic diseases.

Genetic diseases generally fall into one of three categories. One - chromosomal disorders, Two - Mendelian or simple inherited disorders, and Three - Multifactorial disorders. Chromosomal disorders involve the lack, excess or abnormal arrangement of one or more chromosomes. This clearly does not operate in either form of diabetes mellitus. No cytogenetic abnormality has been consistently seen in either form of Diabetes Mellitus. Mendelian or simple inherited disorders are determined primarily by a single mutant gene. These disorders display inheritance patterns which can be classified into autosomal dominant, autosomal recessive or X-linked types. Lets discuss each of these in turn.

The distinction between "dominant" and "recessive" is one of convenience in pedigree analysis and does not imply a fundamental difference in genetic mechanism. The term dominant implies that the mutation is clinically manifest when an individual has one dose of this mutation (or is heterozygous for it), while recessive implies that a double dose (or homozygosity) is required for clinical detection. Genes are never dominant or recessive; their effects, however, produce clinical patterns that are classified as dominant or recessive.

Examples of these two kinds of patterns are illustrated in figures 1 and 2 below. Figure 1 shows the pedigree of an autosomal dominant trait. Note the vertical pattern of inheritance. Figure 2 shows the pedigree of an autosomal recessive trait. Note the horizontal pattern of inheritance.

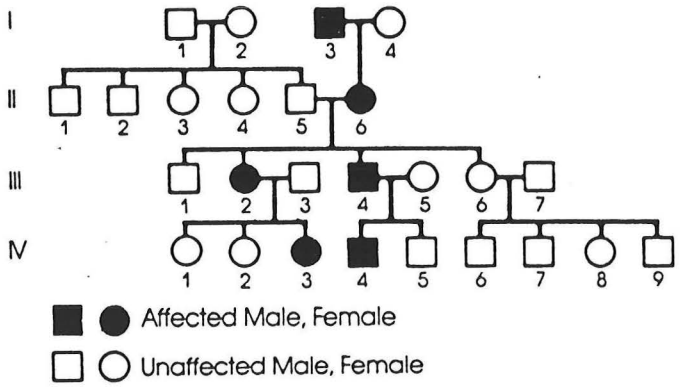

Figure 1. Repetitive pattern of an autosomal dominant trait. Note the vertical pattern of inheritance. From Harrison's Principles of Internal Medicine, 11th edition, p. 291.

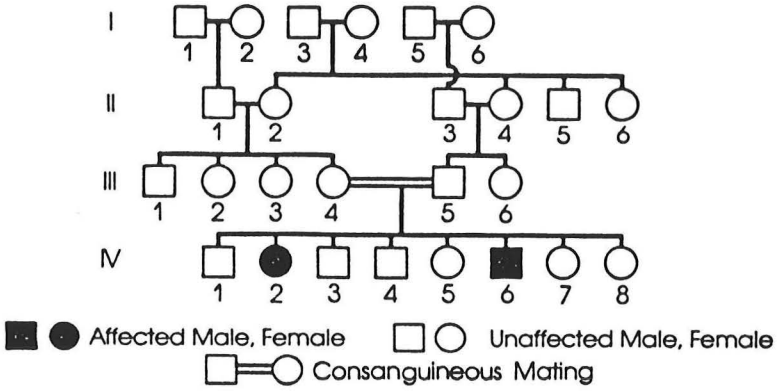

Figure 2. Repetitive pattern of an autosomal recessive trait. Note the horizontal pattern of inheritance. From Harrison, Principles of Internal Medicine, 11th edition, p. 291.

Autosomal dominant disorders have the following characteristics: One, each affected individual has an affected parent. Two, an affected individual will have on average both normal and affected offspring in equal proportions. Three, normal children of an affected individual have only normal offspring.

Autosomal recessive conditions are clinically apparent only in the homozygous state; that is, when both alleles of a particular genetic locus are mutant. The characteristics of autosomal recessive diseases include: One, the parents are clinically normal. Two, only siblings are affected and vertical transmission does not occur; and, Three, males and females are affected in equal proportions.

I need not go over the form of inheritance for X-linked disorders in any detail. Obviously the genes responsible for X-linked disorders are located on the X chromosome. Therefore, the clinical risk and severity of disease is different for the two sexes. Since the female has two X chromosomes, she may be either heterozygous or homozygous for a mutant gene and the trait may therefore demonstrate either recessive or dominant expression. Males on the other hand have only one X chromosome so they can be expected to display the full syndrome whether they inherit the gene regardless of whether the gene behaves as a recessive or dominant trait in the female. Neither form of diabetes suggests an X-linked disorder.

III. THE GENETICS OF IDDM

We know more about the genetics of diabetes than we did twenty years ago although not nearly as much as we would like to know. A major advance came with the recognition that diabetes is not one disease but many. On

purely symptomatic grounds it can be separated into several distinct entities each of which appears to be genetically heterogeneous.

Clinically, diabetes mellitus falls into three broad categories. Primary insulin dependent diabetes (IDDM) is also known as Type I or juvenile onset diabetes mellitus. It affects approximately 0.5% of the population of the United States and represents approximately 10% of all diabetics in the United States. Much more common is primary non-insulin dependent diabetes mellitus (NIDDM) also called Type II or adult onset diabetes. As most clinicians know these terms are relative. IDDM may occur in midlife or even old age. NIDDM may appear in adolescence or occasionally in childhood.

There are other forms of diabetes associated syndromes. Indeed it is said that over 60 such syndromes are associated with varying degrees of glucose intolerance. However, in toto they represent a very small percentage of the total diabetics in the United States.

The rest of this Medical Grand Rounds will deal with the genetics of insulin dependent diabetes mellitus. A major component of the genetics of IDDM's susceptibility has been identified: variation in a gene or genes located near or within the HLA (major histocompatibiliy) complex on the short arm of chromosome 6. As you know the HLA genes are deeply involved in the body's immune responses. Knowing that HLA genes are responsible for a portion of the genetics of IDDM allows us to look at two simple situations and draw certain conclusions which will dominate the rest of our discussion today. First as I have mentioned before, identical twins are concordant for disease only approximately 50% of the time. Thus, it is important to appreciate that some non-genetic factor must be involved in the pathogenesis of this disease, because the non-affected twin in every instance that has been studied carefully is in every way that we can measure perfectly normal. The possible explanations
for this will be discussed below. Thus, in Type I diabetes it should be kept in mind that no genetic based susceptibility test can ever achieve an accuracy of greater than 50%, since people (identical twins) with identical genetic makeups are only 50% concordant.

That the histocompatibility complex is involved in IDDM is unquestioned. We will later discuss the particulars of this association. Right now let us turn to some family studies. In families where there is a sibling with IDDM the chances of another sibling having IDDM are approximately 6%. If, however, one goes through the family and does HLA typing, the following numbers are generated. Siblings that are HLA identical have an approximately 12.5% chance of developing disease (1 in 8). Children that are half HLA identical have approximately a 5% chance of getting disease (1 in 20), whereas individuals who have no HLA sharing with the affected sibling have a 2% chance of getting IDDM (1 in 50).

From these very approximate numbers which I have averaged from several studies we can draw the following conclusions. First HLA alone cannot explain these results. If HLA alone explained these results then HLA identical siblings and identical twins would have the same concordance rate. If the only genetic factors involved in IDDM were the HLA complex, two brothers brought up in the same household or non-identical twins that had the same HLA type would have the same concordance rate as identical twins. Secondly, the fact that even without any HLA sharing, within a sibship 2% incidence of disease still occurs strongly argues that some non-HLA factor can by itself be responsible for IDDM. As I mentioned earlier in this presentation, only 0.5% of the population at large get IDDM. Therefore, if HLA alone were responsible for the disease a sibling with no HLA sharing with a probard should have only a 0.5% incidence of disease or lower!

These two facts - that the incidence of disease in HLA matched siblings is approximately one fourth the incidence in identical twins and that the incidence of IDDM in non-HLA matched siblings is 4 times the incidence of IDDM in the population at large has given credence to the view that a non-HLA linked gene is responsible for a significant portion of the genetics in IDDM.

Indeed, long before the HLA complex was discovered and long before the association of HLA-DR3/4 and/or DQw8 was known, mathematicians studying inheritance patterns in IDDM postulated that three unlinked genes were responsible for the genetic basis of this disease. Twenty five years later we are not able to improve on that prediction.

Let us take a moment to look at the identical twin concordance and speculate as to the possible "non-genetic" factor that could be responsible for the lack of 100% penetrance. Two general theories are held. The first teaches that the non-genetic factor is environmental; that is, even identical twins brought up in the same household do not have identical environments and either through contact with viruses, bacteria or toxins in the environment, the same genetic background will lead to different consequences depending on the environmental insult. A strong argument against this notion is that the concordance of IDDM in identical twins that are reared apart is not significantly different than the concordance rate of identical twins reared together. These studies (although they have been relatively few in number) suggest that it is not an environmental factor unless the factor is common to several parts of the world. Second, with increasing understanding of the mechanisms whereby the T cell repertoire and immunoglobulin repertoire are developed, there has been some speculation that the "non-genetic" or "non-environmental" mechanism for the difference in the concordance rates may relate to the repertoire itself. We all know that immunoglobulin and T cell receptor genes rearrange stochastically (randomly). Thus, two mice from the same inbred strain develop slightly different T cell or immunoglobulin repertoires as it is unlikely that the precise immune receptor gene would undergo rearrangement at precisely the same moment in time in two (even genetically identical) individuals. This would allow two individuals with identical genetic

makeup to develop slightly different immunologic repertoires. Put another way, the number of the combinations of V to D to J gene segments that give rise to the enormous complexity of immune receptor molecules might develop slightly differently in two individuals such that they would at any moment in time have different capabilities to mount or not mount immune responses. Thus, even without an environmental insult, identical twins have a fundamentally different immune repertoire and, as such, one twin could develop self destructive possibilities and autodestruct his or her islets of Langerhans where the other twin could not.

Obviously a combination of these two is quite possible; that is, because of the non-stochastic nature of the development of the T or B cell repertoires a common environmental insult that both twins received at an identical moment in time could lead to different immunologic consequences.

Much of this is obviously handwaving in order to attempt to get around the problems evident from the twin studies. We simply do not understand why twins with identical genetic material are not 100% concordant for disease.

IV. THE HLA COMPLEX

Let us briefly review the HLA complex. The HLA complex is located on the short arm of the human sixth chromosome and includes one to two dozen genes. Because the genes are tightly linked, recombination is uncommon. Consequently, an individual's two HLA complexes (haplotypes) are almost always inherited intact from the two parents. Moreover, the genes are highly polymorphic (have multiple alleles) so that there is little chance that the two haplotypes will contain precisely the same group of alleles. Rather, they typically differ at one or more loci. The haplotypes are inherited in normal mendelian fashion given that each parent has two different haplotypes which we may call 1 and 2 in one parent, 3 and 4 in the other parent - these may be inherited in four different ways: 1/3, 1/4, 2/3, 2/4. Two siblings will inherit the same combination 25% of the time.

The HLA complex contains a number of closely linked genes whose products control a variety of functions especially concerned with the regulation of immune responses and the mediation of immunologic reactions. The complex consists of three regions denoted class I, class II, and class III.

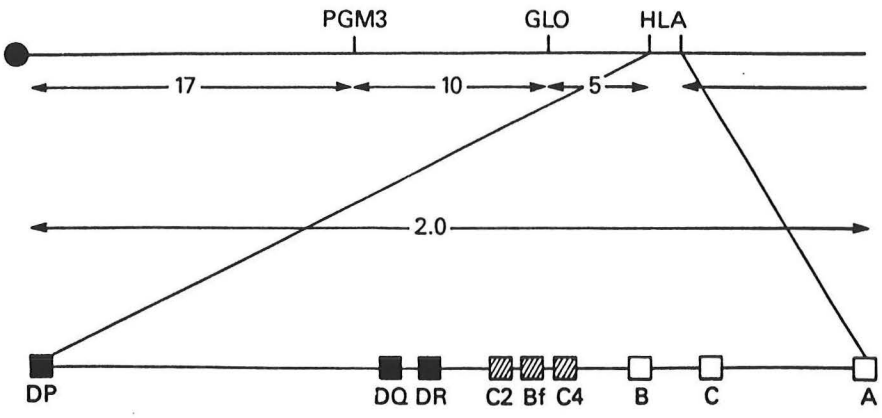

Figure 3. The human HLA complex.

The class I genes HLA-A, -B, and -C encode cell surface antigens that are expressed on all nucleated cells and act as restricting elements in the elimination of virally infected cells by cytotoxic T cells. The class I molecules are comprised of a glycosylated transmembrane 43 Kd polypeptide chain encoded by a class I gene in the HLA complex in association with beta 2 microglobulin, a 12 Kd invariant non-glycosylated polypeptide encoded by a gene on chromosome 15. All three types of interferon (alpha, beta, and gamma) enhance the level of expression of class I antigens.

The class II genes encode cell surface antigens expressed primarily on B lymphocytes, macrophages and dendritic cells all of which are involved in interaction with helper T lymphocytes during immune responses. They are arranged into at least three subregions: HLA-DP, -DQ, and -DR, each of which contain at least one alpha and one beta gene. The class II molecules contain an alpha and a beta chain which are transmembrane disulfide linked polypeptides of 33 and 28 Kd respectively. Class II production can be induced by gamma interferon and tumor necrosis factor (the TNF genes, alpha and beta interferon genes also map to the HLA complex). The class II map may vary in different haplotypes particularly in the DR region in terms of the number of DR beta chain genes. Most haplotypes have two expressed DR beta genes and one or more pseudogenes. The mixed lymphocyte reaction (MLR) detects differences in DR beta and/or DQ alpha or DQ beta, the determinants defined by the MLR are denoted D, those defined serologically are called DR or DQ.

The class III region contains genes for the serum complement component, C2 and factor B of the alternative complement pathway. Two genes for the complement protein C4A (C4A and C4B corresponding to the Rogers and Chido blood groups respectively), and two genes for cytochrome P450 21 hydroxylase (21OHA, and 21OHB) also map in the class III region.

Recently Strominger's group has completely physically linked the HLA-D region with HLA-B (Spies et al., 1989). A 600kb DNA segment from the class III region was overlapped with a series of cosmid clones TNFα, TNFβ, B144, and the major heat shock protein HSP70 were located. Additionally a cluster of genes, BAT-1-BAT-5 (HLA-B-associated transcripts) has been localized in the vicinity of TNFα. BAT6-BAT9 were mapped near C2 and HSP70. Thus, at present, 20 genes have been placed in the class III region. Although the functional properties of most of these genes are yet unknown, they may be involved in some aspects of immunity.

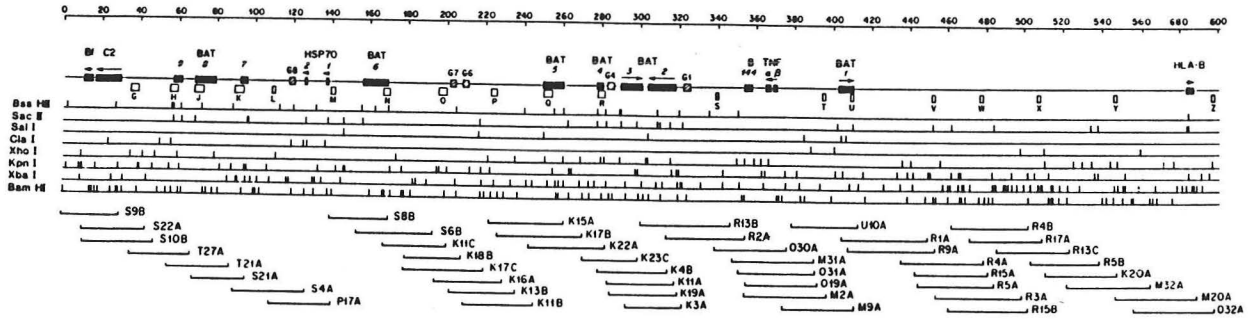

Figure 4. Molecular structure of the 600kb MHC interval between the complement gene cluster and HLA-B. Solid boxes refer to genes. Arrows show direction of gene transcription. From Spies, et al., 1989.

A distinctive feature of the HLA system is the high level of polymorphism exhibited by the class I and class II loci. By using serologic typing methods, at least 25 antigens have been defined for HLA-A, 50 for HLA-B, 10 for -C and 15 each for -DR, -DP, -DQ making this region far more polymorphic than any other known genetic system. The level of heterozygosity at each locus is very high. In no case is there a single very common allele at a locus. Rather there are quite a few alleles with relatively even frequencies. For the HLA-A, -B, -C, -DR, -DQ and -DP loci the distribution of allele frequencies at each locus is more even than and significantly different from the distribution expected under a model where alleles are selectively equivalent in their affect. Some form of selection to maintain variation, possibly of a frequency-dependent nature is the explanation most consistent with the level of genetic variation observed at these loci.

Non-random association (linkage disequilibrium) between certain combinations of alleles at different loci is another predominant feature of the HLA loci. For example, in Caucasians, the antigens A1, B8 and DR3 show significant nonrandom association. Other combinations of alleles at the HLA-A, -B, -C, and -DR loci which are relatively common and show non-random associations are A3, B7, DR2, and A2, Dw62, DR4. Linkage disequilibrium values and patterns that are consistent with past selection of events are observed in certain combinations of alleles.

C2, factor B, C4A and C4B and TNF exhibit polymorphism although to a somewhat less extent than do the class I and class II molecules but there are from four to ten alleles among these loci. The allele frequency distributions for the four complement loci do not show the relative evenness of frequency that is the hallmark of class I and class II loci and show no evidence of selection. Thus, they display evolutionary histories quite different from the class I and II loci despite their close linkage.

Within the last few years, all of the relevant genetic material in this entire chromosomal complex has been physically linked and has been subjected to nucleotide sequence ananlysis. The precise molecular architecture in the HLA region is now known. Almost all of the alleles have been sequenced at the DNA level. And therefore, the extent of polymorphism is fully appreciated. Serologic or cellular reactivities that correspond to these polymorphisms were described several years prior to our understanding of the molecular basis of the polymorphisms although a few new insights were gained by the molecular analysis.

Within the last three years the major new discoveries in this area relate to the widespread use of the polymerase chain reaction and the use of allele specific oligonucleotide probes to analyze polymorphisms in the HLA complex. The use of such allele specific oligonucleotides has had two significant impacts on the field. First it has allowed the widespread application of precise HLA typing in several laboratories independent of sera exchange which, while extraordinarily well done (perhaps better than in any other field in modern biology and medicine), is still more precise by the allele specific oligonucleotide method. Second, and perhaps more importantly, it has allowed the exceptions to be studied in great detail. Individuals, for example, who can not be typed by a defined allele specific probe may have a new allele. Thus their DNA can be amplified and sequenced in order to define the new allele at the molecular level. Thus more alleles than had been appreciated previously have been discovered.

The extensive structural information that has become available in the human HLA complex in the last five years has allowed an appreciation for disease susceptibility that had previously not been possible. Workers in this field have struggled to understand how in different population groups diseases could be "linked" (in reality "associated") with different HLA types. A good example of this is in Rheumatoid Arthritis where in caucasian Americans, the predominant HLA association is HLA DR4 Dw4 and DR4, Dw14 while in Japanese the association is with DR4 Dw15, and in Israelies RA is associated with DR4 Dw10. These seemingly irreconcilable

associations could be explained on two broad theoretical fronts. First the diseases could have separate etiologies. For example, three different viruses affecting three different populations that were present in three different parts of the world could manifest themselves through three different immune receptor molecules. However, since Israelies that move to the United States and Japenese that move to the United States continue to have associations in agreement with their group of origin rather than place of residence, has suggested to many that the disease has the same etiology in all three population groups. A more contemporary view is that a portion of an HLA structure is present in different molecules. This is probably a reflection of extensive gene conversion that has occurred in this complex throughout evolution. Some examples of this are illustrated in figure 5 below.

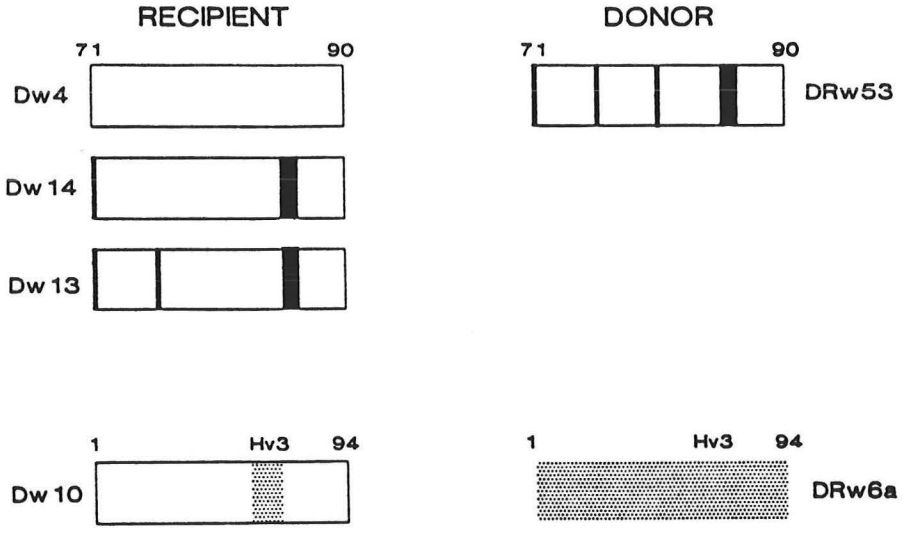

Figure 5. Patchy distribution of specific epitopes in different molecules associated with Rheumatoid Arthritis (Gregersen, et al., 1987).

These insights which have been gleaned from molecular structures suggest that it is not the entire HLA complex or indeed even the entire DR or DQ molecule or even a specific DR or DQ polypeptide chain but rather a small epitope within a particular chain that is responsible for susceptability/resistance. This epitope may occur in different haplotypes (indeed it is quite possible that the epitope could appear in different molecules). Thus, for example, it is conceivable that an epitope could occur in one population in the DR beta 1 chain and in a second population in the DQ beta 1 chain and in a third population in a DP beta 1 chain. The patchy epitope hypothesis explains (based on some newer understandings of roles of HLA class 2 molecules in peptide binding) the distinct genetic patterns of disease association in different population groups.

The fundamental problem of linkage disequilibrium needs to be addressed squarely in this context. Very few genetic loci in biology are subject to the kinds of linkage disequilibrium events that occur in the HLA complex. Thus, it was possible many years ago to appreciate that insulin dependent diabetes mellitus was associated with the HLA complex when all the typing that could be done was at the HLA A and B loci. Because of the associations (linkage disequilibrium) of certain HLA class I and class II genes it was possible by typing any of the class I loci that associations with class II loci were detected. While recombination between the class I and the class II loci is common in the population, it is infrequent enough in family studies to be inconsequential. However, linkage between DR and DQ is quite extraordinary and the preservation of DR and DQ associations is far beyond anything that we understand in modern genetics. Recombinations between these two loci have to my knowledge never been seen in a family study and even within the population recombination is extraordinarily low. Thus, for all practical purposes information gleaned about the genetics of IDDM at the DR locus is immediately transferable to the DQ locus from a genetic perspective. Mechanistically, however, one might be able to gain some increased understanding by appreciating which of the two loci was responsible and perhaps more importantly to allow one to distinguish subtleties of the epitope hypothesis. However, for the purposes of the discussions that we will have today, this distinction is irrelevant.

The mechanisms behind HLA disease associations are largely unknown, apart from C2 deficiency and congenital adrenal hyperplasia (21-OH deficiency). The latter has been shown to be due to a heterogenous collection of molecular defects some of which arise from a substantial deletion of DNA. However, like IDDM the vast majority of HLA associated diseases do not show simple Mendelian segregation, show incomplete penetrance, have possible involvement of multiple HLA predisposing alleles and non-HLA loci and disease pathogenesis and may be genetically heterogenous. The combination of these factors has made the identification of the disease producing factors and mechanisms difficult.

It is not surprising to find so many diseases associated with the HLA system given the direct involvement with many of the genes within this region with immune defense. Class I and II region genes can potentially control the immune responses at several levels. Possible mechanisms for direct involvement of the HLA molecules and disease associations include their roles as immune response genes, receptors for pathogentic organisms, viral modification of HLA antigens, molecular mimicry and interaction with non-immunological ligands. The high level of polymorphism of the HLA complex may be an adaptive response to provide greater protection against pathogens. The complement components could directly influence disease susceptibility given their role in the immune response.

V. THE RELEVANT ANIMAL MODELS

Much has been learned for two animal models that are surprisingly similar to human IDDM. Diabetes-prone BB rats develop spontaneous hyperglycemia and acute acidosis that is fatal unless treated with insulin. Many lines of evidence indicate that the disorder has an autoimmune pathogenesis. Lymphocytic infiltration of the islets of Langerhans (insulitis), selective destruction of insulin secreting beta cells and circulating islet autoantibodies are observed. The disease is

associated with a particular allele of the rat MHC and is prevented by immunosuppression, thymectomy, bone marrow transplantation, and lymphocyte transfusion. Con A activated spleen cells from acutely diabetic BB rats adoptively transfer diabetes to naive recipient rats and also have many abnormalities of cell mediated immunity including severe T cell lymphopenia. Histocompatible diabetes-resistant BB rats have also been developed. Less than 3 % of them develop spontaneous diabetes compared to 40 - 70 % of the diabetes-prone rats, and they appear to have an intact immune system with normal numbers of peripheal lymphocytes. The frequency of diabetes in these histocompatible diabetes-resistant BB rats can be substantially increased by treating them with low dose irradiation or cyclophosphamide.

The NOD (non obese diabetic) mouse spontaneously developes insulin dependent diabetes mellitus characterized by autoimmune insulitis involving lymphocytic infiltration around and into the islets followed by pancreatic beta cell destruction similar to human IDDM. Genetic analysis of breeding studies between NOD and normal mouse strains has demonstrated that two-three different recessive genes on two-three different chromosomes contribute to the development of insulitis. One of the genes is within to the major histocompatibility complex of the mouse. Several groups have demonstrated that they can prevent diabetes in NOD mice by anti-Ia monoclonal antibodies (antibodies to the murine class II molecules) and that protection can be transferred by splenic T cells. Unlike the BB rat, acute T cell lymphopenia is not observed in the NOD mouse. While it has been demonstrated that diabetes appears to be controlled by two to three independent genes or gene complexes one of which is linked to the MHC, the initiation of insulitis is determined by a single gene not linked to the MHC.

It is not my goal in these grand rounds to review these two animal models in detail but to simply point out that in the two relevant animal models of insulin dependent diabetes mellitus, which in both instances appears extraordinarily similar to the human condition, two fundamental observations are clear. 1) <u>In both animal models the evidence for an MHC linked gene is incontrovertible</u> and, 2) <u>in both animal models a minimum of two and probably three unlinked genes best explain the inheritance pattern of the disease</u>. Manipulations of the immune system in the BB rat and the NOD mouse provide considerable impetus to the notion that manipulations to the immune system in man are a potential theraputic target in attempts to prevent IDDM in the human population. In addition, the extensive genetic crosses that have been done between animals with high susceptibility to diabetes and normal animals would suggest a second and indeed third unlinked genetic locus has spurred considerable interest in seeking such additional genetic loci in man. Clues from these two model systems have suggested experiments in man that may make this less than a purely random analysis.

VI. OTHER GENETIC LOCI IMPLICATED IN IDDM

The search for susceptibility loci unlinked to HLA has been motivated by a variety of studies suggesting that HLA-linked predisposition does not explain the total familiarity of IDDM. For example, analysis of linkage between a single IDDM susceptibility locus and HLA resulted in estimates of the recombination frequencies that were unacceptably large given that IDDM-HLA population associations caused by linkage disequilibrium would require a tight linkage but such results could be explained by postulating a second susceptibility locus unlinked to but interacting with the HLA region locus. Similarly as I have emphasized throughout this presentation the proportion of affected sibpairs who shared zero HLA haplotypes is too large to be consistent with estimates of sib versus general population risk in IDDM. These findings support the existence of non-HLA familial factors. However, only very recently has there been some evidence indicating a location for possible non-HLA susceptibility loci.

A. Immunoglobulin Genes

Several investigators have reported data suggesting that genes in the immunoglobulin heavy chain region on chromosome 14q influence IDDM susceptibility in an HLA-dependent manner. For example, there appears to be a weak but significant increase in the frequency of Gm1 and Gm2 in HLA-DR3/DR4 diabetics compared with non-DR3/DR4 diabetics. However, the problem with such interaction studies is that the possibility of Gm X HLA associations in the normal populations have not been examined.

The association with Gm is quite controversial. Some investigators have immediately found the associations in their patient population while others have not. Field (1989) did an extensive sib pair analysis (some of which is shown in table I).

TABLE I

Sharing of Gm and HLA Haplotypes in 31 Affected Sib Pairs

No. of HLA haplotypes shared	No. of GM haplotypes shared			Average percentage of Gm haplotypes shared
	2 (%)	1 (%)	0 (%)	
A) 2	5 (26)	12 (63)	2 (11)	22/38 = 58%
1	3 (25)	5 (42)	4 (33)	11/24 = 46%
B) 2 (DR3/4)	5 (38)	8 (62)	0 (0)	18/26 = 69%
1, or 2 (not DR3/4)	3 (17)	9 (50)	6 (33)	15/36 = 42%

Table I shows the number of Gm haplotypes shared, and the average percentage of Gm haplotypes shared (expected = 50%), in 31 affected sib pairs broken down by A) number of HLA haplotypes shared and B) HLA-identical and DR3/4 vs. other. A previous study suggested that Gm haplotype sharing was increased in affected sib pairs who shared two HLA haplotypes (Field, et al., 1986b). The present data show a similar tendency, particularly if the two HLA haplotypes shared carry DR3 and DR4. Among HLA-identical DR3/4 pairs, the average percentage of Gm haplotypes shared is 69%, compared with 42% among other pairs ($x^2 = 4.6$, $P = .03$). These results are suggestive of a possible HLA-dependent Gm effect on susceptibility to IDDM.

B. Insulin Gene

It has also been suggested on the basis of association studies that the 5' region of the insulin gene on chromosome 11p contributes to IDDM susceptibility (Bell, et al., 1985). But, again, these results could be artifactual. To circumvent these problems, multiplex families with these non-HLA candidate genes were needed. Since sufficient family data typed for all regions of interest did not exist a recent international collaboration was undertaken to generate the data and a significant association between the 5' insulin region alleles and IDDM was reported. Thompson, et al. (1989) concluded that the class 1 allele of the polymorphic region 5' to the insulin gene was directly associated with IDDM. However, others have not been able to replicate these findings. Again Field (1989) did an extensive sib pair analysis. The frequencies of sharing two, one, or no INS haplotypes in 74 affected rib pairs (24%, 47%, 28%) conform closely to the frequency expected given random transmission of INS region genes (25%, 50%, 25%). Thus, these data provide no evidence that genes in the region of the INS locus directly influence susceptibility to IDDM. Dizier, et al (1989) and Cox and Spielman have recently come to the same conclusion (1989).

As an aside A clear cut syndrome of NIDDM is seen with defects of the <u>insulin receptor gene</u> (Terwari, et al., 1989). No such association with IDDM is expected but this has not been tested.

The mechanism behind any possible relationship between HLA and INS region susceptibility is unknown. What is known is that with the possibility of multiple susceptibility loci in the HLA region and with the addition of another susceptibility locus in either the Gm or the INS region we have moved deeper into the forest of complexity. Whatever other regions of the genome in addition to HLA Gm and INS are involved in predisposition to IDDM is still very much an open question.

C. The T Cell Receptor Genes

A critical site in the regulation of the immune response is the formation of the trimolecular complex between the T cell receptor, the class II molecule of the major histocompatibility complex, and antigen. Obviously investigators have studied all three members of this complex. I have detailed for you the implication of the class II major histocompatibility loci in insulin dependent diabetes mellitus. Here we will address the role of the T cell receptor genes.

T cell receptors recognize peptides primarily in the context of the appropriate "self" MHC molecules. This corecognition is termed MHC restriction. The repertoire of T cell receptors is generated by a "random" somatic recombination event. In the normal individual a fine balance is maintained to allow the immune system to distinguish between "self" and "non-self". Because the MHC molecules cannot make this distinction, the T cell provides some of the regulatory mechanisms that prevent self destruction. Such mechanisms are associated with tolerance induction.

We need not review here except in a cursory way the general structure of the T cell receptor as it has been covered in detail in these Medical Grand Rounds previously. There are two distinct T cell receptors: (1) the alpha-beta or "classic" or first T cell receptor and (2) the gamma-delta or "second" T cell receptor. Most of the mechanisms that are available to the immunoglobulin system for generating diversity (junctional diversity, N-region diversity, combinatorial diversity, etc.) occur in the T cell receptor. Somatic mutation however, does not occur in T cell receptors explaining why most T cell responses do not "mature".

In several experimentally induced autoimmune diseases antigens involved in the pathogenesis have been identified and characterized. For example, arthritis in the rat and mouse (native type two collagen in collagen-induced arthritis and mycobacterial heat shock protein in adjuvant arthritis). Secondly, experimental allergic thyroiditis in the mouse. (thyroglobulin), experimental allergic myesthesia gravis (acetylcholine receptor), and finally, experimental allergic encephalomyelitis (mouse and rat mylin basic protein).

In each of these experimental models the T cell receptor genes have been implicated in one form or another in the pathogenesis of the disease, that is, mice or rats that bear specific T cell receptor genes are more susceptible to disease than those with different T cell receptor genes. In these or other animal models each of the various chains have been implicated and there is now abundant evidence that specific immunointervention directed at specific V region genes of the T cell receptor ameliorate most of these diseases. Individuals working in these animal systems and in the human autoimmune diseases quickly turned to the T cell receptor genes hoping that they were the "second locus" in such diverse diseases as multiple sclerosis, rheumatoid arthritis, ankylosing spondylitis, and obviously insulin dependent diabetes. I should say at the onset that most of these studies have been terribly unrewarding.

It is not the intention to describe and review the genetic evidence implicating the T cell receptor in a number of animal models nor is it germain to review the evidence that the T cell receptor is involved in some human autoimmune diseases, as the data remains quite controversial. What I would like to do, however, is discuss the evidence for T cell receptor involvement in insulin dependent diabetes mellitus.

As I have presented at these medical grand rounds in preliminary form in the past, early studies were encouraging. When we had studied 100 patients with IDDM and when three other groups had studied 25 to 35 patients each with IDDM, there was a strong suggestion that polymorphisms in the beta chain of the human T cell receptor were implicated in IDDM. Since that time, three other laboratories have confirmed our initial observations. One laboratory has not been able to duplicate our analysis but we ourselves, having studied an additional 266 patients (to make a total of nearly 400 patients) have been unable to confirm a role for the T cell receptor beta chain complex in IDDM. Additionally, while several other laboratories have reported associations with the T cell receptor alpha chain in IDDM, we have been unable to confirm these findings in our own diabetic population. We and others have been bedeviled trying to understand the difference: the groups of diabetics, the HLA associations, the age of onset of disease, etc. without success. In the most powerful analysis of this kind that I am aware of (sib pair analysis), cosegregation of T cell receptor genes from any chain with IDDM have not been supportive. While several investigators at national meetings have reported such associations typically they were relatively small samples. And as more data was amassed, the results have been disappointing.

These results are extremely discouraging because as mentioned above in the relevant animal models there is a consensus that a second gene is involved in IDDM and in many instances the gene is involved in the formation of one of the two T cell receptors. Whether the analyses that have been done in the human T cell receptor vis a vis IDDM are flawed or there is simply no association remains open. Despite these reservations, I still believe that the T cell receptor plays a critical role in the pathogenesis of this disease. As such, studies are being conducted in our own laboratory which involve a full chromosomal linker scan in our twenty six multiplex families with IDDM in Dallas. Chromosomes 7 and 14 (which encode the T cell receptors) are the first candidate chromosomes we will analyze.

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