THE IMMUNOGENETICS OF INSULIN-DEPENDENT DIABETES

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SUMMARY

Insulin-dependent (type 1) diabetes mellitus (IDDM) is a multifactorial disease with a strong genetic component. The majority of the genetic component can be explained by associations between IDDM and genes in the major histocompatibility complex (MHC). The best single marker for IDDM is based on amino acid polymorphism of the HLA-DQ gene. Current evidence, however, indicates that the MHC susceptibility to IDDM is determined by a combination of HLA class I, II and III genes contained on HLA haplotypes. A non-MHC genetic component to IDDM also exists. To date, the most consistent association is between IDDM and markers of the insulin gene locus.

ENVIRONMENTAL FACTORS

Insulin-dependent (type 1) diabetes mellitus (IDDM) is a multifactorial disease with a strong genetic component. The pathological lesion is autoimmune destruction of the insulin-containing beta cells. The process is likely to be initiated by an environmental factor, perhaps as early as in intra-uterine life. This then leads to progressive beta cell destruction which can go on for 5–15 years before the first symptoms. The clinical presentation with symptoms of hyperglycaemia and/or diabetic ketoacidosis typically occurs in weeks; at this stage, approximately 80% of beta cells are destroyed. What is not known is whether the beta cell can recover from the early stages of destruction. As will be discussed later, there is evidence to indicate that some patients with non-insulin-dependent (type 2) diabetes mellitus (NIDDM) may have gone through an aborted 'type 1' process. What is clear, however, is that the majority with the genetic predisposition do not subsequently develop IDDM (see the monozygotic twin evidence below). In contrast to the rapid progress in identifying the genetic component of IDDM, the search for environmental factors has been enigmatic, with viruses, diet and toxins being implicated.[1] Evidence for viruses being pathogenic for IDDM is indirect except in rare instances of overwhelming viral infection. An interesting example is congenital rubella, which is associated with increased risk of mainly IDDM although NIDDM has also been reported. This *in utero* infection leads to IDDM with a similar age of onset and immunogenetic predisposition to idiopathic IDDM.[2] Examples of toxins include nitrosamines, pyriminil and pentamidine; however, these are again rare causes. Amongst the most frequently implicated dietary factors are dietary proteins, the current favourite being cow's milk.[3]

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THE GENETIC COMPONENT TO IDDM

Indirect evidence implicating genetic factors in IDDM comes from the study of monozygotic twins and family studies. IDDM clusters in families, although only 5% of diabetics have a family history of IDDM. Familial clustering is not proof of a shared genetic determinant as it may equally be due to common environment. In twin studies one identifies a monozygotic (identical) twin who has IDDM and then determines how frequently the co-twin also has the disease. The largest series comes from the studies of Leslie and Pyke who collected a series of 200 twin pairs in which the concordance for IDDM was 30%.[4] These studies are, however, limited as there are no large series of dizygotic (not necessarily identical) twins to act as environmental controls. Furthermore, some monozygotic twins share the same placenta and would, therefore, be exposed to the same intra-uterine environmental factors.

The main evidence for the genetic component to IDDM has come from the demonstration of an association of the disease with genes in the major histocompatibility complex (MHC). In particular, in families with more than one child with IDDM, the diabetic children share the same MHC genes more frequently than would be expected by chance.[5] The MHC is not the complete answer, however, as only 15% of MHC-identical siblings develop IDDM compared with 30% of genetically identical twins. This would, therefore, imply that genes outside the MHC also contribute to the genetics of IDDM.

THE MAJOR HISTOCOMPATIBILITY COMPLEX AND HLA ASSOCIATIONS

The MHC is located on the short arm of chromosome 6 in a region spanning 3500 kilobases (kb). There is little recombination within this region. This leads to the phenomenon of linkage disequilibrium, whereby combinations of alleles at different loci preferentially associate with each other; an example would be HLA-A1, -B8, -DR3, which are found in a higher frequency together (a so-called haplotype) than would be predicted by their individual gene frequencies. The MHC is subdivided into three main regions (Fig. 1). The class I region contains genes for HLA-A, -B, -C, -E, -F and -G genes. HLA-A, -B and -C are expressed on the surface of all nucleated cells. They are composed of three α chains and β_2-microglobulin (the latter coded for by a gene outside the MHC). Given a gene density of 1 in 25 kb in the class III and II regions it is likely that there are many more genes to be found in the class I region than have hereto been identified. The class III region is the most densely mapped region of the MHC. Some of the genes found in this region are those for tumour necrosis factors α and β, heat shock protein 70 (HSP70), complement C4, complement C2, 21-hydroxylase, properdin Bf and the so-called BAT genes. The class II region contains the genes for HLA-DR, -DQ, -DN, -DO and -DP, certain collagen genes and transporter associated peptides (TAP). Class II antigens are expressed on the surface of macrophages, B lymphocytes, activated T helper cells, monocytes, some epithelial cells and melanoma cells. There is a question whether in IDDM class II antigens are aberrantly expressed on the surface of islet beta cells.[6] The HLA class II antigens are comprised of an α and β chain encoded by separate genes, i.e the HLA-DQ molecule is coded for by a DQA1 and a DQB1 gene.

What is the function of HLA molecules? Foreign antigen is endocytosed by an antigen-presenting cell whereupon it is enzymatically degraded and then transported to the cell surface in association with an HLA molecule. At the cell surface an HLA molecule presents the antigen to a T cell receptor, triggering a sequence of events leading to the clearance of antigen and antigen-presenting cell. IDDM is thought to be an autoimmune disease triggered by either a foreign antigen or a self-antigen presented by the beta cell. The beta cell thus becomes the target of immune destruction. HLA associations with IDDM were first noted for the class I antigens HLA-A1-B8 and HLA-B15.[7] With the discovery of the class II antigens, closer associations were found between IDDM and HLA-DR3, -DR4, -DR3/DR4 and -DR2 (DR2 being protective) (Table I).[8–10] The best single markers for IDDM are in the HLA-DQ region and are identified by sequence variation of the gene coding for HLA-DQα (with arginine present in position 52) and HLA-DQβ (with an amino acid other than aspartate at position 57).[11,12] These IDDM-predisposing markers are identified using the polymerase chain reaction and allele-specific oligomer probes. No single marker is found in IDDM subjects throughout the world, although in Caucasoid populations strong associations are found with HLA-DQA1*0301 and HLA-DQB1*0302. There are also interesting differences within Europe, where the prevalence of IDDM ranges from 4.6 (northern Greece) to 42.9 (two regions in Finland) cases per 100 000 per year.[13] In many European populations the strongest HLA-DR association exists with the combination of HLA-DR3 and -DR4.[10] This has led to speculation that IDDM susceptibility molecules may be formed in *trans* (on the same haplotype) as well as *cis* (between haplotypes) configurations, a notion supported by experiments by Nepom who found DQ molecules formed from DR4 DQβ chains and DR3 DQα chains in DR3/DR4 subjects.[14] However in Finland, which has the highest incidence of IDDM in the world, DR3/DR4 is found in only 21.5% of diabetics compared with 4% of controls.[15] This is probably explained by the lower frequency of HLA-DR3 in the background population, and the fact that heterozygote combinations are not so important in this ethnic group in which susceptibility to IDDM is encoded for by genes on a single MHC haplotype.

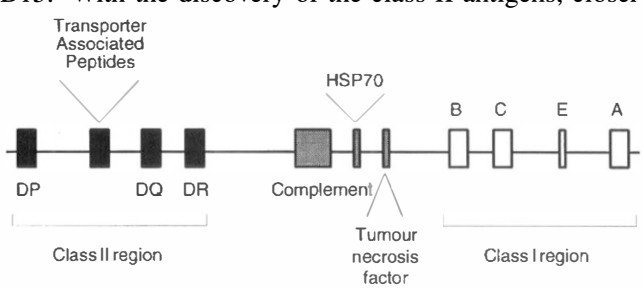

Fig. 1. The human major histocompability complex (3500 kilobases).

Although the study of single-locus HLA markers has provided valuable information at the population level and led to the speculation that the DQ region may encode molecules directly involved in IDDM, for some years it has been apparent that more information is to be derived from the study of whole MHC haplotypes (i.e. combining markers from the class I, II and III regions).[16] A recent example comes from the Finnish DiMe study. Tuomilehto-Wolf and colleagues identified all newly diagnosed cases of IDDM in Finland over a 3-year period; 758 families were recruited into a genetic study.[17] It rapidly emerged that an individual's risk of IDDM was predicted better by single MHC haplotypes than by single markers, including those in the DQ region. These haplotypes have widely varying absolute risks for IDDM, with many possessing the high-risk DQ combinations (Table II).[18] The highest absolute risk of 218/100 000 per year is in individuals carrying the

Table I. HLA-DR phenotype frequencies in IDDM probands and healthy controls (Barts Windsor Study 1983)

HLA-DR antigen	Phenotype frequency (%) IDDM (n = 122)	Controls (n = 110)	Relative risk	95% confidence limits
HLA-DR2	4	28	0.1	0.05–0.3
HLA-DR3	70.5	31.8	5.0	2.9–8.6
HLA-DR4	77.9	33.6	6.8	3.8–12.1
HLA-DR3/DR4	56.5	6.4	14.3	6.2–32.4

Table II. HLA-related haplotypes all characterised by high-risk DQA1 and DQB1 combinations and their absolute risk for IDDM in Finland[18]

HLA haplotype						Haplotype absolute risk (per 100 000 per year)
A	Cw	B	DR	DQA1	DQB1	
2	4	35	4	Arg 52[a]	Non Asp 57[b]	35
1	7	8	3	Arg 52	Non Asp 57	68
3	7	8	3	Arg 52	Non Asp 57	85
2	7	8	3	Arg 52	Non Asp 57	103
3	3	62	4	Arg 52	Non Asp 57	130
24	7	39	4	Arg 52	Non Asp 57	166
2	3	62	4	Arg 52	Non Asp 57	196
2	1	56	4	Arg 52	Non Asp 57	218

[a] Arg 52, an arginine at position 52 of the DQα molecule.
[b] Non Asp 57, an amino acid other than aspartate at position 57 of the DQβ molecule.

haplotype A2-Cwl-Bw56-DR4, whereas another DR4 DQB1*0302 haplotype, A2-Cw4-B35-DR4, which is identical at DQ, has an absolute risk of only 35/100 000 – similar to the background population. In an individual, therefore, the risk of IDDM is determined by the combination of HLA-A, -B, and -C alleles together with the high-risk DR and DQ alleles.

What are the possible genes on the MHC haplotype which are directly involved in the pathogenesis of IDDM? A central role for HLA-DQ molecules in the pathogenesis of IDDM has been hypothesised and backed up by some experimental data. The three-dimensional structure of the HLA class II molecule has been deduced from studies of HLA class I molecules.[19] The floor of the antigen-presenting groove is made of β pleated sheet and the walls of two α helices. In DQ molecules, whilst the β pleated sheet is encoded by both DQA1 and DQB1 genes, the two α helices are encoded separately by DQA1 and DQB1. Amino acid substitutions in the groove due to nucleotide substitutions in the DQA1 and DQB1 genes probably lead to a change in conformation in the antigen-binding groove, thereby affecting antigen recognition and hence the susceptibility to IDDM. Tumour necrosis factor (TNF) has an important role in defence against viruses and is also known to regulate expression of HLA molecules. Subjects with certain MHC alleles are known to secrete different levels of TNFα and TNFβ (unpublished observations).[20,21] Class I molecules could also have a central role in IDDM pathogenesis through their interaction with CD8 cytotoxic T cells. Foulis and colleagues, by studying autopsy pancreases, have noted that in IDDM there is marked hyperexpression of HLA class I molecules which precedes the HLA class II expression.[22] Additional evidence comes from the pancreatic transplantation studies of Sutherland and colleagues in identical twins discordant for IDDM.[23] The twins were selected if the non-diabetic twin had remained so for at least 30 years after the co-twin had developed IDDM, and therefore was most unlikely ever to develop the disease. They transplanted pancreatic tissue from the healthy twin to the diabetic co-twin; however, the previously healthy islet tissue was immediately rejected. Pancreatic histology revealed a heavy lymphocytic infiltration of CD8 (cytotoxic) rather than CD4 (helper) positive cells in the transplanted islets. These transplantation experiments would, therefore, favour a HLA class I restricted process.

Are the HLA associations in diabetes seen only in IDDM? HLA associations have been found in a number of different types of diabetes, including gestational diabetes, fibrocalculous pancreatic diabetes and even in some cases of NIDDM.[24] As these types of diabetes are not characterised by insulin dependence and ketoacidosis, beta cell destruction must have been halted and the diabetes developed after a secondary insult. Thus in the case of fibrocalculous pancreatic diabetes, the HLA predisposition by itself may have led to limited beta cell destruction but not sufficient to cause diabetes.[25] However, with additional pancreatic damage due to chronic pancreatitis, diabetes results.

NON-MHC GENES

The evidence for the involvement of non-MHC genes in IDDM has been discussed previously. In total, the non-MHC genes probably account for approximately 40% of the genetic contribution to IDDM.[26] Of the many candidates tested, the only consistent association is between IDDM and polymorphism in and around the insulin gene on the short arm of chromosome 11. Between 1984 and 1985 several studies demonstrated an association between IDDM and the short class I allele in the 5′ flanking region of the insulin gene.[27,28] This association has recently been re-studied and confirmed using intragenic insulin gene polymorphisms.[29] In the United Kingdom, the strength of the association is similar to that of HLA-DR4, with a relative risk of 4.9 (95% confidence limits 2.7–8.9).[28,30] The literature is less clear as to whether the IDDM-associated insulin gene alleles are also associated with HLA-DR4. Formal linkage of insulin alleles and IDDM is hard to prove because of the major association between HLA and IDDM. Nevertheless, several studies by Owerbach, Julier and Raffell and their respective colleagues have shown an increased frequency of insulin gene alleles in the families of IDDM probands.[29,31,32] The functional link between insulin gene polymorphism and IDDM is harder to understand. The possibilities include that of the primary association being with a locus in linkage disequilibrium with the insulin gene markers and the product of this locus playing a role in IDDM pathogenesis.[30] The alternative hypothesis would be a direct effect related to the insulin gene itself.

Associations of IDDM have also been noted with T cell receptor β chain and immunoglobulin heavy chain polymorphisms, although the results of studies have not been consistent.[33–37] More recent studies might indicate that these represent *a priori* associations with microangiopathic complications. A problem of study design in many of these experiments is that they do not have sufficient power to discriminate an association with IDDM *per se* from an independent association with the complication being studied. We have adopted an alternative approach by studying a NIDDM population in which the genes being studied seem highly unlikely to be involved in NIDDM

pathogenesis *per se*. Associations in South Indian NIDDM subjects were found between proliferative retinopathy and IgA heavy chain gene polymorphism.[38] Similar associations have previously been reported in IDDM subjects, thus confirming that for this locus the primary association is probably with retinopathy. With regard to the T cell receptor, Patel and colleagues suggest that the primary association may be between retinopathy and the aldose reductase gene found on the same chromosome as the gene for the β chain of the T cell receptor.[39]

Key words: Genetics, HLA, Insulin-dependent diabetes mellitus, Insulin gene.

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