DIABETES MELLITUS TYPE I THE PRESENT AND THE FUTURE

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RESUMO

Diabetes Mellitus tipo I: o Presente e o Futuro

A Diabetes Mellitus Insulino Dependente (DID) é uma doença autoimune específica de órgão, resultante da destruição das células B. pancreáticas, mediada pelas células T. São revistos conhecimentos actuais da imunogenética da DID relacionados com o sistema HLA, particularmente os referentes ao polimorfismo da região HLA-D e susceptibilidade genética para a DID. São abordados aspectos de intervenção terapêutica no processo autoimune.

SUMMARY

Insulin dependent diabetes mellitus: The present and the future

Insulin dependent diabetes is an organ specific immunologic disease caused by pancreatic B cell destruction, mediated by T lymphocites Present knowledge about insulin dependent diabetes immunogenetics and their relationship with the HLA system is reviewed, highlighting the polymorphism of the D area of that system and its connectors with diabetes. Possible therapeutic interventions in the autoimmune destructive process are discussed.

Diabetes Mellitus Type I or insulin dependent diabetes (IDD) is one of the most important public health problems in the developed nations. Although not as common as several other disorders, its demanding treatment and multiple and serious complications put a heavy burden on public health systems. In the United States alone, it is responsible for millions of hospitalization days annualy and the cost to the nation runs into many billions of dollares. The fact that it disrupts, often seriously, the capabilities of young individuals at an age when they would be expected to perform at their peak is particularly tragic. Because of this substantial impact on society, there is a great deal of ongoing research attempting to enhance our understanding of its causes and to develop more efficient and convenient treatments.

In this review we will summarize the results of some of these efforts.

PATHOGENESIS OF IDD

Studies conducted during this decade have indicated that insulin-dependent diabetes (IDD) is an organ-specific autoimmune disease with a genetic basis, although is not yet clear which model predicts better the inherited susceptibility[1]. IDD affects about 0,5% of most Caucasian populations, it usually appears before age 20, and is likely the result of a T cell mediated autoimmune destruction of insulin-producing beta cells in the pancreas[2]. Since the finding that certain histocompatibility antigens (HLA — human leukocyte antigens —, or MHC — membrane histocompatibility complex —) were associated with IDD[3,4] and other diseases, immunogeneticists have devoted most of their time to dissect the HLA-disease relationship. Studies of IDD should be framed within the general concept of «organ-specific autoimmunity» and any theory aimed at explaining the genetics of IDD will probably be relevant to other HLA — linked autoimmune diseases. The availability of modern molecular biology methodologies has made possible to approach some intriguing aspects of the immunogenetics of IDD, particularly those related to the HLA system.

1. *The HLA System*

Here we present a review of the current knowledge of the immunogenetics of IDD, with particular emphasis on the HLA-D region encoded genetic polymorphisms. The HLA contribution to genetic susceptibility to IDD is at least 50--75%, considering the new molecular biology findings to be discussed here.

The HLA System comprises, at least, four different regions in the short arm of chromosome 6 (HLA-A, -B, -C, and D) in man. Each of these regions is constituted by several highly polymorphic loci that are in close linkage disequilibrium. The HLA-encoded molecules are membrane glycoproteins grouped under the so-called Membrane Histocompatibility Complex (MHC).

The MHC products are divided in Class I (A, B and C regions) and Class II (HLA-D encoded) molecules. Unlike class I molecules, class II molecules are only expressed by certain cells (specially macrophages and B lymphocytes), which share an antigen-presenting role in the immune system.

The Class II molecules are heterodimeric structures, with two chains of different molecular weight, non-covalently associated[5]. Although the secondary and terciary structures of these molecules are not known, it is thought that the NH2 terminal domains of these chains contribute to form a molecular cleft with antigenic-binding properties.

In general, class I MHC molecules participate in the specific self-restricted recognition of self or foreign epitopes by antigen-specific cytotoxic lymphocytes, and class II molecules in the self-restricted recognition of antigenes by T helper lymphocytes. Thus, Class II MHC molecules bind antigenic peptide fragments and present them to T lymphocytes[6-9]. Although this receptor function is relatively nonspecific, it is not universal. Class II MHC molecules might also be actively involved in transduction processes leading to transcription, synthesis and secretion of Interleukin-l (IL1) during ag/MHC recognition by T helper cells[10]. Indeed class II polymorphism accounts for interindividual differences in LPS--induced IL1 and TNF secretion by human monocytes[11].

The HLA-D region of the Human Histocompatibility Complex is a highly polymorphic system constituted by 14 loci grouped in 6 different subregions (DR, DQ, DP, DX, DO, DZ)[12,13]. The DR subregion contains 3 beta (I, II, and III) and one alpha loci. The beta I and alpha combine to

define the serologically determined (by means of alloantisera and MoAbs) allotypes DR1 through DRw18. The DR beta III locus, in linkage disequilibrium with DR beta I locus, and alpha locus define the DRw52 and DRw53 serotypes, which associate with different DR allotypes. The DR alpha locus is not polymorphic. The DQ subregion contains an alpha and a beta loci, the combination of which gives rise to four serologically determined allotypes (DQw1 through DQw3, and a blank specificity, DQw blank). The chains encoded by both the alpha and beta loci are polymorphic. The DX genes are not expressed and the DO (beta) and DZ (alpha) loci are only expressed at low levels. The DP subregion contains two alpha and two beta loci, although only one alpha and one beta loci are expressed, the combination of which determines the DP allotypes. DP antigens are characterized by the Primed Lymphocyte Typing (PLT test)[14]. The serologically determined DR specificities are supertypic to several cellularly-defined (Dw)-specificities (Mixed lymphocyte reaction, MLR)[14], in the sense that some DR alleles (i.e., DR2, DR4) can be split on the basis of certain Dw specificities[14]. However, most Dw cellular subtypes are defined by polymorphisms in both, DR and DQ loci.

The molecular cloning and sequencing of several HLA--DR, DQ, and DP alleles has revealed that their genetic polymorphisms are basically encoded in hypervariable nucleotide sequences located in the membrane — distal exons of the polymorphic loci (DR beta, DQ alpha and DQ beta, and DP beta)[13,15,24]. HLA-D allelic polymorphism can also be identified by Restriction Fragment Lenght Polymorphism analysis can also be identified by Restriction Fragment Lenght Polymorphism analysis (RFLP). This technique identifies allelic differences in specific endonuclease restriction sites which are in strong linkage disequilibrium with neighbouring nucleotide sequences encoding Class II allelic epitopes. An integrated system has been described which combines the use of exon-specific DR beta, DQ beta and DQ alpha cDNA probes with TaqI-digested genomic DNA targets, which identifies DR and DQ serologic specificities unambiguously at the genotypic level[13,25,26].

Finally, the development of the polymerase chain reaction (PCR)[27-30] has allowed the putative identification of nucleotide sequences encoding epitopes cellularly or serologically defined[31-37]. These molecular studies have shown that although almost half of the 95 amino acid residues of the first domain are polymorphic, most of this polymorphism is clustered into two to four hypervariable regions[38-43].

2. *HLA-D Polymorphism and the genetic susceptibility to IDD*

The understanding of the immunogenetics of IDD has paralleled that of the HLA system, particularly of the HLA-D region; the process is still unfolding. It also seems apparent that other immunologically-related, but HLA-unlinked genes, are important elements in the genetic susceptibility to develop IDD.

The first report associating HLA (HLA-B15) with diabetes was by Singal et al[3]. Later, Nerup et al[4] showed that this association, as well as a second association with B8, applied only to IDD. These associations were later confirmed by many other groups (reviewed in ref. 1). It soon became obvious that these single allelic specificities associated with IDD were in high linkage disequilibrium and were transmited all together (A1, B8 and A2, B15) as an haplotype (low frequency of recombination events during the meiotic crossover)[45-53]. Thomsen et al. (1975)[54] found that the strongest associations of diabetes with HLA were with DR/Dw alleles in the HLA-D region (DR3, DR4), which are in high linkage disequilibrium with the aforementioned Class I alleles (A1-B8-DR3; A2-B18-DR3; A2-B15-DR4)[44-53]. Thomsen et al. (1975)[54] also pointed out that DR3-DR4 heterozygotes had a higher risk for IDD than DR3 or DR4 homozygotes. The existence of a third susceptibility factor (DR1)[55-56] was later described. The understanding of the genetics of IDD has been further complicated by the finding of a negative association between diabetes and the DR2 allele[47,54].

Later on, utilizing cellular reagents, Bach et al. (1985)[58] showed that the HLA-D-associated susceptibility to IDD correlated better with Dw than with DR specificities (Tables 1 and 2). Thus, the positive DR4 association was secondary to an association with Dw4 subtype, and the negative DR2 association was secondary to a significant decrease in the presence of the Dw2 subtype among diabetics, in comparison with controls. PLT reagents prepared against DR2-Dw blank haplotypes of patients defined a new DR2-associated specificity (LdMN2 or Dw21)[59]. Most of the few DR2--positive patients tested were positive for this specificity[56,58].

The study of RFLP patterns by using DR beta[60,61] or DQ beta[62] specific probes showed the existence of heterogeneity in the genetic susceptibility to IDD within specific DR specificities, results that were compatible with the observations of Bach et al. (1985)[58] with respect to DR4 and DR2--associated Dw subtypes. Interestingly, the DQ beta RFLP pattern seen in Dw21 subjects was shown to be nearly identical to that of DR1-Dw1 positive cells[56]. Kim et al. (1985)[63], using a DQ beta probe, have demonstrated a polymorphism within DR4 in which two patterns, DQw3.1 and DQw3.2, can be recognized. The DQw3.1 included RFLPs known to be decreased in diabetes[60-62]. In contrast, the DQw3.2 includes a fragment increased in diabetes[60,62]. Segall and Barbosa (1987)[64] found evidence that the pattern DQW3.1, demonstrated by Kim et al. (1985)[63], appears almost exclusively in the B44-DR4-Dw4 haplotype. Interestingly, this haplotype seems decreased , or at least not increased, in diabetes[64], although most Dw4 haplotypes are increased. A positively associated DQ beta RF is found with other DR4-Dw4 haplotypes (associated with diabetes) as well as with DR4--Dw10 and other DR4 subtype haplotypes not associated with the disease[64]. In the Minnesota diabetic population one of the DR4 subtypes, DR4-Dw4, is positively associated with IDD in Dr4+ patients, while another subtype, DR4-Dw14 is not[58]. Since both subtypes are associated with DQw3.2 in normal controls and diabetics, two factors, a DQw3.2--associated and a DR4-Dw4-associated, might be involved in susceptibility[65].

Recently, Todd et al. (1987)[31] showed that genetic susceptibility to IDD was linked to DQ beta polymorphism rather than to DR/Dw allelic variations. They found that DQ beta chains associated with most of the IDD-associated DR/Dw haplotypes (DR4-DQw3.2, DR3-DQw2, DR1-DQw1.1, DR2-Dw21-DQw1.1, DRw13-Dw19-DQw1.19), encoded an amino acid different than asparagine in positive 57 of the molecule, as opposed to those haplotypes which provide resistance to IDD (DR2-Dw2-DQW1.2, DR2-Dw12--DQw1.12, DRw13-Dw18-DQw1.18, DR4-Dw4-DQw3.1, DR5-Dw5-DQw3.1). These results were concomitantly reported by Erlich and coworkers[32] and generated a great deal of interest. In a sample of IDD patients and normal controls[66], position 57 of the HLA-DQ beta chain was studied with respect to either aspartic acid residue (Asp) or non--Asp (Al, Val, ser) at that position. Non-asp/non-asp was present in 93% of IDD patients and 19% of controls, non--asp/asp in 6% and 46%, and asp/asp in 1% and 35%, respectively. These results provide an estimated relative risk for IDD of 107 in individuals who have the homozygous non--asp residue at position 57 of the HLA-DQ beta chain. However, the enthusiasm for these findings has been tempe-

red by the fact that HLA-associations with IDD do not depend exclusively on DQ beta position 57, but probably on its combinations with allelic variants in other polymorphic chains, as pointed out by Segall (1989)[65] and Sheehy (1989)[67]. Sheehy et al (1985)[65] found that for DR4 subjects, IDD is approximately equally associated with alleles of the DR beta 1 locus (Dw4 and Dw10) and the DQ beta 1 locus (DQw3.2), and that there is a significant statistical interaction between these DR and DQ alleles in IDD. Therefore, the only IDD-associated DR4 haplotypes were those carrying the IDD-associated alleles at both loci[68].

The molecular mechanism by which the amino acid at position 57 of the DQ beta gene product influences the susceptibility to diabetes is not known, but an attractive hypothesis has been proposed. Residue at position 57 could affect the conformation of the antigen-binding cleft at the NH2 terminal domain of the Class II molecules. This, in turn could affect; a) the recognition of specific antigens (in terms of affinity) by the Class II molecule or of the Ag/MHC complex by specific T cell receptors, and b) the selection of specific T cell receptor (TcR) variable genes in fetal development[31,32,68].

In addition to the HLA studies, twin studies have also shown that there is an important genetic component influencing the pathogenesis of IDD. Thirty to 50% of the identical twin pairs are concordant and this percentage rises to 75% when the twins carry the high susceptibility genotype DR3/DR4.

Although these studies have been rewarding and promise an eventual full understanding of genetic susceptibility to IDD they have had little impact on genetic counselling. This is still by and large based on empirical risks. In the United States the risk for siblings of diabetic children is 1-15% (depending on HLA sharing with the proband). Thus, the risk is considerably higher than in the general population (2 to 3 per thousand) but relatively small. The risk for children of diabetic parents is 5% or less when the mother has diabetes and 15% or more when the father is affected.

3. *Autoimmunity and Immune Intervention*

There is a large body of evidence showing that IDD is an autoimmune disease resulting in beta cell damage and failure with subsequent hyperglycemia. This knowledge has resulted in attempts to treat early cases with immunosuppression. Cyclosporine has been partially successful and others, (less toxic drugs such as nicotinamide) are currently being tested.

Prediabetics. Since it is now generally accepted that diabetes includes, at least in some cases, a pre-hyperglycemic stage in which a number of immune abnormalities can be documented, immune intervention might be much more efficacious during this period rather than after hyperglycemia emerges. Hyperglycemia presumably signals destruction of a critical level of beta cell mass with irreversible results in most cases. The fact that cyclosporine seemed more effective in the first four weeks after the onset of diagnosis of diabetes is compatible with this view. Indeed, as stated above, several types of immune intervention in the BB rat, e.g., cyclosporin, are effective as preventive rather than curative measures. However, in humans there has been an understandable reluctance to intervene with potentially toxic regimens in normoglycemic children, especially since the indicators of a prediabetic stage are not 100% reliable. For example, islet cell antibody positive children may convert to negative status and not develop diabetes. Nevertheless, a few relatively simple and low-toxicity protocols have been initiated in high risk islet cell antibody positive siblings of diabetic probands displaying low acute phase insulin secretion after stimulation with IV glucose as well as islet cell and insulin autoantibodies.

A study of the effect of diet, including manipulations of its protein content and insulin, in prediabetic children is also being contemplated at several institutions. In the BB rat a low protein diet has been reported to protect animals from diabetes. Prophylactic treatment of the NOD mouse with insulin before hyperglycemia develops is also effective in reducing the incidence of diabetes, and a similar approach might be attempted in humans. Finally, nicotinamide may also be useful in inducing remissions[70].

It is most important to stress that all these new experimental therapies are strictly research tools and should not be used in clinical practice at this stage. This is particularly true of cyclosporine which can produce serious and irreversible complications including renal disease, the most feared concommitant of IDD.

Microangiopathic complications of diabetes.

Much of the morbidity of IDD stems from the vascular lesions emerging in the eyes and kidneys of most diabetics after 15 to 25 years of disease. Although palliative treatments that can arrest or slow down these complications have been developed in the last 20 years, the ultimate goal remains the prevention of these complications. Animal studies and circumstantial evidence from human studies suggest that hyperglycemia is a major cause of these complications[71]. However, genetic predisposition is likely to be important at least in nephropathy[71,72].

Recently, we have had the opportunity to show that heredity renders some diabetics susceptible to diabetic kidney disease. Concordance rates for diabetic nephropathy were determined in sibships where both proband and sibling(s) had diabetes. The concordance rate in a sibship group where the proband was free of diabetic nephropathy (urinary albumin rate$<$30 mg, day) was then compared to the concordance rate in a sibship group where the proband had required kidney transplantation as treatment of diabetic nephropathy. We found evidence of nephropathy in 17% of the diabetic siblings of probands free of diabetic nephropathy ($n=12$) and in 83% of the diabetic siblings of probands with diabetic nephropathy ($n=29$, $p<0.001$). No significant differences were noted between the sibling groups with respect to duration of diabetes or mean arterial pressure (18 ± 4 years and 97 ± 3 mmHg in the siblings of the probands free of diabetic nephropathy versus 22 ± 2 years and 95 ± 3 mmHg in the siblings of probands with diabetic nephropathy), although hemoglobin A_{1c} was lower in the siblings of probands free of diabetic nephropathy ($8.4\%\pm0.5\%$ versus $9.2\%\pm0.4\%$). Our observations suggest that diabetic nephropathy occurs in familial clusters, evidence consistent with the hypothesis that genetic (or familial) factors are important in susceptibility to diabetic nephropathy[72].

Extensive clinical trials are currently being conducted in the United States to determine the role of the dysmetabolism of IDD in microangiopathy. One of these trials, conducted by one of us (JB) at the University of Minnesota,is already 11 years old and is slated for completion in late 1990[73]. The other, called Diabetes Complication and Control Trial (DCCT) will not be completed for several years.

It is hoped that these long, demanding and very expensive studies will answer some of our major questions, namely, is hyperglycemia the major cause of microangiopathy, and if so, how soon and how close to normal glycemic control has to be implemented in order to minimize or prevent complications? Until this knowledge obtains, one must encourage patients to adhere to the currently available demanding diabetic treatment and to attempt the best metabolic control safely achievable.

The Future

For the next few decades one can confidently predict that our understanding of the autoimmune process in IDD will lead to forms of prevention perhaps applicable to the large majority of individuals at risk and thereby prevent IDD at least to some extent.

Further, it is likely that implantable devices (pumps or other approaches) will make the treatment of IDD far more efficient and also less demanding on the patient. The truly efficient approach will have to involve a «closed loop» system, thereby bypassing the daily participation of the patient in his own care.

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