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Editorial

Insulin-dependent diabetes mellitus: A multistep autoimmune disease with multigene dependence

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Abbreviations: ARG, arginine; ASO, allele specific oligonucleotide; ASP, aspartic acid; CTL, cytolytic T lymphocytes; HSP-65, heath shock protein 65; ICA, islet cell antibodies; ICSA, islet cell surface antibodies; IDDM, insulin-dependent diabetes mellitus; LMP, low molecular weight proteins; NOD, non-obese diabetic; PCR, polymerase chain reaction; TAP, transporter associated with antigen processing.

Insulin-dependent diabetes mellitus, or type I diabetes, is an autoimmune disease characterized by progressive destruction of insulin-secreting pancreatic β-cells leading to impairment of glucose metabolism. IDDM is not an acute process and first clinical signs appear relatively late in the history of the disease, at a stage in which, unfortunately, the anatomical damage of the organ is irreversible. A major histopathologic finding in IDDM is the presence of lymphocytes and monocytes infiltrating the islets of Langerhans [1]. Islet anatomic damage and the progressive loss of β cell function is attributed to the action of these cells and/or their specific products.

The multistep autoimmune aggression is indicated by the pancreas which becomes progressively invaded by blood-derived mononuclear cells, mainly T lymphocytes. However, these cells may remain outside the islets for variable periods of time depending on the severity of the disease. This event is designated as «perinsulitis». Only in a second phase the infiltration among β-cells becomes histologically apparent and the process rapidly progresses towards an «insulitis» with focal destruction of the islets which cannot be regenerated [2]. When the vast majority of pancreatic β-cells are destroyed, alteration of glucose-linked metabolism generates the classical clinical signs of IDDM, including polyuria, polydipsia, weight loss, fatigue and visual disturbances.

A few important laboratory findings can mark the pre-clinical stage di IDDM. Among these, antibodies against both cytoplasmic and cell surface components of β-cells are frequently found (70-80% of subjects), although their biological role in the pathogenesis of IDDM is still uncertain [1]. Nevertheless, anti-islets antibodies are considered invaluable tools for diagnostic and prognostic purposes because only few individuals lacking these antibodies develop frank IDDM.

The etiology of IDDM is certainly multifactorial involving both environmental and genetic factors. Many forms of virus infections, particularly congenital rubella and Coxackie virus infections, have been implicated as triggering events of the autoimmune process. Diet, certain drugs, and climate conditions have also been advocated as additional factors to explain particularly high incidences of IDDM in certain ethnic groups. The apparent difficulty in defining a specific agent as the major etiologic factor and the growing evidence of the association between IDDM and certain alleles of the HLA class II region have contributed to focus the interest of many investigators, particularly immunologists, on the genetic aspects of this disease.

HLA class II region encompasses about 1200 kilobases of DNA and includes at least three subregions, designated DR, DP and DQ each containing at least a pair of expressable alpha (A) and beta (B) genes whose highly polymorphic products are assembled as an α-β heterodimer and exported to the cell surface. In physiological conditions only a restricted number of cell types can express the class II genes [3]. Subjects which are DR3 and/or DR4 are

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the ones most frequently found among diabetic patients [4]. Further studies have demonstrated that it is the allelism at the DQ, more than at the DR, locus to show the highest linkage to susceptibility [5].

In recent years, the introduction of very sophisticated molecular techniques such as the polymerase chain reaction combined with the possibility to synthesize allele specific oligonucleotide probes, has allowed typing of HLA alleles at genomic level without the need of time consuming, often approximate, serological typing [6]. Further improvements of ASO typing have been recently described which render it simpler and technically amenable to clinical routine HLA molecular typing [7]. By PCR and ASO typing it has been possible to assess that the presence of Aspartic Acid in position 57 of the DQ beta chain was positively associated to resistance whereas a non-ASP residue in the same position was associated to susceptibility [8,9]. After preliminary findings suggesting that ASP residue in itself was protective even in heterozygous conditions [9], it became apparent that this was not the case. Indeed, in the Caucasoid population up to 30-40% of IDDM patients were ASP/non-ASP heterozygous. Moreover, the non-ASP susceptible alleles could also vary among Caucasoid groups being in certain statistics more frequent the DQB1 0302 [9], and in others the DQB1 0201 [10].

Because of the heterodimeric nature of the functional class II molecules, investigators have recently begun to study the DQA1 locus to assess, as for the DQB1 locus, whether a particular allelic distribution existed in IDDM patients. It has been observed that the presence of an Arginine residue in position 52 of the DQA1 alpha chain is indeed associated to susceptibility [11]. As was found for non-ASP/non-ASP situation in the DQB1 beta chain, the association between homozygous ARG/ARG condition and IDDM was statistically significant. However, the non-ARG/non-ARG condition was not protective, as was the ASP/ASP [12]. Moreover we recently found (Tosi G., et al., manuscript in preparation) that 30-40% of IDDM patients were ARG/non-ARG heterozygous; interestingly, the large majority of patients heterozygous for either DQA1-position 52 or DQB1-position 57 presented an homozygous susceptible allele at the DQB1 or DQA1 locus, respectively. Thus, in this group of IDDM patients at least one «susceptible» α-β heterodimer could always be formed. The fundamental role played by the HLA class II molecules in the homeostasis of the immune system, namely as receptors for antigenic peptides to be presented to regulatory T helper cells, suggests that the observed genetic linkage of IDDM to HLA class II DQ locus may result from a direct participation of DQ molecules in the presentation of autologous peptides to autoreactive T cells. An alternative hypothesis has been recently proposed to account for both the supposed recessive behavior of IDDM-predisposing DQ alleles (a concept that should be reassessed in the light of the results mentioned above) and the low representation of DQ-restricted T cells in the periphery. Following this hypothesis DQ molecules could play an important role in shaping the repertoire of T-cell receptors during thymic maturation. The presence of certain DQ alleles could be instrumental in deleting a higher number of T cell clones with DQ-restricted specificities. As a consequence, the selected T cell repertoire will compensate with a higher number of DR and/or DP restricted T-cell clones among which the tendency toward autoreactivity can be overrepresented [13]. To date, however, no strong functional or biochemical evidence for the role either in antigen presentation in the periphery or in intrathymic repertoire selection has been obtained.

A different hypothesis which considers the DQ locus associated with IDDM not because of its products but because of linkage disequilibrium with other loci, can also be formulated. Within this framework likely candidates could be the newly described, relatively non polymorphic, HLA loci between DP and DQ subregions, designated TAP-1 and TAP-2 (transporter associated with antigen processing) and LMP-2 and LMP-7 (low molecular weight proteins). The products of these genes seem to be involved in the intracellular protein degradation (LMP-2 and LMP-7) [14] and transport of peptides (TAP-1 and TAP-2) [15] to intracellular components where peptides can be bound by HLA class I molecules. Given the importance of HLA class I molecules in presenting intracellular peptides to CTL, quantitative and/or qualitative alterations in the intracellular degradation and transport of potentially antigenic peptides could result in activation of autoreactive CTL and subsequent immunologically mediated tissue damage.

However, the HLA class II genotype is not sufficient to fully explain the genetic susceptibility to IDDM. Indeed even in the case of monozygotic twins the concordance for risk of disease is only 40-50%. In other siblings this values drops 10-13% [4]. Therefore, there must be other genetic elements non-MHC linked which play a role in the susceptibility phenotype. An extensive genetic study has been recently performed by several groups in the «NOD» (non-obese diabetic) mouse, which spontaneously develops, particularly in the female, a disease closely resembling the IDDM in man. In addition to the known and strongest genetic linkage to MHC, par-

ticularly related to onset and severity of IDDM [16], other loci in distinct chromosomes have been identified which contribute to the susceptibility trait (see table I). Some of these loci are involved in the early phases of the pathogenesis of IDDM, as predisposing for example to perinsulitis (Bcl-2-linked) [17], or to insulitis and early diabetes (Idd-3, Idd-4, Idd-5) [18,19]. None of these loci, however, is by itself sufficiently associated to overt diabetic state without the MHC susceptible trait (Idd-1).

Table I
Susceptibility loci involved in the pathogenesis of IDDM in NOD mice.

Pathological phase	Locus	Chromosome	Ref.
Perinsulitis	Bcl-2-linked	1	17
Insulitis	Idd-5	1	19
	Idd-3	3	18
	Idd-1	17	16
Diabetes	Idd-1	17	16
	Idd-3	3	18
	Idd-4	11	18
	Idd-5	1	19

Very little is known on the molecular nature of the autoantigen involved in the pathogenesis of IDDM in humans. Antibodies against a variety of antigens have been detected in IDDM patients including anti-insulin, anti-cytoplasmic islet cell antibodies, anti-64kd islet protein recently identified as the GABA-synthesizing enzyme glutamic acid decarboxylase, heath shock protein 65, etc. However, as outlined above, a clear pathogenetic effect has not been demonstrated in any of this cases [1]. Probably many of these antibodies are synthesized in response to antigenic material derived from the continuous destruction of β-cells and the consequent liberation in the extracellular space of intracellular proteins and thus are the result more than the cause of the disease.

The disease is primarily a T cell-mediated disease. The most abundant cellular type infiltrating the islet is constituted by $CD4^+$ T cells and, at least in the NOD mouse, the disease can be transferred by this cell subpopulation [20]. Regulatory $CD4^+$ T cells usually have an helper phenotype and are required for functional maturation of effector cells such as B cells and $CD8^+$ cytolytic T lymphocytes. It is generally believed that CTL are the major effector cells implicated in the destruction of pancreatic β-cells. However, the question is still open whether classical $CD8^+$ or more rare $CD4^+$ CTL are involved in the tissue damage [20,21]. Recently, Irun Cohen et al. have demonstrated that helper T-cell clones isolated from spleen cells of NOD mice and specific for certain epitopes of HSP-65, could both transfer the disease in normal mice and, interestingly, vaccinate susceptibile mice if introduced in inactivated form [22]. These findings strongly suggest a direct implication of $CD4^+$ T cells with specificity for an intracellular normal component, such as HSP-65, in the pathogenesis of IDDM in NOD mice and may suggest that equivalent self reactive T cell clones exist in man. Further support for this result was recently obtained by Nakano and coworkers [23] who showed that T-helper clones with specificities for distinct antigens of β cells could produce insulitis in $I\text{-}E^+$ (Eα-transgenic) NOD mice which never develop insulitis.

The apparent multiplicity of antigens involved in the pathogenesis of IDDM is also paralleled by the apparent lack of a restricted repertoire of T cell receptor Vα and Vβ genes which can be found in T cell clones infiltrating the pancreas of NOD mice [23,24].

The new and rapidly accumulating information on genetic and immunologic aspects of IDDM are providing a still complex but more rational scenario on the pathogenesis of the disease. Among the various possible hypotheses we would like to suggest the following scenario (see figure 1). Still elusive etiologic agents trigger, in a genetically predisposed individual, local recruitment of inflammatory cells. Initiation and maintenance of perinsulitis is probably dictated by a susceptible gene (or genes) distinct from those necessary for initiation and maintenance of insulitis «sensu stricto». Insulitis is therefore envisaged as a subsequent obligatory step toward overt diabetes. Insulitis, however, can remain clinically silent for months or years, or can even be arrested at this phase if HLA-linked susceptible genes are not present. If these are present, the antigenic peptides, as products of limited and aspecific tissue damage caused by the ongoing inflammatory process (in which macrophages and various interleukins play an important role), can effectively be presented to autoreactive T cells. The two necessary immunological conditions are thus the presence of a restricted element to which autologous peptides can bind with sufficient affinity, and a T-cell repertoire specific for pancreatic β-cell antigens that has not been deleted during thymic maturation. Specific β-cell destruction begins and is self-sustained by continuous recruitment and further proliferation of autoreactive T-cell clones with distinct antigenic

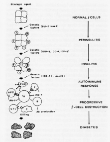

Figure 1. - An hypothetical model of the various steps involved in the pathogenesis of IDDM. For description of the model see the text.

specificities. Secretion of lymphokines and particularly of IFN-γ in the area of tissue damage certainly contributes to increase the expression of HLA class II genes in antigen-presenting cells. This population can be represented by canonical APC, but also by non canonical APC, for example by β cells themselves, that may acquire this capacity because of «de novo» expression of HLA class II genes under the effect of IFN-γ. The end result is a progressive, highly specific destruction of β cells and consequent clinically overt IDDM.

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