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Lecture

Type 1 diabetes mellitus as a polygenic multifactorial disease: immunopathogenic mechanisms of beta-cell destruction

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Type 1 diabetes mellitus is a multifactorial autoimmune disease for which susceptibility is determined by genetic, environmental and immunologic factors (1). Genetic studies have shown that this is a polygenic disease involving one major locus (IDDM1=HLA) and an undefined number of loci with minor effect (2). Among these, besides the IDDM2 (INS) locus that has been conclusively shown to be implicated in the disease, the strongest evidence so far available is that on the IDDM12 locus. This corresponds to a region homologous to that harboring the IDD5 locus in the mouse and it includes genes such as CTLA-4 and CD28 known to be involved in the regulation of the T-cell response to antigenic stimulation. In particular, a microsatellite on the 3' UTR region of CTLA-4 and a G-A dimorphism in the first exon of the gene have been shown to be both linked and associated with the disease.

Humoral and cellular autoimmunity in type 1 diabetes

A number of studies have shown that the destructive process in autoimmune diabetes specifically directed against islet β-cells, can last for years before the appearance of clinical symptoms and is characterized by the presence of circulating autoantibodies and of T-lymphocytes directed against islet molecules (3). The information so far available on the disease pathogenetic process has clearly indicated that the progressive autoimmune islet β-cell destruction is a T-lymphocyte-mediated event. However, despite the pathogenic importance of the T-cell response in type 1 diabetes, the target molecules of such a response remain largely uncharacterized, especially in humans. The autoantigens so far identified in autoimmune diabetes have been discovered starting from disease-associated autoantibodies that, although representing important tools for the prediction of future diabetes development, have no direct pathogenic role since they do not cause any islet damage (4). Interestingly, molecules such as insulin and GAD have been shown not only to be target of autoantibodies, but also of autoreactive T-lymphocytes both in man and in an animal model of autoimmune diabetes such as the NOD mouse. In addition to insulin and to GAD, molecules expressed in insulin secretory granules and in mitochondria with a molecular weight around 38kD may be important target autoantigens in the T-cell response associated with type 1 diabetes. As far as the pathogenetic role of T-lymphocytes in diabetes pathogenesis is concerned, it has been elucidated, at least in part, only in animal models of islet autoimmunity, while no data is so far available in man, due to the impossibility to isolate islet-infiltrating lymphocytes from human pancreas. For the same reason, the available information on the pancreatic islets at the disease onset in man is still rudimentary, with data obtained mainly at autopsy from patients who died soon after diagnosis. Consequently, much of what we know about the etiology and pathogenesis of autoimmune diabetes comes from studies on small animal models (such as the NOD mouse) which spontaneously develop a disease with many of the features of the human disorder. In the NOD mouse, a complex pathogenetic picture has

emerged (5), involving distinct phases and multiple lymphocyte subpopulations. It has been suggested that disease progression is regulated at two checkpoints: 1) at 3-5 weeks of age, islet infiltration (insulitis) begins; 2) at 12-15 weeks of age, active destruction of β-cells starts, leading rapidly to disease onset. As far as the immune-cells involved in diabetes pathogenesis is concerned, a consensus is emerging that CD8+ cells seem to initiate the process, while CD4+ lymphocytes have a major role in the islet destructive process, according to the results of adoptive transfer experiments. However, the role of other cells clearly present in the insulitis process, such as macrophages and dendritic cells, has to be determined: with regard to this, it has been proposed that potentially pathogenic CD4+ cells can interact with these Antigen Presenting Cells (APC) and, as a result of such interactions, activated T cells may directly kill the islet β-cells (e.g. through Fas/FasL interaction) or may activate the cytocidal functions of macrophages.

Being type 1 diabetes a T-cell mediated disorder, several studies have aimed at the characterization of the cell-mediated immune response in islet autoimmunity (6). Research protocols performed primarily in NOD mice have attempted to determine whether the repertoire of islet-infiltrating T cells exhibits Vα or Vβ restriction. To date, no sign of such a restriction has been found in islet-infiltrating lymphocytes; however, it is of interest to note that a Vα13 restriction has been reported in pathogenic CD4+ T-cell clones in the NOD mouse. These clones are directed against the insulin B-chain and display a novel Vα-sequence that was termed Vα13.3. In man, a preferential Vβ7 usage was detected in two cases, suggesting a potential role of an unidentified superantigen in disease pathogenesis. As far as the characterization of T-cell clones is concerned, a number of such clones have been isolated from islet-infiltrating T-cells in NOD mice. These clones have been shown to be directed either against insulin or GAD or carboxypeptidase-H, and in some cases are able to transfer disease in recipient animals. Usually, these are CD4+ clones with a pattern of cytokine production of Th1-cells (IL-2, IFNγ and TNFα).

Most investigators now agree that both CD4+ (helper) and CD8+ (cytotoxic) T-cells are required for maximum development of diabetes although B cells, macrophages and dendritic cells have important pathogenetic functions. In NOD mouse and BioBreeding (BB) rat, macrophages and/or dendritic cells are the first cell types to infiltrate the pancreatic islets. The presentation of β-cell autoantigens by macrophages and/or dendritic cells to CD4+ T-helper cells, in association with MHC class II molecules, is considered the initial step in the development of type 1 diabetes. Activated macrophages and CD4+ release cytokines, such as IL1-β, TNF-α and INF-γ, inducing the migration of CD8+ peripheral T-cells to the inflamed islets. The activated cytotoxic CD8+ T-cells then damage β-cell by releasing perforin, granzyme and by Fas-mediated apoptosis (7).

The relative importance of CD4+ and CD8+ T-cells in the pathogenesis of diabetes is still controversial; some authors have proposed that CD8+ are sufficient for priming and expansion of autoreactive CD4+ T-cells, while others have shown that cloned autoaggressive CD8+ T cells are able to cause diabetes by themselves (8).

Apoptosis and type 1 diabetes

Apoptosis or Programmed Cell Death is a highly regulated and evolutionary-conserved form of cell death, which plays a crucial role in the development and homeostasis of multicellular organisms (9). Dysregulated apoptosis has been shown to represent the basis of several diseases including cancer and autoimmune syndromes. In organ-specific autoimmune diseases the role of apoptosis is double, representing the mechanism of target cell death and being involved in the selection of T-lymphocytes, which are the mediators of autoimmune tissue destruction. Fas system is involved in the elimination of normal activated peripheral T-cells. Mature lymphocytes constitutively express Fas, activation by antigen induces Fas ligand (FasL) expression: Fas-FasL interaction determines the killing (by apoptosis) of Fas-expressing cells. Fas-mediated death plays an important regulatory role in autoimmune disorders of lpr (lymphoproliferation) and gld (generalized lymphoproliferative disease) mice that show, respectively, mutations in Fas and FasL genes. Initial findings hypothesized a fundamental ro-

le of death receptor Fas in β-cell destruction, confirmed by observation that NODlpr did not develop diabetes or insulitis, nor it was possible to transfer diabetes to NODlpr recipient.

Both necrosis and apoptosis have been proposed as cell death mechanisms in autoimmune diabetes, with the latter being a highly regulated process initiated by a variety of intra- and inter-cellular signals and involved in homeostasis of target cells as well as of immunocompetent cells. Regardless from the cell type involved, apoptosis is a multi-step process which starts with an extracellular death signal (induction phase), activating one or more signal transduction pathways (effector phase) that converge to few final death pathways (degradation phase). These final pathways are usually executed by proteases called caspases and by caspase-activated DNAse (CAD). Death receptors play a central role in apoptosis initiated by death signals (10); among such receptors, those belonging to the TNF receptor gene superfamily are the best characterized, and include Fas (CD95), TNF receptor 1 (TNFR1), death receptor 3 (DR3), DR4 and DR5. Once activated by their specific ligands, these receptors, through adapter molecules (FADD for Fas and TRADD for TNFR1), activate down-stream caspases that can determine the apoptotic death within hours. In addition to death receptors and their downstream caspases, other molecules, usually associated with mitochondria, are actively involved in apoptosis regulation. These include anti-apoptotic protein bcl-2 and pro-apoptotic bax, whose relative ratio has a profound influence on the commitment of a given cell to die or survive; furthermore, release of cytochrome-c from the mitochondria rapidly leads the cell to apoptotic death, since once in the cytoplasm, cytochrome-c forms an essential part of the "apoptosome" together with caspase-9 and Apaf-1. In autoimmune diseases, Fas- and TNFR-dependent pathways have been proposed as the main mechanisms of target cell death.

Cytokines and β-cell death

A great body of evidence (11) indicates that pancreatic β-cell destruction results from a disorder of immunoregulation and that cytokines secreted by infiltrating cells (CD4+ and CD8+ T lymphocytes, B lymphocytes, NK and macrophages) might have an important role in the pathogenesis in Type 1 diabetes. In NOD mouse, macrophages are the first inflammatory cells to infiltrate the islets, where they may engulf apoptotic β-cells. A current hypothesis is that the pathogenic immune response is mediated by a Th1 subset of T cells, whereas the protective response is mediated by a Th2 subset of T cells. Th1 and Th2 cells produce a different cytokine pattern. Th1 cells secrete IL-2, INF-γ and TNF-β and activate cell-mediated immunity, whereas Th2 cells secrete IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13 and stimulate humoral immunity. Many studies support the hypothesis that Th1-cytokines are β-cell destructive whereas Th2-cytokines protect β-cell by suppressing Th1-cytokines expression (12). As a matter of, both IL-4 and TGFβ, when transgenically expressed in the pancreatic β-cells of NOD mice, prevent autoimmune diabetes.

In addition, macrophages produce IL1(α and β), TNF-α and IFN-α that are referred as proinflammatory cytokines. Correlation has been observed between β-cell destructive insulitis and expression of proinflammatory cytokines. Several cytokines potentially involved in the pathogenesis of Type 1 diabetes have been found to be expressed in the insulitis lesion in NOD mouse, BB rat and man; although this observation does not directly prove the pathogenic role of these cytokines. It has been observed that, IL1-β, TNF-α and INF-γ, in combination, induce functional and morphological damage β-cells in both rodent and human islets, in a dose and time dependent manner.

One of the mechanisms proposed (13) is that, as observed in rodent islets, incubation of human islets with a combination of IL1-β+TNF-α+INF-γ determines up-regulation, in β-cells and macrophages, of the expression of inducible nitric oxide synthase (iNOS) and the subsequent synthesis of the radical nitric oxide (NO) as well as of other free radicals such as peroxynitrite ($ONOO^-$) and superoxide (O_2^-) that contribute to β-cell damage. Nitric oxide impairs β-cell function by blocking the enzyme aconitase and by inducing DNA strand breaks. Previous reports showed that NO could induce Fas expression on β-cells, so that, they might be destroyed by T-lymphocytes.

A recent study has shown that iNOS-deficient mouse islets, treated with a combination of IL1-β+ TNF-α + INF-γ, expressed Fas mRNA in response to cytokines similarly to wild-type islets and the murine NIT-1β-cell line. In addition, the accumulation of Fas-specific mRNA, in islets following exposure to proinflammatory cytokines, correlated directly with an increased surface expression of Fas comparably in wild-type, iNOS-deficient islets and NIT-1. This result shows that Fas mRNA transcription and Fas protein expression in murine islets are independent of NO production.

On the basis of the involvement of death receptors in β-cell destruction, several observations (14) have pointed out that TNF-α could have a diabetogenic role in the elimination of β-cell. TNF-α is an inflammatory cytokine principally secreted by macrophages and activated CD4+ T cells during autoimmune and infection diseases; it is able to induce cell death in many tumor cells and can modulate insulitis in NOD mouse.

A pivotal role of this cytokine in the disease process comes from several observations: TNF is produced in inflamed islet in NOD mouse; neutralizing anti-TNF monoclonal antibodies prevented both insulitis and disease; treatment with recombinant TNF (rTNF) had different effects, depending on age of NOD mouse. Administration of rTNF at birth accelerated disease onset while the same treatment, in adult, delayed it.

Similar results have been obtained by experiments performed with transgenic NOD mouse expressing TNF in pancreatic β-cells: the early expression of TNF-α induced an early disease onset while late expression of this cytokine resulted to be protective.

These findings show that TNF has complex effects on the development of the autoimmune response.

Several studies suggest that TNF is directly toxic to β-cells although, it seems that this cytokine potentiates its toxic effect in synergy with IL-1β and IFN-γ to β-cell elimination (15).

A recent report seems to clarify the role of TNF-α. The authors have observed that TNFR1-deficient NOD mouse developed insulitis and the proportion of CD4+ and CD8+ was similar to that in normal control NOD, but more importantly, progression to diabetes was completely abrogated. Transfer of spleen cells from diabetic normal control NOD mice into TNFR1-deficient recipient, significantly delayed diabetes and transfer of perforin-deficient NOD spleen cells into TNFR1-deficient recipients failed to induce diabetes.

These experiments would highlight the diabetogenic role of TNF-α/TNFR1 system that acting in synergism with perforin system is able to determine β-cell death. In addition, these findings argue against the involvement of Fas system in the beta cells elimination in Type 1 diabetes.

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