

THE ORIGIN OF TYPE 1 DIABETES: AN AUTOIMMUNE DISEASE?

C. BOITARD

Type 1 diabetes mellitus is an autoimmune disease targeted on the β cell. Its incidence is regularly increasing so that some countries like Finland are experiencing very high levels (50/10⁵ per year). It is a multifactorial disease characterised by the development of an abnormal immune response against beta cells of pancreatic islets of Langerhans, in a predisposed genetic background, which is barely identified. The hypothesis has been raised that environmental factors might trigger the disease. This is a highly multigenic disease. Autoimmune reaction is targeted against multiple auto-antigens expressed by β cells and mostly non specific of these cells. Several good animal models of this disease are available, such as the NOD mouse and the BB rat.

The origin of the disease raises two major questions. The first one is related to the respective role of Langerhans islet abnormalities and immune system abnormalities in its onset. The second one states that no environmental factors may really intervene in its onset. Such an hypothesis is opposed to the classical scheme of autoimmune diseases triggered by an environmental factor, responsible for the rupture of tolerance of lymphocytes towards self antigens expressed by a target, such as the β cell.

■ DIABETES: AN AUTOIMMUNE DISEASE

Transfer experiments performed in animal models have established that type 1 diabetes can be transferred to naive animals (for instance recipients with the NOD

genetic background but presenting with the SCID or RAG mutation and devoided of lymphocytes) by T lymphocytes from diabetic animals. Transfer is also possible with bone marrow lymphocytes or islet-infiltrating T lymphocytes. More recently, it has been shown that diabetes can be induced in naive NOD recipients by injecting cells lines of CD4⁺ T cells that are specific of different self-antigens expressed by β cells (glutamate decarboxylase, I-A2, insulin, HSP 65).

Other experiments has shown that autoimmune disease cannot be summarised by the activation of effector lymphocytes. Cotransfer experiments have demonstrated the existence of regulatory cells in non diabetic animals or in diabetes-prone animals, able to slow down the development of diabetes. Several kinds of regulatory cells have been identified, such as CD4⁺ T cells in the thymus or the spleen of non diabetic animals, particularly young NOD mice; once injected in irradiated NOD recipients or SCID NOD recipients, these cells can prevent the induction of diabetes by the transfer of spenic T cells procured from diabetic animals. In a recent model developed in collaboration with Docteur Philip AVNER (Institut Pasteur), we obtained congenic mice with the NOD background, but harboring different fragments of a small telomeric region of chromosome 6 from C3H mice. We observed that some of these congenic mice were partially protected from diabetes. Among two protected strains, we have demonstrated the presence of regulatory CD4⁺ T cells expressing either the α chain of the high affinity interleukin-2 receptor (CD25), or the adhesion molecule CD62L+. Different populations of regulatory cells able to block the development of diabetes have been defined in the NOD mouse model in addition to the previously mentioned CD4⁺ T cells, such as TNK cells and CD4⁺ Th2 cells. Agnès Lehuen has established that the defect in TNK cells in NOD mice was corrected in transgenic mice expressing the invariant α chain of T receptor in the NOD genetic background, which is characteristic of T NK cells. A correlation between the degree of T NK cell restoration and the degree of protection was observed in

 : C. Boitard, Unité 561, Hôpital St-Vincent de Paul, 82 avenue Denfert Rochereau, 75014 Paris.
e-mail: boitard@cochin.inserm.fr

Received : April 8, 2002

Unité 561, Hôpital St-Vincent de Paul, Paris.

ALFEDIAM Strasbourg February 27, 2002

these transgenic mice. Besides, it has been shown that the activation of T cells with the Th2 phenotype in the NOD mouse, characterised by the production of IL-4, IL-5, IL-6, IL-10 and IL-13, could protect against the development of the disease.

Data are now available in humans that indicate the existence of some of these regulatory cells at a low level among patients with recent onset type 1 diabetes, but also among patients with long-standing diabetes (CD4+, CD25+ T cells), as well as in relatives of type 1 diabetic patients (NK T cells). By contrast, the number of these cells is normal in non diabetic controls and in type 2 diabetic patients.

■ THE ONSET OF THE AUTOIMMUNE DISEASE

The main question of the mechanisms leading to the rupture of tolerance of lymphocytes towards antigens expressed by β cells during type 1 diabetes remains unresolved. The hypothesis of a primary islet abnormality or, on the other side, of a primary defect of the immune system, and the hypothesis of the role of an environmental factor triggering the disease are still unclear. Circulating T cells expressing a β -cell antigen-specific receptor can be detected in every individual. However, these cells are physiologically tolerant, i.e., they do not get activated against their specific target. Several experimental models have established that it was possible in normal animals to induce an autoimmune diabetes through modifications involving the immune system exclusively.

In a transgenic mouse model, Nora Sarvetnik was able to establish that the β -cell targeted expression of a cytokine gene, γ interferon, can lead to insulinitis an autoimmune diabetes mediated by β -cell antigen-specific T cells. She also has shown in an other model that the expression of the same transgene under the control of a different promotor, i.e., the promotor of the α chain of the acetylcholine receptor, did not induce diabetes but rather a myasthenia due to the overexpression of the γ -interferon gene in the neuromuscular junctions. Thus it is an exclusive defect of the immune system that triggers the autoimmune disease in this model.

Experiments performed in the NOD mouse have also demonstrated that disease could be linked with hematopoietic stem cells exclusively. C3H/HeN mice devoided of functional T cells (bearing the nude mutation) develop an autoimmune diabetes when they are reconstituted with NOD mouse bone marrow cells. On the other hand, it has been shown that NOD mice reconstituted with hematopoietic stem cells from murine strains not predisposed to diabetes (Balb/cnu/nou (NOD \times C57Bl/6) F1) were protected from the disease.

In human diabetes, the striking remanence of the disease in patients transplanted with the hemipancreas

of their monozygotic twin that is discordant for the disease, in some instances over 20 to 30 years following the onset of diabetes, thus at a stage where the totality of β cells has been destroyed for numerous years, clearly indicate the high recurrence of the disease and its dependancy upon primary defects in the immune system of type 1 diabetic patients. Characterization of CD8+ T cells that recognise antigens presented by class I histocompatibility molecules such as β cells, allowed us to show that several decades following the onset of type 1 diabetes, among subjects treated with insulin for years, proinsulin peptides-specific T cells could be detected, including cells specific for C-peptide determinants or for the leader region of proinsulin. This indicated the absence of any relationship of these detected T cells with the exogenous injected insulin, and thus the long term remanence of the autoimmune disease.

■ ENVIRONMENT OR GENES?

One of the major surprises brought by the studies performed in animal models is the absence of any dependence of the disease towards environmental factors. In the NOD mouse model as well as in the BB rat model, disease can be observed under conditions where animals are protected against any specific pathogens, or even under gnotobiotic conditions. Thus in these models, the disease is not triggered by environmental factors. Noteworthy, in both models, and particularly in the NOD mouse model, every animal raised under the same conditions and from the same litter does not get the disease. Only 90% of female and 10 to 30% of male NOD mice will develop diabetes whereas mice bear the same genetic background and are influenced by the same environment. This observation is similar to the finding of the frequent discordance, among humans, of diabetes incidence in monozygotic twins (50-70%). Thus it seems that under the usual conditions, the NOD mouse as well as the BB rat develop the disease for the sole reason of the association of genes constituting a predisposing background. However it is possible to induce an acute disease following a viral infection. In the BB rat model, the infection of rats with the Kilham virus induces in diabetes-resistant BB rat strains an acute diabetes. The interpretation of these data is complex, in that the infection of diabetes-prone BB rat strains does not induce an acute diabetes and does not modify the evolution of the disease in these animals. In the NOD mouse model, the injection of cyclophosphamide, an immunosuppressant, triggers an acute diabetes, but no pathogen has been identified so far, able to reproduce the action of cyclophosphamide. In this model, by contrast, it has been shown that the infection with different pathogen agents, for instance the choriomeningitis lymphocytic virus at birth or at few weeks of age, could induce a protection towards the

triggering of the disease. These data gathered in animal models underline the complexity in the analysis of epidemiological studies performed in humans, as these are able to suggest the abnormal prevalence of some infections among subjects with the disease, without allowing to clearly indicate that such identified infectious agents have a protective or inductive effect towards the development of diabetes.

The animal situation thus features the identification of a series of defects in the immune system, unable when analysed individually to contribute to the development of the disease, which in fact may result from their combination. Thus defects in hematopoietic stem cells, abnormalities in the maturation of regulatory cells (TNK cells, TCD4, CD25+ and/or CD62L+ cells) that have been previously detailed, have been shown in the NOD mouse, as well as numerous other abnormalities: apoptosis defects in lymphocytes, defects in the negative selection of T lymphocytes in the thymus, defects in T cell response through their antigen receptor, defects in the production of Th2 cytokine (IL-4), defects in the maturation and functional abnormalities of antigen presenting cells (macrophage, dendritic cell).

Genetic studies (genom scan) performed in the human disease as well as in the BB rat or the NOD mouse models indicate the unexpected multiplicity of genetic markers linked with the disease (> 15). In the majority of the suspected genetic regions, genes implicated in the predisposition to diabetes are unknown. The two or three genes that have been identified so far in man and in mouse are characterised by the fact that they do not correspond with mutations such as those described in monogenic genetic diseases, while they most probably represent variants coding for molecules with a normal function, but only different from the variants present in non predisposed individuals. It is possible that each genetic variant which association constitutes the genetic background defining the predisposition to diabetes gives a functional mark to the immune system, thus favoring the development of the disease. It is possible that the association of these variants allows the development of the disease in the majority of individuals in a stochastic way, indepen-

dently from the involvement of any environmental factor. It is also possible that whatever determines the development of the disease on a predisposed genetic background is dependant of environmental factors that have a protective role towards the disease. Such hypotheses do not rule out that, under certain circumstances, an environmental agent may acutely trigger the disease. In experimental models, the introduction of cyclophosphamide in the NOD mouse, the infection with the Kilham virus in the BB rat, or the introduction of major mutations (such as the invalidation of a gene) can induce an accelerated diabetes. This is the case in the NOD mouse when the gene coding for a coactivation molecule of T cells (CD28) or its counterpart on antigen presenting cells (B7.1) is inactivated under the NOD genetic background.

However, genetic data obtained in humans indicate the possible role of islet abnormalities in the development of the disease. Thus, this is the case of the identification, in addition to major histocompatibility complex class 2 genes (IDDM1), of a second susceptibility gene directly influencing the expression of the insulin gene in response to glucose, a VNTR localised 5' from the insulin gene (IDDM2). Yet, it has been underlined that this genetic variant was susceptible to influence the insulin gene expression in the β cells (which data are contradictory), but also in the thymus where the expression of the insulin gene directly influences the selection of T lymphocytes that will constitute the peripheral pool of T cells of an individual. In a recent model where we have invalidated the expression of the gene of one of the two isoforms of murin insulin in the NOD genetic background, proinsulin, we observed an acceleration and an increase of diabetes prevalence. We have shown that this acceleration intervenes while the absence in the expression of the proinsulin 2 gene in the thymus was responsible for the peripheral passage of T lymphocytes that are specific for an insulin peptide that are not observed in conventional NOD mice. This may be the indication that, while influencing the selection of peripheral T lymphocytes repertoire, the genetic variants of the insulin gene can act on the predisposition towards type 1 diabetes.