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Beta-cell Activity and Destruction in Type 1 Diabetes

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ABSTRACT

Type 1 diabetes is the result of a chronic inflammatory process that causes elimination of insulin-producing beta-cells, resulting in insulin deficiency and hyperglycemia. The destruction is thought to be mediated by an autoimmune process involving cytotoxic T cells recognizing beta-cell autoantigens in the context of MHC class I-peptide complexes. Autoantibodies against insulin, glutamic acid decarboxylase (GAD) and and ICA 512 protein tyrosine phosphatase are frequently found. At the clinical onset of diabetes, some beta-cells remain and after initiation of insulin treatment, most patients enter a period of remission, a phenomenon that may reflect diminished autoimmune activity in the islets. There is evidence to suggest that a further loss of beta-cells can be curtailed, and that patients, who maintain endogenous insulin production, have better glycemic control and less risk of complications. This is the basis for our current research.

We are characterizing the remission phenomenon in epidemiological studies in order to identify determinants of beta-cell survival. In randomized, prospective multicenter trials, we are evaluating the benefit of beta-cell secretory rest for rescue of insulin production in patients at onset of clinical disease. In experimental studies, we are investigating expression and regulation of the key molecules of an autoimmune process in the islets. Further, selective beta-cell damage is induced in rat islets and measures to enhance beta-cell resistance and repair are being examined. We have recently identified a remarkable, beta-cell protective effect of K_{ATP}-channel opening.

BACKGROUND

Beta-cell mass at onset of clinical disease

Type 1 diabetes is considered an autoimmune disease, resulting in complete and irreversible loss of the insulin-producing beta-cells (3,10,15). The destruction leading to diabetes seems to follow markedly different temporal courses in patients. Individuals at high risk of developing overt disease may even recover beta-cell

function when observed prospectively (25,38,41). There are several reports, including recent studies (12), on the presence of apparently intact islets in the pancreata of patients who have died shortly after the onset of disease. In studies of streptozotocin-treated baboons, where correlations were made between in vivo measurements of beta- cell function and in vitro estimates of beta-cell mass, 40–50% of beta-cells were found to be present at a time when the insulin response to a glucose load was essentially zero (39). Furthermore, islets isolated from diabetic NOD mice with recent onset of diabetes can regain function during culture in vitro (45).

Patients with residual insulin production have been shown to achieve better metabolic control and have a lower risk of long-term complications (18,40,47). In the DCCT trial, patients with detectable C-peptide levels at onset of disease were randomized to conventional (insulin twice daily) or intensive (insulin four times daily or more) insulin treatment and followed for up to 9 years. The patients on intensive treatment developed fewer vascular complications and had lower levels of HbA1c and maintained higher C-peptide serum concentrations compared with patients on conventional treatment in whom endogenous insulin secretion disappeared. Thus, a number of findings suggest that the remaining islet cell mass at the time of diagnosis of clinical Type 1 diabetes may be considerably larger than previously thought, and underline the value for maintaining residual beta-cell mass. Altogether, this provides strong support to efforts trying to rescue beta-cells (14).

An antigen-driven endocrine disease

Autoantibodies against insulin, glutamic acid decarboxylase 65 (GAD) and ICA 512 are found in 60–85% of patients with Type 1 diabetes (3,10,15,49). Antigen-specific cytotoxic T cells are thought to be responsible for the destruction of beta-cells and the presence of CD8 positive T cells in islets from autopsy specimens (24) support such a view. A role of antigen-specific lymphocytes is supported by the reports of transfer of disease after bone marrow transplantation (32,50) as well as the recurrence of disease in pancreas transplanted obtained from identical twins or HLA-identical siblings (46).

In recent years, we and others have identified the autoantibody targets of several endocrine organs (e.g. the thyroid, the parietal cells, the adrenal cortex, the islets of Langerhans) and shown the antigens to be membrane-bound enzymes such as thyroid peroxidase (13), H^+,K^+-ATPase (29), 21-hydroxylase (51), glutamic acid decarboxylase (4), IA-2/ICA 512 protein tyrosine phosphatase (44), and/or secretory products such as intrinsic factor, thyroglobulin, and insulin. These findings imply that the activity of an endocrine cell is of importance in driving the autoimmunity.

In experimental models of Type 1 diabetes (NOD mouse, BB rat), prophylactic insulin treatment prevents or delays the onset of disease (2,20), and adoptive transfer of disease in NOD mice can be prevented by insulin treatment (48). In humans, intensive insulin treatment at the onset of disease has been shown to prolong the period of remission. Furthermore, glucose upregulates islet cell autoantigen expres-

Ongoing studies

Clinical

- Remission after new onset of Type 1 diabetes
- Diazoxide treatment at onset of childhood Type 1 diabetes
- Octreotide or diazoxide treatment at onset of Type 1 diabetes in adults
- Experimental
- Expression and production of islet cell autoantigens
- Immunological response to experimental beta-cell damage
- Development of beta-cell protection

sion and the amount of antigen is related to the rate of insulin secretion (8,23). Moderately elevated glucose concentrations have also been shown to sensitize beta-cells to experimental damage in vitro by streptozotocin (16), or to cytokines such as interleukin-1 (42). The accumulated evidence thus suggests that destruction of the islets of Langerhans by an autoimmune process is closely dependent on the functional activity of the insulin-producing beta-cells.

The remission phenomenon

After commencing insulin therapy, a majority of patients with Type 1 diabetes enter a period of partial remission, characterized by a reduced requirement for exogenous insulin and a near normal glycemic control (1,35). It has been shown that the ambient glucose concentration regulates islet cell autoantigen expression (8,31,37) and that this process is increased by stimulation of insulin secretion (7). Possibly, the remission reflects a reduced destruction of the beta-cells, brought about by the normalization of glucose levels following the initiation of exogenous insulin therapy (beta-cell rest) (28). This concept was tested in a clinical trial, in which 3 months of supple-mentary treatment with diazoxide, a K+-channel opener, inhibiting the release of insulin, was found to improve the endogenous insulin production of patients with newly onset of disease (6).

Remission after newly onset of disease

To further characterize the period of clinical remission in adult patients with Type 1 diabetes, two studies are being performed on a national basis and in cooperation with the Diabetes Incidence Study in Sweden group. One study is retrospective and will describe the course of clinical remission in more than 600 adult patients from all over Sweden with Type 1 diabetes diagnosed in 1992 or 1993 with the aim of identifying clinical and laboratory variables predictive for the occurrence and length of remission. Patient record forms are analyzed retrospectively with emphasis on C-peptide and islet autoantibodies, before and during remission.

The second study is a nationwide Diabetes Incidence Study in Sweden started in 1999. In 200 patients, data concerning body mass index, duration of diabetic symp-

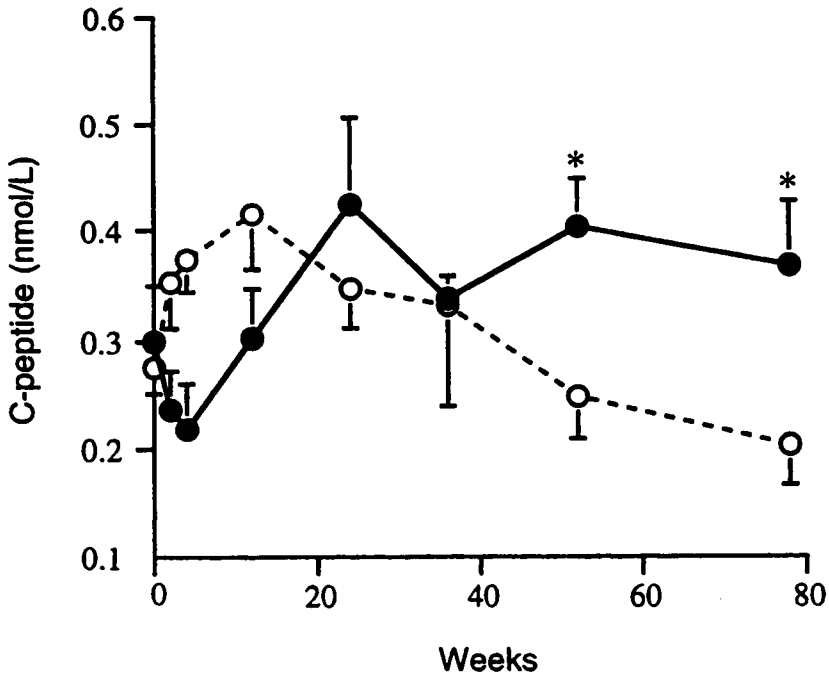

Fig 1. C-peptide levels in recently diagnosed adult Type 1 diabetic patients, treated with either placebo (open circles) or diazoxide supplementation (closed circles) in combination with multiple insulin injections, during the first three months after diagnosis. Values are means + SEM for 10 patients in each group (From 6).

toms, blood glucose, HbA1c, serum bicarbonate, fasting C-peptide and autoantibodies (GAD and ICA 512/IA-2) are collected at diagnosis of clinical disease. The patients are then followed for three years with analysis of C-peptide, autoantibodies and HbA1c every third month. Doses of insulin and body mass index are also recorded.

Diazoxide treatment at onset of childhood Type 1 diabetes

In the first clinical trial of beta-cell secretory rest in adults with recent onset Type 1 diabetes, 3 months of supplementary treatment with diazoxide was found to result in an enhanced endogenous insulin production compared to placebo even as late as 18 months after clinical diagnosis (6). This indicated that it is possible to intervene in the disease process even at clinical onset. Therefore a trial with the same protocol in children (7-16 years of age) was initiated in 1996. A total of seven pediatric centers in Sweden are participating in a study of 60 children. The study is now entering its final phase and the results are expected at the end of 2000.

Diazoxide or octreotide treatment at onset of Type 1 diabetes in adults

In a search for drugs that can lower beta-cell activity and induce beta-cell secretory rest, we have examined the effect of octreotide, a somatostatin analogue, on serum

C-peptide concentrations in patients with Type 1 diabetes, and compared its effect with that of diazoxide. The study showed that both drugs, through different pathways, are capable of lowering insulin output. Diazoxide blocked glucagon-stimulated insulin release more potently than octreotide, whereas the opposite was found for meal-stimulated insulin release (5).

Ninety adult patients (16–40 years of age) with newly onset Type 1 diabetes are now being randomized to 6 months of treatment with diazoxide or placebo orally or subcutaneous injections with the somatostatin analogue, in addition to insulin therapy. The patients, recruited from eight participating regional diabetes centers, will be followed for 2 years with basal serum concentrations of C-peptide as primary endpoint. Metabolic control (HbA1c, glucose profiles), islet cell antibodies (GAD and ICA 512/IA-2 autoantibodies determined by radioligand assays), blood glucose variability, frequency of hypoglycemia, insulin requirement, insulin sensitivity, and quality of life will also be evaluated throughout the trial.

Regulation of the expression and production of islet autoantigens

Since insulin, GAD and ICA 512 are the key antigens for autoantibody formation, it is conceivable that these proteins are important also for generation of autoantigen-specific cytotoxic T cells. The critical role of both antigen concentration and the number of MHC molelcules on the antigen-presenting cells in determining the magnitude of T cell proliferation has been previously demonstrated (27,36). Both the density of the peptide MHC ligand and the affinity of the T cell receptor for the ligand affect the strength of the signal that is delivered in the activation of T cells. The amount of MHC class I-peptide complexes is a critical determinant also of target cell sensitivity to effector T cell cytotoxicity (9).

Against this background, we have investigated the expression of antigens of autoimmune diabetes, glutamic acid decarboxylase (GAD), ICA 512 protein tyrosine phosphatase (ICA 512) and insulin, in isolated rat islets by immunohistochemistry and by mRNA measurements, using a quantitative solid-phase miniseqeuncing RT-PCR technique. The results indicate that exocytosis of insulin is associated with conformational changes in the GAD and ICA 512 molecules, which possibly have immunological significance, and that the long-term control of these antigens is closely linked to insulin production. Islets contain approximately 100-fold more insulin mRNA than GAD and ICA 512 mRNAs 108 molecules vs. 10^6 and 10^6 per µg total RNA, respectively (Figure 2). The large number of insulin mRNA molecules, and the much lower levels of the GAD and ICA 512 mRNAs, as well as MHC class I mRNA, suggests that insulin is the autoantigen most likely to be responsible for peptide loading onto MHC class I and recognition by putative beta-cell-specific cytotoxic T cells.

Selective beta-cell damage – studies of an immunological response in vivo

Streptozotocin is a beta-cell toxin which, when given to a sensitive mouse strain (C57BL/Ks) in multiple small doses induces beta-cell damage that is followed by an immunological reaction with further destruction leading to overt diabetes (34).

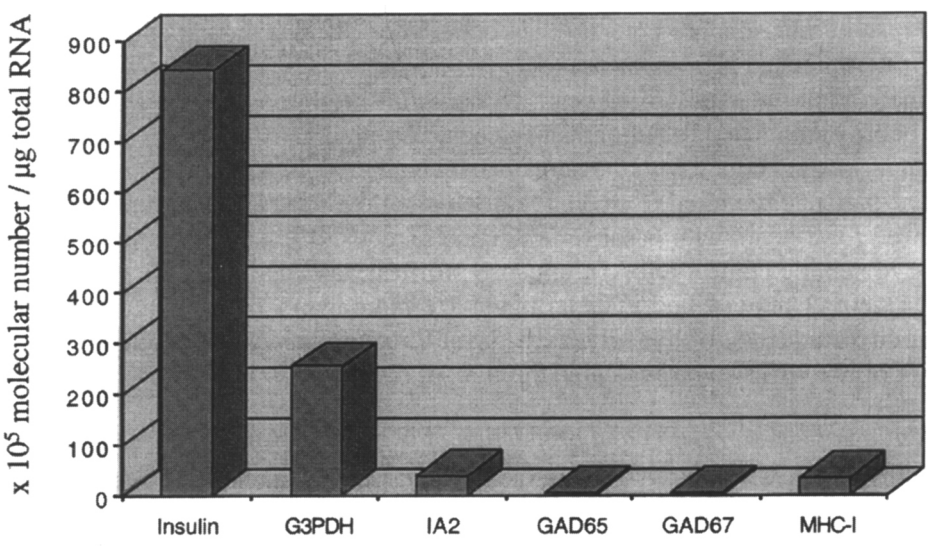

Fig. 2. Abundance of mRNAs in rat islets cultured in 5 mM glucose for 3 days.

Activated immune cells are thought to contribute to the development of the diabetes in these mice. A role in the process for MHC class I restricted T cells has remained, however, a matter of debate. Therefore we have examined, by confocal microscopy, the pancreatic expression of MHC class I protein, insulin, ICA 512 protein tyrosine phosphatase in mice given streptozotocin on 5 consecutive days (33). The animals became hyperglycemic from day 7 and onwards. A loss of ICA 512 from the central portions of the islets was noticeable on day 3. On day 7, an increase in MHC class I expression, confined primarily to immune cells in the exocrine pancreas and the peri-insular areas, was detected. Later several MHC class I/glucagon and some MHC class I/insulin double-positive cells were found. The insulitis was maximal on day 14 and declined thereafter.

The induction of MHC class I expression in endocrine cells, occurring only subsequent to the cellular infiltration, and when the animals were diabetic, indicates that the immune component of the disease does not depend upon MHC class I restricted cytotoxic T cells, but rather comprises a non-antigen specific process.

Selective beta-cell damage – development of protective strategies by studies in vitro

Since we have found diazoxide to have a protective effect in vivo in patients with new onset of disease, we are interested in examining the effect of the compound in promoting beta-cell resistance to destruction and carring out studies with cultures of isolated rat islets of Langerhans.

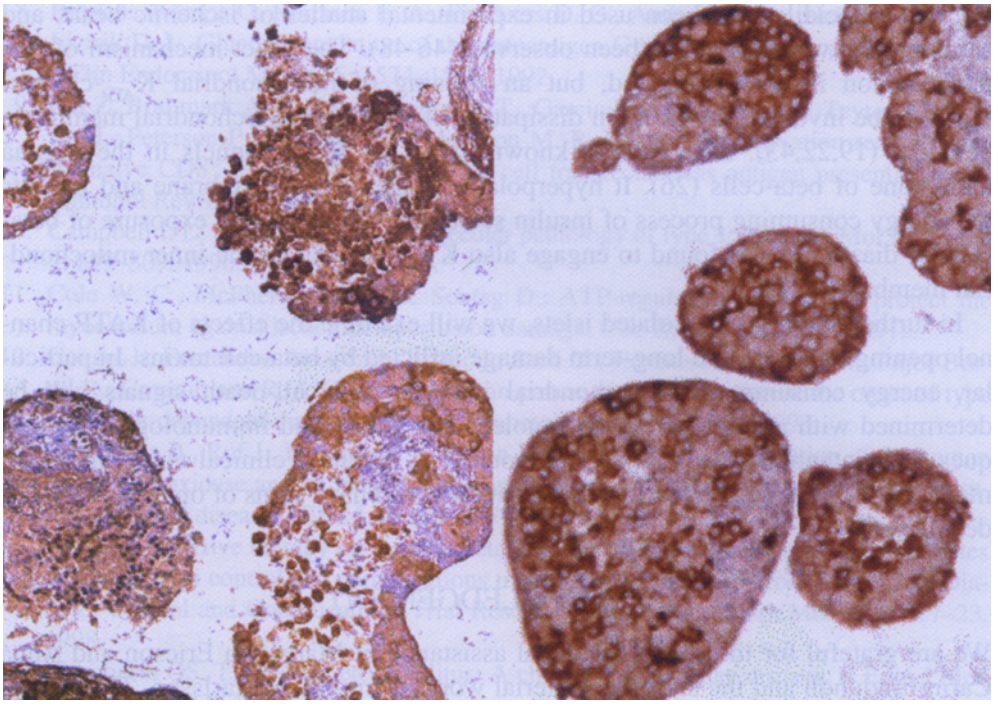

Fig. 3. Protection against damage. Rat islets two hours after exposure to streptozotocin in the presence (right) and absence of diazoxide (left).

Strepotzotocin and alloxan are strong, selective beta-cell toxins and the compounds have been extensively employed in studies of diabetes in experimental animals. We examined (30) the influence of two K_{ATP}-channel openers, diazoxide and an analogue, NNC 55-0118, on experimental beta-cell damage induced by streptozotocin (STZ; 0.5 mM), an agent known to cause energy depletion and beta-cell death (52). Rat pancreatic islets were exposed to diazoxide or NNC 55-0118 for 30 minutes and further incubated for 30 minutes after addition of STZ. The islets were then washed and incubated for 24 hours. Islets exposed to STZ alone showed extensive morphological damage, reduced glucose oxidation, low insulin content, and severely impaired glucose-stimulated insulin release and proinsulin biosynthesis. In contrast, islets incubated with diazoxide + STZ or NNC 55-0118 + STZ appeared morphologically intact at the 0 hour point. At 2 hours, the shape of the islets showed some irregularity (Figure 3) and this became more apparent at 24 hours, in particular with the islets incubated in the lower concentration (0.03 mM) of the channel openers. STZ-treated islets in the presence of the channel openers (0.03–0.3 mM) showed not only dose-dependent preservation of the morphology, but also improved glucose oxidation rate, insulin content and secretion. NNC 55-0118 was capable of fully counteracting the impairment of islet function induced by STZ.

It is of interest, that diazoxide and other K_{ATP}-channel openers, such as cromaka-

lim and pinacidil, have been used in experimental studies of ischemic heart, and cardioprotective effects have been observed (46-48). The exact mechanism of this phenomenon is not understood, but an opening of mitochondrial K_{ATP}-channel seems to be involved, resulting in dissipation of the inner mitochondrial membrane potential (19,22,43). Diazoxide is known to act on K_{ATP}-channels in the plasma membrane of beta-cells (26). It hyperpolarizes the plasma membrane and inhibits the energy consuming process of insulin secretion (17). Recently, exposure of beta-cells to diazoxide was found to engage also K_{ATP}-channels in the inner mitochondrial membrane (21).

In further studies with isolated islets, we will examine the effects of KATP-channel opening on short- and long-term damage inflicted by beta-cell toxins. In particular, energy consumption, mitochondrial integrity and cell-death signals will be determined with a range of cellular, molecular biology and immunological techniques. Our intention is to validate compounds of potential clinical value in animal models prior to clinical trials involving patients who have signs of ongoing beta-cell destruction.

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