Transplanted islets from lpr mice are resistant to autoimmune destruction in a model of streptozotocin-induced type I diabetes

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The mechanism by which β-cells die during autoimmune diabetes has remained a subject of intense investigation. The loss of β-cells in the disease is T cell mediated and thought to result from a number of different insults including apoptosis induction through the death receptor CD95. However, the role of CD95 in autoimmune diabetes, studied primarily in the non-obese diabetic (NOD) mouse model, has been controversial. We have used an alternative model of autoimmune diabetes triggered by repeated low doses of streptozotocin. In this model, islet grafts from C3H mice that carry the lpr mutation, and therefore lack the ability to undergo apoptosis through CD95-CD95L interaction, were completely protected when grafted in autoimmune diabetic mice despite periinsulitis (infiltration of T cells) which however did not progress to islet destruction. In contrast wild-type grafts were rapidly eliminated in autoimmune recipients. Our data provide strong support for a major role of CD95 in the destruction of islets in autoimmune mice.

Keywords: apoptosis; CD95; lpr; type I diabetes

Introduction

Autoimmune or type 1 diabetes, a major disease in the U.S., is a T cell-mediated autoimmune disease in which the insulin-secreting β-cells are selectively destroyed leading to the loss of insulin production and hyperglycemia following years of insulitis, a pathogenic infiltrate within the pancreatic Islets of Langerhans. Both CD8+ and CD4+ T cells contribute to the process, and several mechanisms of β-cell death are most likely involved. Since type 1 diabetes is a T cell mediated autoimmune disease, β-cells can potentially die by two mechanisms, apoptosis induced through the death receptor CD95 after triggering with CD95 ligand (CD95L) and the perforin/granzyme pathway with some contribution of the TNF-RI. While perforin deficient non-obese diabetic (NOD) mice develop severe insulitis but rarely become diabetic; NOD-lpr/lpr mice with a defect in the expression of CD95 develop neither insulitis nor diabetes. NOD mice expressing signaling deficient CD95 with the mutation found in lpr mice only in β-cells under control of the rat insulin promoter (RIP) (NOD-lpr/tg) are significantly protected against diabetes upon adoptive transfer of diabetogenic splenocytes from diabetic NOD mice. Furthermore, CD4+ T cells from transgenic mice expressing the highly diabetogenic β-cell specific 4.1 T cell receptor can kill β-cells from wild-type but not from lpr mice and only when wild-type β-cells were treated with IL-1β, IL-1α and IFNγ, cytokines that upregulate CD95, which is characteristic for the progression of the disease. These experiments suggest an involvement of the CD95/CD95L system in the apoptosis observed in β-cells. However, a number of studies have raised concerns about the significance of CD95 as a major mediator of β-cell loss during diabetes. It was suggested that a specific property other than lack of CD95 expression of lymphocytes from lpr mice caused them to be resistant to autoimmune destruction. CD95 was shown to only play a minor role in the autoimmune destruction of β-cells upon transplantation of islets from NOD lpr mice into diabetic NOD mice and a recent study did not find a role for CD95 in a MHC class II dependent transgenic model of autoimmune diabetes. The reason for these discrepancies is not known at present but the situation is complicated by the fact that both the CD95 receptor and its ligand are expressed on β-cells, autoimmune T cells, natural killer cells and inflammatory cells. The majority of the data on the role of CD95 in type 1 diabetes were obtained using adoptive transfer of diabetogenic splenocytes from diabetic into nondiabetic mice. While transfer...
of diabetogenic T cells is a good way to study the mechanism of islet cell destruction it has little direct therapeutic relevance. We therefore chose to address the role of CD95 in diabetes by transplanting islets into diabetic mice. We now provide evidence for a strict requirement for the CD95-CD95L pathway in the pathogenesis of type 1 diabetes in the model of chemically (multiple low dose streptozotocin) induced autoimmune type 1 diabetes in mice.12

Material and methods

Animals

C3H/HeJ and C3Hlpr/lpr were purchased from Jackson Labs, ME. C3H/HeJ males 8 weeks of age were used as recipients while 6 week old male C3H/HeJ and C3Hlpr/lpr mice were used as islet donors. All animals were housed in specific pathogen-free conditions at the University of Chicago Animal Facility. They were fed standard laboratory food and given water ad libitum and were maintained in agreement with the National Institute of Health guidelines for use and care of laboratory animals. Streptozotocin (STZ) (Pharmacia, Kalamazoo, MI, USA) solution at 5 mg/ml was prepared in sterile PBS. Recipients were made autoimmune diabetic by administration of five low dose (80 mg/kg body weight) injections daily. Once blood glucose levels were elevated (>300 mg/dL) for more than 48 hrs in no case did we see recovery of endogenous insulin secretion within 7 days of continued monitoring.

Islet isolation and transplantation

Donor pancreata were perfused in situ through the common bile duct with collagenase P (0.375 mg/ml; Roche, Basel, Switzerland). Pancreata were harvested after perfusion and were incubated at 37°C for 10 minutes. Islets were released from the pancreata by gentle shaking. After being washed twice with Hank’s balanced salt solution (HBSS), islets were further purified on a discontinuous Ficoll gradient. After centrifugation, the islets were harvested from the 1.096/1.069 gradient interface, washed twice in HBSS, and collected under the microscope. A total of 300 islets were transplanted under the renal capsule of each recipient. Graft survival was monitored by serial blood glucose measurements. Graft rejection was defined as a rise of blood glucose concentration to more than 300 mg/dL on 2 consecutive measurements after a period of normoglycemia. All transplanted mice achieved normoglycemia for at least a week.

Histology

For immunostaining, 5 µm-thick cryostat sections of pancreata fresh-frozen in OCT compound (Sakura Finnetek, Torrance, CA) were fixed in acetone at −20°C for 3 min., air-dried for 2 hrs, stained for 1 hr at room temperature with guinea-pig anti-insulin (Dako), mouse anti-CD8 and anti-CD4 (BD Biosciences) Abs, washed, and stained with goat anti-guinea pig (Chemicon) and goat anti-IgG conjugated with horseradish peroxidase (Caltag, South San Francisco, CA) and color developed with diaminobenzidine. The slides were counter-stained with hematoxylin for nuclear staining and evaluated by a pathologist blinded to the type of transplant received by the mice. At least 5 of the mice were analyzed.

Results and discussion

The model of multiple low dose injections of STZ (MLD STZ) has been extensively characterized.12 It has been shown that low level apoptosis in the β-cells induced by MLD STZ effectively primes autoreactive CD8 T cells.13 We first titered STZ given at five time low dose intervals in C3H mice. At 40 mg/kg about 40% of the mice developed diabetes after 14–18 days. At 80 mg/kg 80% of the mice became diabetic within 14–18 days. At 100 mg/kg 90% of the mice were diabetic after 7–9 days. Mortality of mice injected with 120 mg/kg increased so that we discontinued testing this concentration. By performing immunohistochemistry we confirmed that most islets in mice made diabetic with MLD STZ of 80 mg/kg were destroyed and showed decreased staining for insulin and infiltration of CD8+ and CD4+ T cells within the islets (Figure 1) when compared to untreated nondiabetic mice. Consistent with previous reports,12,13 the diabetes observed in these mice therefore appeared to be autoimmune driven. To directly determine the contribution of CD95 to the destruction of β-cells in this model we grafted 300 wild-type or lpr islets under the kidney capsule of low dose STZ induced autoimmune diabetic C3H recipient mice, and monitored blood glucose levels as an indicator for graft rejection/survival. All of the eight recipients that received C3H lpr islets maintained graft survival and were therefore normoglycemic until the last day of blood glucose measurement i.e. 2 months after the day of transplantation, whereas all recipients of wild-type islets became diabetic before day 18 after transplantation (Figure 2). Although the number of transplanted mice was relatively small the significance of the difference in graft survival time between mice transplanted with islets from wild-type versus lpr mice was high (p = 0.0003).

We recovered the grafts by unilateral nephrectomy 65 days after transplant and assessed the islet integrity in both the wild-type and lpr grafts by histology. Histological examination revealed infiltration of both CD4+ and CD8+ cells under the kidney capsule of mice harboring both wild-type and lpr islets (Figure 3 center and bottom row). No lymphocyte infiltrates were detected in the