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In Situ β Cell Death Promotes Priming of Diabetogenic CD8 T Lymphocytes¹

Yiqun Zhang,* Bronwyn O’Brien,‡ Jacqueline Trudeau,†† Rusung Tan,† Pere Santamaría,§ Jan P. Dutz²*

CTLs are important mediators of pancreatic β cell destruction in the nonobese diabetic mouse model of type 1 diabetes. Cross-presentation of Ag is one means of priming CTLs. The death of Ag-bearing cells has been implicated in facilitating this mode of priming. The role of β cell death in facilitating the onset of spontaneous autoimmune diabetes is unknown. Here, we used an adoptive transfer system to determine the time course of islet-derived Ag presentation to naïve β cell-specific CD8 T cells in nonobese diabetic mice and to test the hypothesis that β cell death enhances the presentation of β cell autoantigen. We have determined that β cell death enhances autoantigen presentation. Priming of diabetogenic CD8 T cells in the pancreatic lymph nodes was negligible before 4 wk, progressively increased until 8 wk of age, and was not influenced by gender. Administration of multiple low doses of the β cell toxin streptozotocin augmented in situ β cell apoptosis and accelerated the onset and magnitude of autoantigen presentation to naïve CD8 T cells. Increasing doses of streptozotocin resulted in both increased pancreatic β cell death and significantly enhanced T cell priming. These results indicate that in situ β cell death facilitates autoantigen-specific CD8 T cell priming and can contribute to both the initiation and the ongoing amplification of an autoimmune response. The Journal of Immunology, 2002, 168: 1466–1472.

Type 1 diabetes mellitus (DM) is an autoimmune disorder in which the pancreatic β cells are destroyed by the cellular immune system (1, 2). The nonobese diabetic (NOD) mouse is a useful and well-studied animal model for human type 1 DM. Adoptive T cell transfer studies using T cells from prediabetic NOD mice have demonstrated that transfer of diabetes into immunodeficient mice requires both CD4 and CD8 T cells (3–6). CD8 T cells are known to participate in pancreatic β cell destruction (7–9). The importance of CD8 T cells in diabeticogenesis is underscored by the fact that neither DM nor insulitis develop in NOD mice deficient in MHC class I expression and CD8 T cells (10, 11). Recent data suggest that CD8 T cells play a pivotal role in the earliest initiative phases of autoimmune pancreatic β cell destruction (12, 13).

The activation of naïve (resting and previously unactivated) T cells requires activation of the TCR and the simultaneous delivery of a costimulatory signal by a specialized APC. Using adoptive transfer of naïve CD4 T cells specific for a pancreatic β cell Ag, it has been demonstrated that these cells can be primed in the pancreatic lymph nodes as early as, but not before, 14 days of age (14). Similarly, CD8 T cells responding to transgenically overexpressed Ags in the pancreas cannot be primed in mice of 5–10 days of age (14, 15). The timing and mechanism by which CD8 T cells are primed to pancreatic β cell Ag in spontaneous autoimmunity remains to be determined. Cross-presentation of Ag, where tissue-specific Ag is processed by APCs and presented to T cells via class I MHC, is one means of priming CD8 T cells (16). Cross-presentation of cellular Ags expressed by tumors (17), viruses (18), and self (19), as well as of soluble Ags, has been described (20). Cell death, specifically by apoptosis, can provide an excellent source of cellular Ag for cross-presentation (21, 22). Cell death, by either apoptosis (23) or necrosis (24), can also provide cell-derived signals that activate APCs and promote immune responses. Therefore, we hypothesized that β cell death could promote the initiation and/or progression of spontaneous autoimmune diabetes.

The aims of the present study were first to delineate the timing of CD8 T cell priming to pancreatic β cell-associated Ag in NOD mice and, second, to determine whether β cell death can promote the priming of CD8 T cells to a diabetogenic self-Ag. We used an adoptive transfer system using CD8 T cells derived from a mouse line that transgenically expresses a TCR of a diabetogenic T cell clone (8). This clone uses a TCR rearrangement expressed by a preponderance of CD8 T cells found in early insulitic lesions of NOD mice (13). The results of our investigations show that priming of diabetogenic CD8 T cells occurs in the pancreatic lymph nodes and is undetectable before 3–4 wk of age and then progressively increases with age to reach maximal levels at 8 wk of age. An increased incidence of apoptosis in the pancreas after the administration of low doses of the β cell toxin streptozotocin (STZ) augmented CD8 T cell priming and allowed it to occur at ages when it does not occur spontaneously. These results suggest that β cell death precedes the priming of T cells in autoimmune diabetes and implicate in situ β cell death in the facilitation of autoantigen-specific CD8 T cell priming in autoimmunity.

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3 Abbreviations used in this paper: DM, diabetes mellitus; NOD, nonobese diabetic; STZ, streptozotocin; CM, complete medium; DC, dendritic cell; PARP, poly(A)DP ribose polymerase.
Materials and Methods

Mice and cell lines

NOD mice were obtained from Taconic Farms (Germantown, NY) and bred in a specific pathogen-free environment. The 8.3-NOD mice, expressing the rearranged TCR genes of the diabetogenic CTL clone NY8.3, have been previously described (8) and were bred at the University of Calgary (Calgary, Alberta, Canada). C57BL/6 mice were obtained from The Jackson Laboratory (Bar Harbor, ME). All animal experiments were approved by the Animal Care Committee of the University of British Columbia. EL4 thymoma cells and E.G7-OVA cells (EL4 cells transfected to express OVA) (20) were obtained from the American Type Culture Collection (Manassas, VA) and cultured in complete medium (CM) consisting of RPMI 1640 medium (Invitrogen, Burlington, Ontario, Canada) supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), 5 × 10⁻³ M 2-ME, and 10% FCS.

Adoptive transfers and cytofluorometric analysis

Naïve 8.3 CD8 T cells were isolated from 4- to 5-wk-old 8.3-NOD mice. Single-cell suspensions were prepared from peripheral lymph nodes and spleen, and CD8 T cells were purified using immunomagnetic cell separation kits (Miltenyi Biotec, Auburn, CA) according to the manufacturer’s specifications. Purified 8.3 CD8 T cells were labeled with 5 μM CFSE (Molecular Probes, Eugene, OR) in PBS for 10 min. The labeled cells (10 × 10⁶) were transferred to NOD recipients by tail vein injection (for 8-wk-old animals) or i.p. (for younger animals). Three-channel FACS analysis was performed on a FACScalibur flow cytometer (BD Biosciences, San Jose, CA) using Cell Quest software (BD Biosciences). Briefly, 200,000 cells were analyzed after staining with appropriate Abs or tetramers. 5′-OVA tetramers were synthesized as described (25). Abs to CD44, CD25, and TCR Vα 8.1/8.2 were purchased from Cedarlane (Hornby, Canada) and CD4 was purchased from BD Pharmingen (San Diego, CA). The Abs to CD44, CD25, and TCR Vα 8.1/8.2 recognize the Vβ8.1/Vβ8.2 chains of CD8 T cells. In vitro stimulation of purified 8.3 CD8 T cells was conducted in the presence of 1 μg/ml of the indicated peptides and CM using irradiated (3000 rad) syngeneic spleenocytes as APCs.

Immunizations and cytotoxicity assay

C57BL/6 mice (5–6 wk old) were immunized in the flank by s.c. injection with 100 μg of OVA (Sigma-Aldrich, St. Louis, MO) in PBS with or without 5 × 10⁵ EL4 thymoma cells that had been either irradiated or freeze thawed for four cycles. The gamma irradiation consisted of 20,000 rad (for younger animals). The 8.3-NOD mice were immunized with 100 μg OVA (Sigma-Aldrich, St. Louis, MO) in PBS with or without 5 × 10⁵ EL4 thymoma cells that had been either irradiated or freeze thawed for four cycles. The gamma irradiation consisted of 20,000 rad (for younger animals). Three-channel FACS analysis was performed on a FACScalibur flow cytometer (BD Biosciences, San Jose, CA) using Cell Quest software (BD Biosciences). Briefly, 200,000 cells were analyzed after staining with appropriate Abs or tetramers. 5′-OVA tetramers were synthesized as described (25). The Abs to CD44, CD25, and TCR Vβ 8.1/8.2 were purchased from Cedarlane (Hornby, Canada). The Abs to CD4 and CD25 were purchased from BD Pharmingen (San Diego, CA). The Ab to TCR Vβ 8.1/8.2 recognizes the Vβ8.1/Vβ8.2 chain used by the 8.3 TCR (8). In vitro stimulation of purified 8.3 CD8 T cells was conducted in the presence of 1 μg/ml of the indicated peptides and CM using irradiated (3000 rad) syngeneic spleenocytes as APCs.

Statistical analysis

Groups were compared using one- or two-tailed Student’s t tests.

Results

Proliferating 8.3 CD8 T cells are detected in NOD pancreatic lymph nodes after adoptive transfer

Cytotoxic T cells are known to infiltrate pancreatic islets, where they participate in pancreatic β cell damage (7–9). A significant fraction of CD8 T cells in NOD mice express highly homologous TCR α-chains (Vα17 and Jα26 elements, joined by the N-region sequence M-R-D/E) (7, 13, 27) and are cytotoxic to β cells in the presence of 2-kDa MHC class I molecule. The 8.3-NOD mice were generated using the TCR α and β genes of one such H-2Kd-restricted β cell cytotoxic CD8 T cell clone isolated from the islets of an acutely diabetic NOD mouse (8). We first purified 8.3 CD8 T cells from 8.3-NOD mice using either negative or positive selection. Phenotype analysis using the activation markers CD69, CD25, and CD44 revealed that our positive selection protocol yielded both resting and naive CD8 T cells. By combinatorial peptide library screening, an agonist peptide (NRP-A7, KYNKANAFL) for the 8.3 TCR has been identified (28). The purified cells up-regulated early activation markers in response to agonist peptide but not to a control peptide (TUM, KYQAVTTTL) (Fig. 1; Ref. 29). Adoptive transfer of fluorochrome-labeled Ag-specific T cells has been used as a method to track the activation of T cell in vivo and can be used to determine the location and timing of cognate Ag presentation (30). Using this method, it has been determined that diabetogenic CD4 T cells encounter cognate Ag in the pancreatic lymph nodes in adult but not in neonatal (10-day-old) animals, were injected i.p. with STZ (Sigma-Aldrich), freshly prepared in distilled water and adjusted to 30 mg/ml, and each animal was subsequently confirmed to be diabetic if the glucose exceeded 14 mmol/L. Animals were considered to be overtly diabetic when two consecutive blood glucose measurements exceeded 14 mmol/L.

FIGURE 1. The 8.3 CD8 T cells are naive and respond to mimotope in vitro. The 8.3 CD8 T cells were purified and analyzed immediately ex vivo (solid line, unstimulated) or cultured for 24 h in the presence of irradiated syngeneic spleenocytes and an irrelevant peptide (dotted line, TUM, KYQAVTTTL) or a known agonist peptide (gray dotted line, NRP-A7, KYNKANAFL). Live cells were gated for CD8 and TCR Vβ 8.1/8.2 positivity. Expression of activation markers CD69, CD25, and CD44 was then analyzed by fluorescence cytometry. Data are representative of two independent experiments.
mice (14). Similarly, transgenic expression of OVA in pancreatic islets leads to OVA-specific CTLs in adult but not in 10-day-old mice. To determine whether diabetogenic CD8 T cells recognizing an endogenous pancreatic β cell Ag are primed in the pancreatic lymph nodes, we labeled purified naive 8.3 CD8 T cells with the fluorescent dye CFSE. We then adoptively transferred 10 × 10^6 of these cells into 8-wk-old NOD mice by tail vein injection. Within 72 h after transfer, cells that had proliferated at least once (as indicated by serial CFSE dye dilution) were detected in the pancreatic lymph nodes but not in the mesenteric lymph nodes or spleens of these mice (Fig. 2). Thus, naive 8.3 CD8 T cells proliferate and are first activated (primed) in the pancreatic draining lymph nodes of prediabetic NOD mice.

Time course of 8.3 CD8 T cell priming in NOD mice

NOD mice develop an inflammatory infiltrate of the islets (insulitis) consisting of T and B lymphocytes, macrophages, and dendritic cells (DCs). Onset of diabetes in female NOD mice occurs from 12 wk of age onwards (2). The spontaneous onset of insulitis seems to be a fixed checkpoint at approximately days 18–21 (31). It has been shown that diabetogenic CD4 T cells are primed in the pancreatic lymph nodes before islet infiltration (14). To establish the time course of priming of naive diabetogenic CD8 T cells in NOD mice, we adoptively transferred CFSE-labeled naive 8.3 CD8 T cells into male and female NOD mice aged 1–8 wk. The percentage of CFSE-labeled cells that had undergone at least one cell division was then quantified (Fig. 3). The priming of diabetogenic CTLs in the pancreatic lymph nodes was negligible (3% ± 0.9% of adoptively transferred cells at 1 wk, n = 8) until 4 wk of age (at which time it was 11% ± 6%, n = 7). Priming then progressively increased until 8 wk of age (when it reaches a maximum of 42% ± 11%, n = 6). Autoantigen presentation to diabetogenic CD8 T cells between 4 and 8 wk of age was not influenced by gender, as shown by the fact that priming of adoptively transferred cells into male and female NOD recipients was similar. Thus, as is the case for diabetogenic CD4 T cells, there is a developmental regulation of the presentation of endogenous pancreatic β cell Ags to diabetogenic CD8 T cells with minimal presentation of Ag before 3–4 wk of age. Interestingly, this period of activation follows a period of remodeling and physiologic apoptosis of the pancreatic islets that peaks at 2 wk of age (32, 33). Thus, in situ β cell death precedes the priming of diabetogenic CD8 T cells. Whether this local wave of cell death plays a role in the initiation of autoimmunity (33) or whether Ag presentation is developmentally blocked to minimize autoimmunity is not clear (16).

Cell death enhances CTL priming to nominal Ag

Cell death by either apoptosis or necrosis can make cellular contents available for uptake by professional APCs and may thus facilitate the priming of cells to self-Ag. Cell death may also act as a stimulus to create mature immunogenic DCs (24, 34). To determine whether cell death has adjuvant properties in addition to providing a means of accessing cell-associated Ag, C57BL/6 mice were immunized with the nominal soluble Ag OVA in the presence or absence of either apoptotic or necrotic cells. We chose to use OVA as the nominal Ag because its immunodominant CTL epitope, SIINFEKL, in the context of the H-2Kb class I MHC molecule, is well defined (35). CTL responses were monitored by the enumeration of OVA (SIINFEKL)-specific CTLs using fluorescence cytometry and Kb-OVA multimeric complexes. CTL responses were confirmed using a standard chromium release assay (Fig. 4). Whereas C57BL/6 mice immunized with soluble protein did not mount significant CTL responses, mice immunized with soluble OVA co-injected with either irradiated (apoptotic) or freeze thawed (necrotic) EL4 thymoma cells mounted good CTL responses. These responses were not due to tumor-specific factors because similar results were obtained with the co-injection of autologous irradiated or freeze thawed fibroblasts (data not shown). These results suggest that cellular material released by either apoptotic or necrotic cells can potentiate the priming of CTLs to soluble co-administered Ag.

The β cell toxin STZ enhances the priming of 8.3 CD8 T cells in 2-wk-old NOD mice

Next we wanted to know whether increased cell death at the tissue level could promote the priming of autoreactive CD8 T cells. STZ, a d-glucopyranose derivative of N-methyl-N-nitrosourea, is cytotoxic to pancreatic β cells. When delivered at high doses (200 mg/kg), STZ causes complete β cell destruction and insulin depletion. At low concentrations and when administered in multiple low doses, STZ can initiate a delayed diabetic state in susceptible
strains that is largely dependent on immune mechanisms (36–38). High doses of STZ in vitro can cause necrosis of β cells (39). Low doses of STZ, however, initiate an apoptotic pathway of β cell death. Multiple low doses of STZ have been shown to increase in situ apoptotic cell death in the pancreas (26). Because cell death may increase cellular Ag availability and have adventitiously properties, we treated 2-wk-old mice with STZ and assessed β cell-specific T cell priming in the pancreatic lymph nodes. Mice were treated with either STZ (40 mg/kg) daily for 3 days or with citrate buffer alone before adoptive transfer of naïve 8.3 CD8 T cells. Adoptively transferred cells were analyzed 4 days later (Fig. 5). Priming was detected by CFSE dilution and up-regulation of the early activation marker, CD69. Although there was minimal priming in 2- to 3-wk-old animals treated with citrate buffer alone (n = 5), both proliferation and CD69 up-regulation of 8.3 CD8 T cells were observed in the pancreatic lymph nodes of mice treated with STZ (n = 11). Comparison of the means of CFSE-labeled cells having undergone at least one division revealed that STZ significantly enhanced priming (p = 0.007). Similar results were found using 4-wk-old mice (n = 5 for both test and control groups; p = 0.016). These results suggest that STZ enhances the priming of diabetogenic CD8 T cells to endogenous pancreatic β cell Ag and allows this priming to occur at a time when there is normally minimal Ag presentation and priming.

**CTL priming to a β cell autoantigen correlates with β cell death**

STZ-induced pancreatic β cell toxicity is dependent on initial glucose transporter-2-mediated STZ transport into the cell (40, 41) and subsequent poly(A)DP-ribose polymerase (PARP)-dependent depletion of NAD+ within the β cells (42–44). Because susceptibility to low-dose STZ is strain specific, we first determined whether 4-wk-old NOD mice given five daily doses of STZ develop accelerated diabetes. Seven of 10 STZ-treated mice developed diabetes (blood glucose ≥ 14 mmol/L over two consecutive readings) at age 6 wk. Nine of 10 developed diabetes by age 7 wk and 10 of 10 did so at 8 wk, compared with zero of 10 citrate buffer-treated mice (Fig. 6.). A delay of 1–2 wk between STZ administration and diabetes onset suggests an immune contribution to diabetes development and is consistent with the delayed onset observed in standard susceptible strains. Our finding that multiple low-dose STZ can accelerate the onset of diabetes in NOD mice is in agreement with previous observations (45). To confirm that the administered STZ was inducing apoptotic cell death of pancreatic β cells, 2-wk-old mice were treated with three repeated injections of STZ using varying doses. Pancreatic tissue was then removed, processed for histology, and double stained for insulin to label β cells and by the TUNEL technique to identify apoptotic cells. The number of apoptotic pancreatic β cells correlated positively with the dose of STZ (Fig. 7). Identically treated littersates received naïve 8.3 CD8 T cells after the three injections of STZ to assess levels of priming in the pancreatic lymph nodes. Again, priming increased with increasing doses of STZ, and the percentage of proliferating cells correlated with the incidence of apoptotic pancreatic β cells in situ. We conclude that low doses of STZ induce apoptotic death of pancreatic β cells. Furthermore, the magnitude...
of in situ cell death correlates with CD8 T cell priming to a natural pancreatic β cell self-Ag in the pancreatic lymph nodes. To determine whether increased β cell death at an early time point could result in the priming of diabetogenic T cells, 9-day-old NOD mice were next treated with a single injection of STZ at a dose of 80 mg/kg. Adoptive transfer of CFSE-labeled 8.3 CD8 T cells was performed the next day, with analysis 4 days later. Although this treatment was toxic to the mice, in the two of five mice that survived this treatment, 14 and 34% of adoptively transferred cells detectable in the pancreatic lymph nodes had proliferated. We conclude that chemically induced pancreatic β cell death can facilitate priming of diabetogenic CD8 T cells at a time point when it does not occur naturally and before the peak of physiologic apoptosis.

**Discussion**

This is the first study in which adoptive transfer of naive self-Ag-specific CD8 T cells has allowed determination of the location and timing of priming to self in the NOD mouse model of type 1 diabetes. We first observed significant priming of adoptively transferred naive CD8 T cells in the pancreatic lymph nodes of NOD mice at 4 wk of age. Previous studies have shown that this priming is the result of cross-presentation of islet Ags by professional APCs and is not due to direct presentation by β cells (19, 46). Consistent with previous reports, we find that there is a developmental regulation of priming to Ags present in the pancreata of NOD mice (14, 15). Developmental regulation of priming exists in both NOD mice and in nonautoimmune mouse strains and is specific for the pancreas. Although priming of OVA-specific CD8 T cells to transgenically expressed Ag in the pancreas does not occur in the pancreatic lymph nodes in 10-day-old mice, it does occur in the lymph nodes draining the kidneys, which also express the Ag (14). This suggests organ-specific mechanisms for the developmental regulation of Ag presentation. The priming of naive diabetogenic CD8 T cells that we noted in NOD mice precedes the commonly observed onset of insulitis in genetically unmanipulated mice (47). This is also subsequent to a natural increase in the incidence of apoptotic pancreatic β cells in the pancreas that peaks at 2 wk of age (32). Acceleration of diabetes and insulitis in NOD mice over-expressing TNF-α on β cells is preceded by increased apoptotic death of these cells (47). It is possible that this endogenous β cell death subsequently allows the priming of naive diabetogenic CD8 T cells. We chose to explore this possibility in subsequent experiments.

The observation that priming does not differ between male (which have an incidence of diabetes of ~30%) and female (80% of which will develop diabetes) NOD mice suggests that the timing of presentation of self-Ag does not identify mice that will later develop diabetes. This finding is consistent with the observation that the onset of insulitis does not differ between male and female NOD mice. Therefore, the difference between male and female NOD mice (with respect to their equal priming ability and differing susceptibilities to diabetes development) must reside either within the avidity/affinity of self-reactive T cells generated or in the existence of differential suppressor or homeostatic mechanisms to control these self-reactive T cell populations. It recently has been shown that the progression of benign to destructive insulitis is characterized by an increase in the avidity/affinity of diabetogenic CD8 T cells for a mimotope target (48). Furthermore, CD8 T cells that exhibit high avidity for a model pancreatic Ag can be isolated from NOD mice bearing the model Ag on the pancreas. In contrast, BALB/c mice, bearing the same model Ag on the pancreas, retain only low-avidity CD8 T cells for the immunodominant epitope (49). Although priming to self-Ag must occur to initiate autoimmunity, these observations underscore the importance of events subsequent to the priming of autoreactive T cells in the development of a full autoimmune phenotype.

Apoptotic cell death can enable Ag uptake by professional APCs (21, 22). Cell death can thus enhance the availability and
quantity of self-Ag to be sampled and detected by the immune system. For instance, apoptotic cells derived from the gut can be transported to the T cell areas of the mesenteric lymph nodes by a subpopulation of DCs (50). Such sampling of apoptotic cells has been associated with tolerance induction. An apoptotic cell load can also promote the maturation of DCs (23). Indeed, injection of large numbers of apoptotic cells has been associated with the induction of autoimmune responses (51). Therefore, we sought to determine whether cell death can also enhance immune responses to co-administered Ag in an adjuvant-like fashion. Our results show that co-injection of dead cells with soluble Ag does indeed result in the enhanced generation of effector cytotoxic CD8 T cells specific for the co-injected Ag. The mechanism of this adjuvancy is still unclear, but the release of endogenous heat shock proteins from necrotic cells (52) and the release of cytokines and other “danger” signals from cells undergoing apoptosis may be contributory.

Whereas priming of diabetogenic CD8 T cells is negligible in 2-wk-old animals, it is clearly detected in 4-wk-old animals. Enhanced priming at 4 wk of age compared with 2 wk of age may be attributable to multiple factors. Activation of APCs by interaction with CD4 T cells and subsequent DC licensing after CD40-CD40 ligand interactions is one such possibility (53–55). However, neither co-injection of naive diabetogenic CD4 T cells nor co-injection of an activating CD40 Ab resulted in enhanced or earlier CD8 T cell priming in 2-wk-old mice (data not shown). This suggests that other mechanisms, such as Ag availability or the presence of currently undefined signals that promote DC maturation, are operant. Autoreactive CD8 T cell priming was found to occur subsequent to the natural increase in β cell death in the pancreas (32). Whether this sequencing is causally related is unclear. Because cell death can both increase sampling of self-Ag and have adjuvant properties, we next determined whether increasing cell death by administration of a pancreatic β cell toxin could enhance priming. STZ has been shown to increase the incidence of pancreatic β cell apoptosis both in situ (26) and in a β cell line in vitro (39). The resistance of PARP-deficient mice to the β cell cytotoxic effects of STZ (42–44) suggests that a primary mode of action of this toxin is via PARP-mediated NAD^+ depletion, which subsequently induces β cell death. Our results demonstrate that multiple low doses of STZ facilitate priming of diabetogenic CD8 T cells in 2-wk-old mice, at an age when negligible priming otherwise occurs.

Because of the chemical reactivity of STZ, it has been suggested that one possible mechanism by which STZ may accelerate diabetes is through the generation of neoantigens. As the 8.3 CD8 T cell recognizes a self-Ag, our finding suggests that STZ enables and enhances the presentation of a chemically unmodified self-Ag. This is consistent with recent studies using mice that overexpress CD80 and a model Ag derived from lymphocytic choriomeningitis virus on pancreatic β cells (56). The ability to enhance priming of 8.3 CD8 T cells with STZ at and before 2 wk of age suggests that developmental expression of the autoantigen may not be a limiting factor. Rather, the processing and display of the Ag may be modulated. Using increasing concentrations of STZ, we confirmed that a consequence of administration of this agent was a corresponding increase in apoptotic β cell death in situ. The positive correlation between in situ cell death of pancreatic β cells with priming of a diabetogenic CD8 T cell in the pancreatic lymph nodes is consistent with the hypothesis that cell death promotes self-Ag processing. Such cell death may also release factors that promote either T cell priming or APC maturation. As multiple low doses of STZ accelerate immune-mediated diabetes in NOD mice (Ref. 45 and Fig. 6), such priming likely results in activation and not tolerance induction. Although we detected an increase in apoptotic cell death after low-dose STZ administration, we cannot rule out a concomitant increase in necrotic cell death.

Tissue damage induced by activated cytotoxic T cells can promote priming to tissue Ags (57). Autoantibodies to epitopes generated by granzyme B released during cytotoxic cell assault are a unifying theme in many autoimmune diseases (58). Although these observations argue for the importance of cellular cytotoxic mechanisms in the development of autoimmunity, these observations do not explain how the initial wave of CTLs may become activated. It has been proposed that increased cell death of keratinocytes after UV exposure may be one trigger for systemic autoimmunity in individuals predisposed to photosensitive lupus erythematosus (59, 60). Recently, the in vivo prevention of apoptosis using the irreversible caspase inhibitor carbobenzoxyvalylalanylaspartyl-(β-O-methyl)fluoromethylketone has been shown to decrease the severity of glomerular disease in a mouse model of systemic lupus erythematosus (61). Our present study is the first to link increased parenchymal cell death (in the absence of CTL assault or viral damage) to the increased priming of autoreactive T cells in the draining lymph nodes of affected tissues and the subsequent acceleration of spontaneous autoimmune diabetes. Our findings suggest that strategies aimed at decreasing pancreatic β cell death may delay the onset of autoimmunity. Clinical trials of one such strategy, using nicotinamide, are already underway (62). Nicotinamide can prevent cyclophosphamide-induced pancreatic β cell apoptosis (63) and can delay or prevent spontaneous diabetes in NOD mice (63, 64). Our observations suggest that one mechanism contributing to the protective actions of nicotinamide may be to diminish T cell priming by decreasing in situ β cell death. This hypothesis is currently being tested in our laboratory. The early timing of the β cell-specific T cell priming that we have observed would suggest that such strategies, to be optimally effective, may need to be initiated in very young predisposed individuals several years before the clinical onset of diabetes.

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