Perspectives in Diabetes
Neonatal β-Cell Apoptosis
A Trigger for Autoimmune Diabetes?

Jacqueline D. Trudeau, Jan P. Dutz, Edith Arany, David J. Hill, Warren E. Fieldus, and Diane T. Finegood

In neonatal rodents, the β-cell mass undergoes a phase of remodeling that includes a wave of apoptosis. Using both mathematical modeling and histochemical detection methods, we have demonstrated that β-cell apoptosis is significantly increased in neonates as compared with adult rats, peaking at ~2 weeks of age. Other tissues, including the kidney and nervous system, also exhibit neonatal waves of apoptosis, suggesting that this is a normal developmental phenomenon. We have demonstrated that increased neonatal β-cell apoptosis is also present in animal models of autoimmune diabetes, including both the BB rat and NOD mouse. Traditionally, apoptosis has been considered a process that does not induce an immune response. However, recent studies indicate that apoptotic cells can do the following: 1) display autoreactive antigen in their surface blebs; 2) preferentially activate dendritic cells capable of priming tissue-specific cytotoxic T-cells; and 3) induce the formation of autoantibodies. These findings suggest that in some circumstances physiological apoptosis may, in fact, initiate autoimmunity. Initiation of β-cell–directed autoimmunity in murine models appears to be fixed at ~15 days of age, even when diabetes onset is dramatically accelerated. Taken together, these observations have led us to hypothesize that the neonatal wave of β-cell apoptosis is a trigger for β-cell–directed autoimmunity. *Diabetes* 49:1–7, 2000

A poptosis, also known as programmed cell death, is a normal process contributing to tissue turnover during development and in the adult. During development, apoptosis is involved in diverse processes such as negative selection in the thymus, deletion of structures that are needed exclusively during one stage of development, and sculpting various parts of the body (1). In addition, apoptosis is involved in tissue homeostasis and eliminating nonfunctional, superfluous, or harmful cells. In the adult, in whom cell proliferation is estimated to occur at a rate of 100,000 cells per second, apoptosis acts as a counterbalance to maintain tissue size (2). Apoptosis also accounts for many cell deaths in response to cellular stress, such as viral infection or exposure to cytotoxic compounds, and may function in the pathogenesis of degenerative diseases (3).

The contribution of apoptosis to normal tissue homeostasis has generally been underappreciated because, even at high rates, it is "histologically inconspicuous" (4). Although cells undergoing apoptosis display distinct morphological features, rapid clearance of apoptotic cells generally means that very few cells can be classified as apoptotic at any given time (1). In the developing rat thymus, it has been estimated that ~97% of newly formed thymocytes die by apoptosis (5). However, through histological analysis, only 0.2% of cells can be identified as apoptotic at any given time, suggesting that apoptotic cells are cleared within minutes (4). Even in the kidney, where 50% of perinatal cells are thought to die by apoptosis and clearance occurs within 1–2 h, no more than 3% of cells can be identified with the characteristic morphology of apoptotic cell death (4). Because apoptosis is difficult to detect, the contribution of apoptosis to remodeling in the neonate has been documented in only a limited number of tissues. Neonatal increases in apoptosis have been detected in the nervous system (6–8), kidney (4), cardiac myocytes and arteries (9,10), male germ cells (11), and adrenal cortex (12).

In 1995, Finegood et al. (13) described a postnatal wave of β-cell death in the developing rat pancreas using a mathematical model of β-cell mass dynamics. The rate at which the β-cell mass grows is determined by the rate of new cell formation and the rate of cell loss. New cells form by replication of existing β-cells, or through differentiation of precursors, a process referred to as neogenesis. Cell deletion can occur by apoptosis or necrosis, a form of cell death associated with extreme stress of the cellular microenvironment (14). To understand how replication, neogenesis, cell death, and the growth of the β-cell mass are interrelated, we modeled β-cell turnover with the mass balance equation:

$$REP + NEO - DEATH = d(\beta\text{-cell number})/dt$$

in which REP is the rate of β-cell replication, NEO is the rate of β-cell neogenesis, DEATH is the rate of β-cell death, and d(β-cell number)/dt is the rate of change of the number
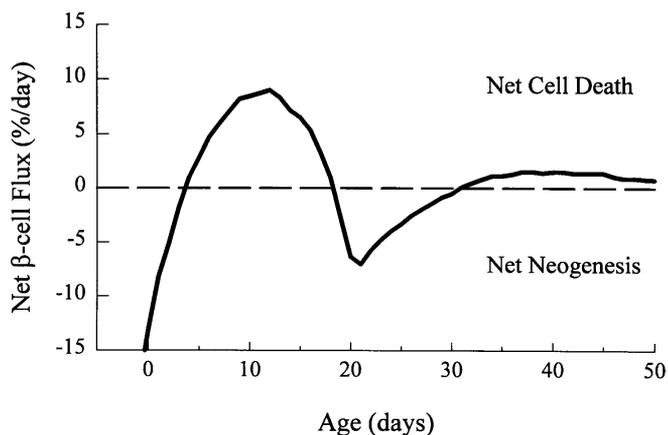
FIG. 1. Predictions of net cell death and net neogenesis as a function of age in normal Sprague-Dawley rats (13). The y-axis denotes "net β-cell flux," which equals the rate of cell death minus the rate of neogenesis. A positive value indicates that total cell death is greater than the formation of new cells by neogenesis. A negative value indicates that neogenesis is greater than total cell death. The growth of the β-cell mass is determined by this net cell flux together with the rate of β-cell replication (not shown).

of β-cells, estimated from the β-cell mass and average β-cell size over time (13).

In Sprague-Dawley rats, β-cell mass increases almost linearly until ~100 days of age, except for a plateau in the rate of growth from 5 to 20 days of age (13). β-Cell replication is initially ~10% per day at birth and falls exponentially to 2–3% per day in adult rats. Through application of the above model to these data, we predicted that the plateau in β-cell growth was due to a wave of β-cell death with a peak rate of cell loss of 9% per day at 12 days of age (Fig. 1). Since the rate of neogenesis is unknown, this estimate is "net" cell death (DEATH – NEO), and the absolute rate of cell death (DEATH) may be higher.

Subsequently, the model-based prediction of a neonatal wave of β-cell death was proven valid through direct histochemical detection of apoptotic cells. Using propidium iodide staining to morphologically identify apoptotic cells, we detected an increased number of apoptotic β-cells in neonatal Sprague-Dawley rats compared with adult animals (15). The apoptotic index peaked at 13 days of age (3.5 ± 0.4%), coincident with the plateau in β-cell mass growth and the peak of β-cell death that was predicted by our model. The proportion of apoptotic β-cells was elevated in all neonatal animals (for example, 1.5 ± 0.2% at 2 days of age) as compared with adult rats (0.4 ± 0.1% at 3 months of age). In a separate study using Wistar rats, Petrik et al. (16) demonstrated a wave of apoptosis in neonatal islet cells using terminal dUTP nick-end labeling (TUNEL). The number of apoptotic islet cells peaked at 14 days of age (13.2 ± 3.4%) compared with 5.9 ± 1.2 and 0.2 ± 0.1% in 4-day-old and adult animals, respectively. These histological indices of apoptosis provide qualitative validation of the model-based prediction of the neonatal wave of β-cell apoptosis. They cannot, however, provide a quantitative assessment of the rate of apoptosis, since the rate at which apoptotic β-cells are cleared is unknown. The reason for the discrepancy in apoptotic indices between studies is also not known, but it may be due to differences in

the clearance rate of propidium iodide and TUNEL-stained apoptotic cells, or the time over which each of these markers is visible.

Together, these studies have established that a neonatal wave of β-cell apoptosis exists in normal developing rats, peaking at ~12–14 days of age. As in other tissues, this wave of cell death is not easily detected. However, a dramatic increase in cell death must occur before weaning to account for the rate at which the β-cell mass proliferates and grows. Our model suggests that at least 60% of preexisting β-cells die during this remodeling phase. The rapidity with which apoptotic cells are cleared limits our ability to morphologically detect this wave of β-cell apoptosis. However, even with this limitation, a doubling in the number of apoptotic β-cells is detectable between 1 and 2 weeks of age.

The functional significance of this remodeling phase during the neonatal period is unknown. In other systems, such as in male germ cells, cardiac myocytes, or the adrenal cortices, similar waves of neonatal apoptosis contribute to tissue remodeling coincident with changing function or cellular demand (9,11,12). In the pancreas, the wave of β-cell apoptosis occurs just before weaning and may prepare the β-cell mass for changing functional demands.

COULD THE HIGH RATE OF NEONATAL β-CELL APOPTOSIS HAVE OTHER CONSEQUENCES BEYOND TISSUE REMODELING?

Infiltration of immune cells into the islets of Langerhans appears to be fixed at ~15 days of age in NOD mice and transgenic NOD models with accelerated onset of autoimmune diabetes (17–20). This infiltration is coincident with the neonatal wave of β-cell apoptosis in normal rats. Although apoptosis has traditionally been considered a noninflammatory event, many studies have now shown that immune responses may result from the apoptotic process. In this article, we consider the hypothesis that the neonatal wave of β-cell apoptosis may trigger β-cell–directed autoimmunity. The high rate of apoptosis during this time could provide a source of antigen for presentation to the immune system, which, in genetically susceptible individuals, results in priming of autoreactive β-cell–specific T-cells.

DOES A NEONATAL WAVE OF β-CELL APOPTOSIS EXIST IN AUTOIMMUNE ANIMAL MODELS OF DIABETES?

To establish the mechanism by which neonatal β-cell apoptosis may contribute to autoimmunity, we first sought to characterize β-cell turnover during the neonatal period in animal models of spontaneous autoimmune diabetes, the BB rat and NOD mouse. In the BB rat, autoimmune diabetes develops in ~70–80% of the diabetes-prone strain (BBdp), characterized by an invasive infiltration of mononuclear cells into the islets, a process termed insulitis (21). Using our model-based approach, we have demonstrated that a wave of apoptosis exists in the neonatal BB rat (Fig. 2) (22). The wave of β-cell apoptosis is predicted to occur around 5–25 days of age, with a magnitude that is similar in the BBdp and the diabetes-resistant (BBdr) strains.

The NOD mouse develops autoimmune diabetes, which occurs in 80–90% of females and 10–40% of males by 4–6 months of age. Infiltration of the islets by mononuclear cells becomes obvious at ~5 weeks of age (23). Using the TUNEL method, we (E.A., D.J.H.) observed a neonatal wave of β-cell
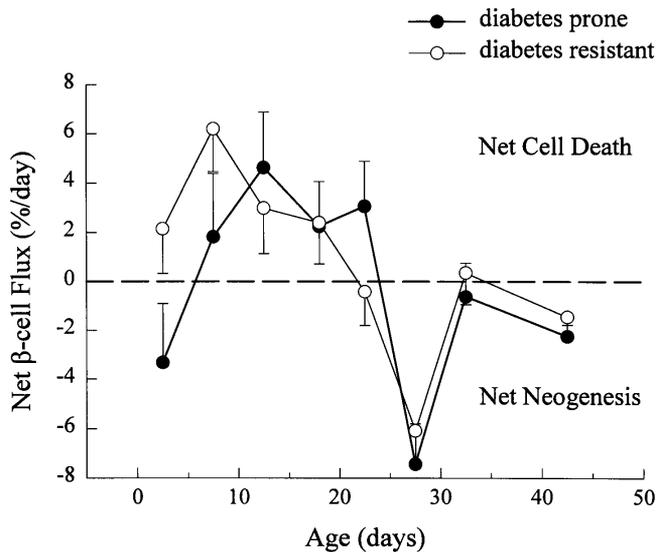
FIG. 2. Net β-cell death and net neogenesis in young diabetes-prone (BBdp) and diabetes-resistant (BBdr) BioBreeding rats. Data were generated based on β-cell mass and replication rates using the mathematical model of Finegood et al. (13). No significant differences were found between BBdp and BBdr rats at any age. No insulitis was detected before 25 days of age, and very mild peri-insulitis was present between 25 and 50 days of age in diabetes-prone animals.
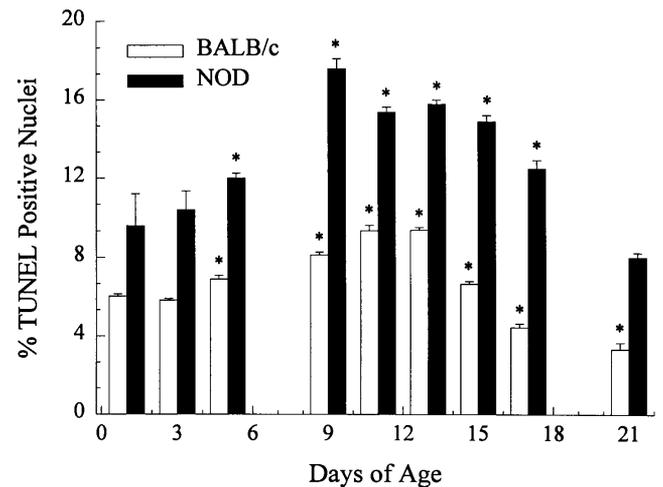

FIG. 3. Islet cell apoptosis (% TUNEL-positive nuclei) as a function of age in female NOD and BALB/c mice. Sections were stained with the ApopTag Peroxidase Detection Kit (Intergen, Purchase, NY) for detection of apoptotic nuclei and counterstained with Carazzi's hematoxylin. Five sections per animal (n = 5 at 1–17 days of age, n = 3 at 21 days of age) were systematically counted for all TUNEL-positive and TUNEL-negative islet cells. There was a significant difference (P < 0.001) between BALB/c and NOD mice at each age examined. *Significant difference from 1 day of age (P < 0.001).

apoptosis with an increase in the number of apoptotic cells beginning at 5 days of age in both NOD and control BALB/c mice (Fig. 3). Interestingly, the apoptotic index in the BALB/c mice was considerably less than in the NOD mice throughout the neonatal period, suggesting that the rate of apoptosis in this model of autoimmune diabetes is increased.

These two studies demonstrate that a neonatal wave of β-cell apoptosis does exist in autoimmune animal models of diabetes. Using two very different approaches in two distinct rodent models, we have demonstrated that β-cell apoptosis increases during the second week of life and decreases around the time of weaning. Using our model-based method, BBdp and BBdr rats were found to have similar waves of apoptosis. In contrast, the apoptotic index was increased in diabetes-prone NOD mice as compared with normal BALB/c mice. Whether differences in the actual rate of β-cell apoptosis exist between animals that develop autoimmune diabetes and normal strains, or whether differences exist between BB rats and NOD mice, remains to be clarified.

COULD APOPTOSIS BE RESPONSIBLE FOR THE INITIATION OF AUTOIMMUNITY?

Apoptosis has been traditionally thought of as a noninflammatory process. The apoptotic pathway involves membrane blebbing, followed by shrinkage of the cytoplasm, nuclear condensation, DNA fragmentation, and dissociation from surrounding cells. Many membrane-bound apoptotic bodies are formed and subsequently phagocytosed by neighboring cells or specialized phagocytes, without discharge of the cellular contents into the surrounding environment. Thus, the apoptotic pathway is thought to prevent the induction of an immune response (14). In contrast, cell death by necrosis involves swelling of cellular organelles and bursting of the plasma membrane leading to spillage of cellular content (1).

Necrosis is usually associated with a local inflammatory response that includes activation of macrophages, priming and proliferation of T- and B-cells, and secretion of cytokines that augment the inflammatory process.

Recent evidence, however, has demonstrated that apoptotic cells can induce immune responses. In vitro culture of apoptotic cells with dendritic cells resulted in presentation of antigen and stimulation of both major histocompatibility complex (MHC) class I–restricted CD8^{+} T-cells (24,25) and MHC class II–restricted CD4^{+} T-cells (25,26). In another study, macrophages were also able to present antigen from apoptotic cells and activate CD8^{+} T-cells (27). Uchimura et al. (28) found that incubation of apoptotic cells with peritoneal macrophages resulted in greater secretion of proinflammatory (T_H1) cytokines when compared with incubation with non-apoptotic cells, although Fadok et al. (29) found that incubation of macrophages with apoptotic cells provoked secretion of anti-inflammatory (T_H2) cytokines.

In vivo studies also support a causal relationship between the presence of apoptotic cells and activation of an immune response. Mevorach et al. (30) recently demonstrated that systemic exposure of normal mice to syngeneic apoptotic thymocytes induced the production of autoantibodies involved in the autoimmune disease systemic lupus erythematosus (SLE). Autoantibodies from the sera of patients with SLE often recognize epitopes that result from apoptosis (31). Casciola-Rosen et al. (32) linked apoptosis and autoimmunity with the observation that antigens for autoantibodies in SLE could be found in clusters in the surface blebs of two distinct populations of apoptotic keratinocytes. Kurts et al. (33) demonstrated that T-cell–mediated tissue destruction, which likely occurred by apoptosis (34), revealed otherwise unavailable antigens resulting in stimulation of naïve T-cells. Finally, it has been suggested that exposure of phospholipids during the apoptotic process is the antigenic stimulus driving the pro-

duction of antiphospholipid antibodies (35). Together, these studies provide compelling evidence to suggest that cell death via the apoptotic pathway can initiate immune responses, and, in susceptible individuals, could invoke an autoimmune response.

COULD β-CELL APOPTOSIS LEAD TO THE INITIATION OF AUTOIMMUNE DIABETES?

Several studies have demonstrated that β-cell apoptosis precedes the development of insulitis in models of type 1 diabetes. O'Brien et al. (36) quantified β-cell apoptosis in mice injected with multiple low doses of the β-cell toxin streptozotocin (STZ). β-Cell apoptosis increased and initially peaked 5 days after the first day of treatment. This first wave of apoptosis preceded the appearance of insulitis, which began on day 9. β-Cell apoptosis peaked again on day 11 when lymphocytic infiltration of the islets was maximal. These data suggest that STZ-induced β-cell apoptosis triggered an immune response that led to additional β-cell death, although the specificity of this response has been questioned (37,38). O'Brien et al. (39) found also that β-cell apoptosis preceded insulitis in NOD mice. In addition, β-cell apoptosis has been observed before the appearance of insulitis in transgenic mouse models of accelerated diabetes (18,19). Transgenic overexpression of tumor necrosis factor-α (TNF-α) in β-cells was associated with β-cell apoptosis at 7–10 days of age, a few days before T-cell infiltration (18). β-Cell apoptosis also preceded insulitis in NOD mice overexpressing diabetogenic T-cells (19). Although these studies do not demonstrate a causal link between β-cell apoptosis and initiation of insulitis, the temporal association provides support for our hypothesis that β-cell apoptosis may trigger autoimmune diabetes.

Additional support for the temporal association between the neonatal wave of β-cell apoptosis and subsequent initiation of insulitis is derived from animal models of accelerated autoimmune diabetes. Accelerated models have been established by forced expression of islet-specific T-cells (40,41), by transgenic expression of the co-stimulatory molecule B7 (42) or TNF-α (18) in islets, and by treatment with cyclophosphamide (43) or anti-CTLA-4 (20). In these models, the time required for disease development is greatly reduced, with the onset of hyperglycemia occurring as early as 17 days of age (20). Despite rapid disease development, the time at which initiation of insulitis occurs appears to be fixed at ~15 days of age (18–20), although this has not been specifically examined in all models. That the initiation of insulitis does not occur before ~15 days of age in any of these rodent models of spontaneous disease suggests that a critical event occurs at this point in time. The neonatal wave of β-cell apoptosis is concomitant with this critical period, suggesting that it may be the initiating event for development of autoimmunity.

WHY DOESN'T β-CELL–DIRECTED AUTOIMMUNITY BEGIN BEFORE 15 DAYS OF AGE?

To initiate an autoimmune attack in genetically susceptible individuals, β-cell autoantigens must be available to antigen-presenting cells (APCs). APCs, with the appropriate co-stimulatory molecules, can then prime T-cells and induce their proliferation (44). Autoimmunity cannot be initiated if there is insufficient or unavailable antigen, if no APCs are present, if the APCs are unable to present antigen, or if T-cells are incapable of being activated. Some insight into these possibilities

is gained from recent work by Höglund et al. (45), who demonstrated that T-cells from 10-day-old mice are fully functional. β-Cell–specific CD4+ T-cells from 10-day-old transgenic mice with a high proportion of T-cells bearing β-cell–specific T-cell receptors (BDC 2.5 TCR) were successfully primed when transferred into adult mice lacking conventional T-cells (Cα°/NOD). In contrast, T-cells from adult BDC 2.5 TCR transgenic mice transferred into 10-day-old Cα°/NOD recipients did not proliferate in the pancreatic lymph node. This finding suggests that in the pancreas of 10-day-old animals, there is either insufficient or unavailable antigen to stimulate T-cells, or that APCs are unavailable or unable to present antigen. Yet, by 15 days of age, T-cells in the pancreatic lymph node are activated and the first signs of islet insulitis are detected (45). The activation of T-cells between 10 and 15 days of age suggests that during this window, either antigen is made available to functional APCs, or APCs become functional.

We gain further insight from additional experiments by Höglund et al. (45), which suggest that APCs in 10-day-old animals are capable of presenting antigen. Naïve CD8+ T-cells specific for ovalbumin (OVA) were transferred into 10-day-old transgenic mice expressing the OVA protein on the rat insulin promoter (RIP-OVA). These mice express significant amounts of OVA protein on β-cells and in the kidney (46). Proliferation of T-cells was detected in lymph nodes draining the kidney, suggesting that APCs of the 10-day-old animal are able to prime CD8+ T-cells. Surprisingly, no proliferation of T-cells was detected in lymph nodes draining the pancreas, suggesting that regional differences in APC function or organ-specific differences in availability of antigen for presentation exist. Collectively, these data suggest that autoimmunity is not initiated before 15 days of age because there is insufficient or unavailable antigen to stimulate β-cell–specific T-cells, rather than because there is a lack of T-cell or APC function or availability. The neonatal wave of apoptosis that occurs during this critical time could be the source of previously unavailable antigen.

COULD DEVELOPMENTAL APOPTOSIS ACCOUNT FOR ORGAN-SPECIFIC DIFFERENCES IN AUTOANTIGEN AVAILABILITY?

Developmental waves of apoptosis have been observed in the neonatal kidney (4). In the medullary papilla, <1% of cells appeared apoptotic at 2 days of age. The apoptotic index increased to 3.2% at 6–7 days of age and then fell to <0.1% by 14 days of age. In the nephrogenic zone, the apoptotic index was highest (2.7%) in the late-stage embryo and fell to <0.2% by 14 days of age. Thus, in contrast to the pancreas, developmental apoptosis in the kidney peaks before 10 days of age. If autoantigen derived from developmental apoptosis is required for T-cell priming, then T-cell proliferation will occur in the kidney, but not in the pancreas, at 10 days of age. As noted, when naïve OVA-specific T-cells were transferred into 10-day-old RIP-OVA transgenic mice, they were found to proliferate in the lymph nodes draining the kidney, but not the pancreas. Thus, organ-specific developmental apoptosis may account for differences in autoantigen availability and consequent T-cell priming.

Thus, considerable evidence supports a link between β-cell apoptosis and the initiation of autoimmunity. β-Cell apoptosis precedes insulitis in spontaneous, induced, and accelerated
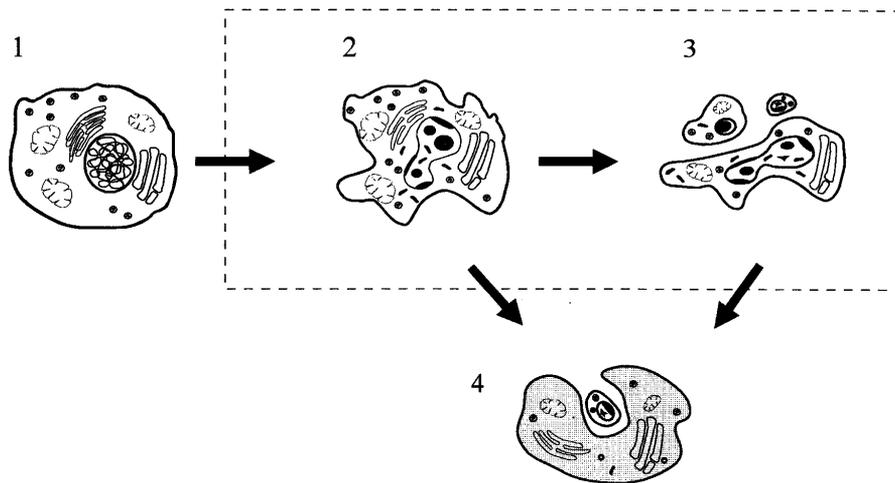
FIG. 4. Morphological recognition of apoptosis at various stages in the apoptotic pathway. 1: Normal cell morphology. 2: The initial stages of apoptosis, including blebbing of the plasma membrane, chromatin condensation, and DNA fragmentation. 3: Cell fragmentation into many apoptotic bodies that are often very small and chromatin dense. 4: Finally, apoptotic cells are engulfed by phagocytes and degraded. Phagocytes are capable of recognizing apoptotic cells during early (2) and late (3) apoptosis. The number of apoptotic cells evident at any given time by light microscopy with traditional histochemical detection is dependent on both the rate of apoptosis (1→2) and the rate of clearance (2→4, 3→4). Cells are generally visible during the stages outlined in the dashed box. An increase in the rate of apoptosis and/or a decrease in the rate of clearance increases the number of apoptotic cells that are identified using these methods.

models of autoimmune diabetes. In spontaneous murine models, initiation of autoimmunity appears to be fixed at ~15 days of age, subsequent to the peak in the neonatal wave of β-cell apoptosis. Developmental waves of apoptosis are not unique to the pancreas, but their timing appears to be organ specific, and organ-specific differences in developmental apoptosis correlate with differences in T-cell priming. Together, these observations suggest that developmental waves of apoptosis, in particular the neonatal wave of β-cell apoptosis, may provide the autoantigen necessary for initiation of a β-cell–directed autoimmune response.

WHY DOESN'T EVERYONE GET AUTOIMMUNE DIABETES?
The neonatal wave of β-cell apoptosis is present in both diabetes-prone and diabetes-resistant animal models. If the neonatal wave provides the antigen necessary for priming β-cell–specific T-cells, why don't all animals develop insulitis?

The rate of apoptosis is elevated throughout the neonatal period as compared with adult life. Yet, the initiation of insulitis appears to be coincident with the peak of the neonatal wave of β-cell apoptosis, suggesting that the rate of apoptosis may be important. We observed a 1.5- to 3-fold greater number of TUNEL-positive β-cells in NOD as compared with BALB/c mice during the neonatal period (Fig. 3), suggesting that in this model, the rate of β-cell apoptosis may account for the development of autoimmunity in NOD mice. The importance of the apoptotic rate is consistent with the findings of Rovere et al. (25), who demonstrated that apoptotic cell numbers affected immune responses. Co-culture of apoptotic cells with dendritic cells at a 5:1 ratio resulted in activation of both CD4+ and CD8+ T-cells, accompanied by secretion of the proinflammatory (T_H1) cytokines interleukin-1β and TNF-α. Activation of T-cells and the accompanying cytokine secretion were not detected with an apoptotic:dendritic cell ratio of 1:1.

Although apoptotic rate may be important, the finding of increased TUNEL-positive β-cells in NOD mice (Fig. 3) con-

trasts with our observation of similar waves of β-cell apoptosis in diabetes-prone and diabetes-resistant BB rats (Fig. 2). This apparent paradox might be due to differences in the mechanism of disease initiation between BB rats and NOD mice, but it may also result from differences in methodology. TUNEL positivity is generally assumed to reflect the rate of apoptosis. However, the time over which morphologic evidence of apoptosis is visible depends not only on the rate at which apoptosis occurs, but also the rate at which the apoptotic debris is cleared (Fig. 4). Therefore, an increase in TUNEL positivity does not necessarily reflect an increase in apoptosis, but may reflect a decrease in clearance. Figure 5 illustrates that the increase in TUNEL positivity in NOD mice as compared with that in BALB/c mice can be accounted for by modeling a 50% decrease in clearance rate.

In contrast to the NOD mouse, we found no difference between diabetes-prone and diabetes-resistant BB rats using a model-based method for estimating the rate of β-cell death. The model-based method is not dependent on morphological evidence of apoptosis and therefore is independent of the clearance rate of apoptotic debris. Thus, if the neonatal wave of β-cell apoptosis is the same, and defective clearance of apoptotic debris is what distinguishes diabetes-prone from diabetes-resistant animals, then our observations in the BB rat and NOD mouse are consistent.

Apoptotic debris is cleared through phagocytosis (47). Phagocytes capable of clearing apoptotic β-cells include macrophages, dendritic cells, and perhaps neighboring β-cells (47). The relative contribution of each cell type to clearance of the debris from the neonatal wave of β-cell apoptosis is unknown. Consistent with the idea that defective clearance may explain differences in the development of autoimmune diabetes, defective phagocyte function has been observed in both NOD mice and BB rats. Defects observed in macrophages of NOD mice include reduced recognition of opsinized particles (48), decreased cytokine secretion and MHC class I expression (49), and less effective processing and
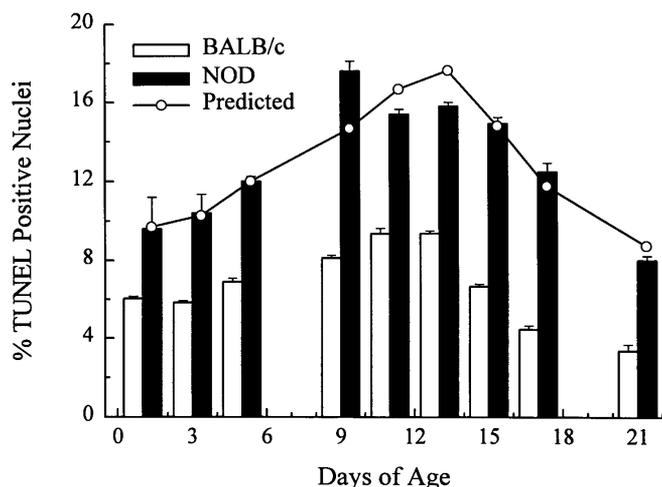
FIG. 5. The number of TUNEL-positive islet cells in NOD mice can be predicted by a 50% decrease in clearance rate of apoptotic cells. The number of TUNEL-positive islet cells is determined by the balance between the rate of apoptosis and the rate of clearance of the apoptotic cells. The rates of apoptosis and clearance in normal BALB/c mice were estimated from the time course of BALB/c TUNEL positivity (□). By assuming a similar rate of apoptosis in NOD and control BALB/c mouse islets, and a 50% decrease in the rate of clearance of the TUNEL marker, the predicted TUNEL staining in NOD mouse islets (–○–) is similar to the observed results (■).

presentation of antigenic material (50). NOD macrophages have also been shown to produce unusually high levels of prostaglandin-E2, which leads to inhibition of macrophage and dendritic cell function (51). In addition, NOD mice have fewer macrophages than normal mice (49). Aberrant dendritic cell function has also been observed in BB rats (52,53). Defective clearance of apoptotic debris during the neonatal wave of β-cell apoptosis provides a mechanism whereby increased TUNEL positivity would be observed. Delayed phagocytosis of apoptotic debris may result in the release of potentially immunogenic cellular contents as apoptoic cells undergo secondary necrosis (14). The presence of an increased apoptotic load in combination with defective APC function may lead to presentation of β-cell epitopes in an immunogenic fashion, and a subsequent immune response. We speculate that altered phagocytic function in neonatal diabetes-prone animals may be a critical factor in determining whether the β-cells will be targeted for autoimmune attack. Defective clearance of apoptotic cells has been implicated in the etiology of the autoimmune disease SLE (54).

SUMMARY

This article considers the role of neonatal β-cell apoptosis in triggering autoimmune diabetes. We have previously demonstrated that a neonatal wave of β-cell apoptosis exists in normal rats (13). A similar neonatal wave of β-cell apoptosis exists in the NOD mouse and BB rat, peaking at ~13 days of age (Figs. 2 and 3). Initiation of insulitis in accelerated murine models of autoimmune diabetes appears to be fixed at ~15 days of age, immediately following this marked increase in β-cell apoptosis (18–20). Although apoptosis has traditionally been considered an innocuous event that does not involve an inflammatory response, there are many studies that clearly show that apoptosis can invoke a rapid

response from the immune system (25–33,35). Together, these data suggest that the neonatal wave of β-cell apoptosis may provide the autoantigen necessary for triggering β-cell–directed autoimmunity.

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