

Autoimmune Destruction of Pancreatic β Cells

Ji-Won Yoon* and Hee-Sook Jun

Type 1 diabetes results from the destruction of insulin-producing pancreatic β cells by a β cell-specific autoimmune process. β Cell autoantigens, macrophages, dendritic cells, B lymphocytes, and T lymphocytes have been shown to be involved in the pathogenesis of autoimmune diabetes. β Cell autoantigens are thought to be released from β cells by cellular turnover or damage and are processed and presented to T helper cells by antigen-presenting cells. Macrophages and dendritic cells are the first cell types to infiltrate the pancreatic islets. Naive $CD4^+$ T cells that circulate in the blood and lymphoid organs, including the pancreatic lymph nodes, may recognize major histocompatibility complex and β cell peptides presented by dendritic cells and macrophages in the islets. These $CD4^+$ T cells can be activated by interleukin (IL)-12 released from macrophages and dendritic cells. While this process takes place, β cell antigen-specific $CD8^+$ T cells are activated by IL-2 produced by the activated T_H1 $CD4^+$ T cells, differentiate into cytotoxic T cells and are recruited into the pancreatic islets. These activated T_H1 $CD4^+$ T cells and $CD8^+$ cytotoxic T cells are involved in the destruction of β cells. In addition, β cells can also be damaged by granzymes and perforin released from $CD8^+$ cytotoxic T cells and by soluble mediators such as cytokines and reactive oxygen molecules released from activated macrophages in the islets. Thus, activated macrophages, T_H1 $CD4^+$ T cells, and β cell-cytotoxic $CD8^+$ T cells act synergistically to destroy β cells, resulting in autoimmune type 1 diabetes.

Keywords: autoimmune diabetes, destruction of β cells, β cell autoantigens, T cells, macrophages/dendritic cells

INTRODUCTION

Type 1 diabetes results from insulin deficiency caused by the loss of insulin-producing pancreatic β cells,^{1–3} generally develops in the young, and accounts for approximately 5%–10% of the diabetic population worldwide. The development of type 1 diabetes is the consequence of progressive β cell destruction by autoimmune processes during an asymptomatic period that often extends over many years. Genetic susceptibility is believed to be a prerequisite for the development of type 1 diabetes.^{4,5} However, studies on the development of the disease in identical twins

showed that their concordance rate is only 40%,⁶ suggesting that environmental or nongenetic factors contribute to the development of the disease. Histologic analysis of the pancreas from patients with recent-onset type 1 diabetes revealed an infiltration of the islets of Langerhans by mononuclear cells,⁷ which were later identified as T and B lymphocytes, monocytes/macrophages, and natural killer (NK) cells.^{8,9} As well, circulating islet-reactive autoantibodies¹⁰ and islet-reactive T cells have been found in patients with type 1 diabetes.^{11–14}

The pathogenesis of autoimmune type 1 diabetes has been extensively studied using 2 animal models, the nonobese diabetic (NOD) mouse and the diabetes-prone BioBreeding (DP-BB) rat, which have greatly enhanced our understanding of the pathogenesis of human type 1 diabetes. Although the exact mechanisms involved in the initiation and progression of β cell destruction are still not clear, it is generally believed that β cell autoantigens, macrophages, dendritic

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cells, B lymphocytes, and T lymphocytes are involved in the β cell-specific autoimmune process.¹⁵⁻¹⁹ β Cell autoantigens are processed by macrophages, dendritic cells, or B cells in the pancreatic islets and presented to autoreactive CD4⁺ T cells in the peripheral lymphoid system. These autoreactive CD4⁺ T cells are activated and secrete cytokines, which can activate β cell-specific cytotoxic CD8⁺ T cells. The activated T cells are recruited to the pancreatic islets and produce cytokines, which further activate macrophages and other T cells, contributing to the destruction of β cells (Fig. 1). In this review, we briefly discuss the possible roles of islet cell autoantigens, macrophages, and T cells in the autoimmune destruction of pancreatic β cells.

ISLET CELL AUTOANTIGENS

Pancreatic β cell autoantigens are the targets of immune-mediated destruction of β cells. Therefore, antigen-specific immune reactions are believed to be involved in the β cell destruction. One of the most common immunologic markers of humans and animals with autoimmune diabetes is the presence of autoantibodies and

autoreactive T cells directed against β cell autoantigens. These autoantigens include insulin,²⁰ glutamic acid decarboxylase (GAD)65,²¹ tyrosine phosphatase, insulinoma antigen (IA)-2 and IA-2 β ,^{22,23} carboxypeptidase-H,²⁴ islet cell antigen (ICA)-69,²⁵ GM gangliosides,²⁶ a 38-kd autoantigen,²⁷ and SOX13.²⁸ Autoantibodies against these β cell autoantigens are not believed to have a pathogenic role. However, the risk of developing diabetes is strongly related to the number of autoantibody markers, that is, the presence of 2 or more autoantibodies gives a higher probability of developing the disease than the presence of a single autoantibody.²⁹

Glutamic acid decarboxylase (GAD)

GAD, which is a synthetic enzyme of the inhibitory neurotransmitter γ -aminobutyric acid, has been the most extensively studied β cell autoantigen. Sera from type 1 diabetic patients was found to precipitate a 64-kd protein, which was later identified as GAD. GAD65 antibody is found in 70%–75% of type 1 diabetes patients and 1%–2% of healthy individuals.³⁰ The anti-GAD autoantibodies in type 1 diabetes patients are predominantly directed to a conformational epitope of

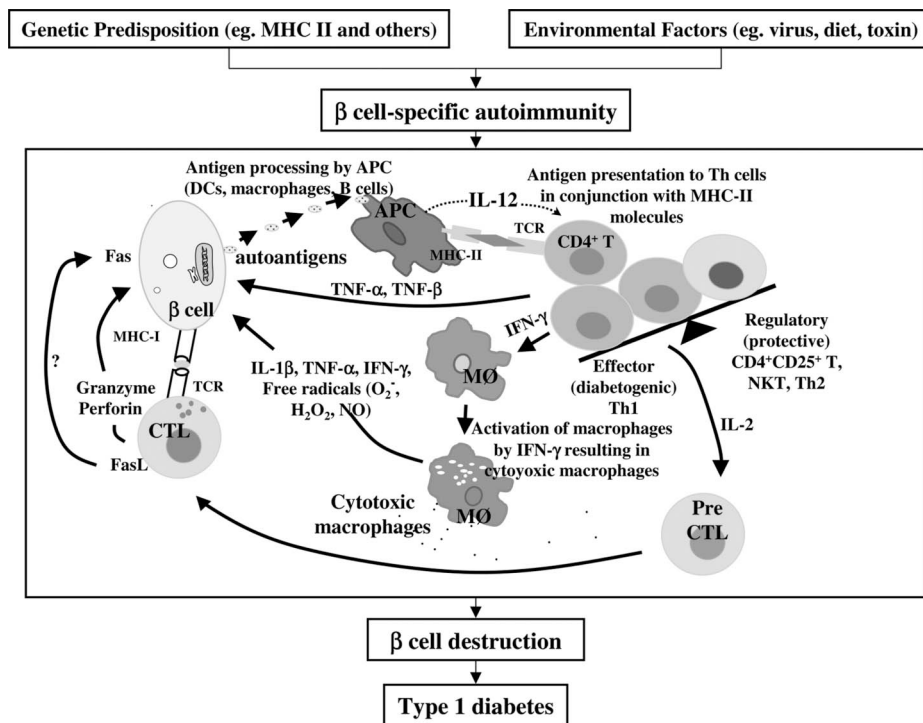


FIGURE 1. Etiology of type 1 diabetes. Genetic predisposition appears to be a prerequisite for the development of type 1 diabetes. Environmental factors such as viruses, toxins, and diet may be involved in the clinical expression of genetic susceptibility. Once β cell-specific autoimmunity has developed, autoimmune-mediated destruction of β cells results in the onset of type 1 diabetes. Autoantigens released from β cells are processed by antigen-presenting cells (APCs) and presented to helper T cells (Th cells) in association with MHC class II molecules. IL-12 released from APCs activates T_H1-type CD4⁺ T cells, causing the immune balance between effector and regulatory cells to breakdown. T_H1 cells produce IL-2, which activates β cell-specific precytotoxic T cells (Pre CTL) to become cytotoxic (CTL), and IFN- γ , which may

cause macrophages (M ϕ) to become cytotoxic. These cytotoxic macrophages release β cell-cytotoxic cytokines including IL-1 β , TNF- α , and IFN- γ , and free radicals. T_H1 cells also secrete cytokines that are directly cytotoxic to β cells. β Cell antigen-specific CD8⁺ cytotoxic T cells (CTL) recognize antigens expressed on β cells in association with MHC class I molecules. These CTLs release granzyme and perforin (cytolysin), which are toxic to β cells. In addition, Fas- and TNFR-mediated apoptosis are involved in β cell destruction. In this way, macrophages, T cells, and cytokines synergistically act to destroy β cells, resulting in the development of autoimmune type 1 diabetes.

GAD, and the major antigenic region in humans has been identified as the middle and carboxyterminal region of GAD65.^{31,32}

Although the presence of anti-GAD65 antibodies is a highly predictive marker for the development of type 1 diabetes in humans, contradictory results have been reported regarding the correlation between the presence of anti-GAD antibodies and diabetes in NOD mice. One study found that anti-GAD65 antibodies were detected in the early stages of the disease,³³ whereas another study found that the presence of anti-GAD65 antibodies was not a prerequisite for the development of diabetes in NOD/Lt and NOD/Wehi mice, which have a higher and lower incidence of diabetes, respectively, than NOD mice.³⁴ The latter study suggested that a strong humoral response to GAD may actually be associated with less destructive pathology, as indicated by the negative correlation between insulinitis and anti-GAD antibody levels.

It was found that the initial immune response directed against pancreatic islets in NOD mice is a T_H1 response against a confined region of GAD (amino acids 509–528 and 524–543) and that later responses are directed against another region of GAD and other autoantigens.³⁵ Immunization of NOD mice with purified GAD resulted in the tolerization of GAD-reactive T cells and blocked the T cell response to other β cell antigens, thus preventing diabetes.³⁵ There is also direct evidence that GAD-reactive T cells are diabetogenic in NOD mice.^{36,37} GAD-specific $CD4^+$ T cells have also been observed in recent-onset type 1 diabetic patients and their relatives at risk to develop diabetes.^{38–40} In addition to $CD4^+$ T cells, MHC class I human lymphocyte antigen (HLA)-A*0201-restricted $CD8^+$ cytotoxic T cells reactive against GAD were identified in recently diagnosed diabetic patients and in high-risk subjects, but not in healthy control subjects expressing HLA-A*0201.⁴¹ These results suggest that GAD may be an important target antigen and GAD-reactive T cells may play a pathogenic role in the destruction of pancreatic β cells in animal models and in human type 1 diabetes. However, contradictory results regarding the role of GAD in the pathogenesis of type 1 diabetes have been reported. Some GAD-reactive T cells do not have the ability to induce diabetes, and T cell responses to GAD-derived peptides were observed in mice resistant to type 1 diabetes,^{42,43} suggesting that peripheral tolerance to GAD is not associated with protection from diabetes. In addition, molecular mimicry between GAD and Cocksackie B4 virus has been hypothesized for the development of type 1 diabetes, but controversies still exist.^{44,45}

To investigate the role of GAD in the pathogenesis of autoimmune diabetes, several lines of transgenic mice have been established in which the expression of GAD

has been manipulated. Hyperexpression of GAD65 in β cells of NOD mice resulted in a lower incidence of diabetes in one line of transgenic mice and no difference in the incidence of diabetes in another transgenic line, as compared with nontransgenic control NOD mice. A quantitative difference in the expression of GAD between the 2 lines might have accounted for prevention of diabetes.⁴⁶ A transgenic NOD mouse line that expresses GAD65 in all tissues showed an accelerated onset and increased incidence of diabetes as compared with control NOD mice.⁴⁷ Interestingly, β cell-specific suppression of GAD65 and GAD67 expression prevented insulinitis and diabetes in anti-sense-GAD transgenic mice backcrossed with NOD mice.^{48,49} These results suggest that the expression of GAD in pancreatic β cells is involved in the modulation of β cell-specific autoimmunity. Recently, it was reported that expression of a modified form of GAD under the control of the invariant chain promoter in NOD mice induced tolerance to GAD65, but failed to prevent insulinitis and diabetes,⁵⁰ suggesting that inhibition of the generation of GAD65-reactive T cells may not be the mechanism for the prevention of diabetes in GAD-suppressed NOD mice. However, GAD67-reactive T cells were not tolerized in this study. Because GAD67 is the dominant form in mice and any level of GAD expression in NOD mice results in the development of diabetes, further study on the tolerization of both GAD65 and GAD67-reactive T cells remains to be done to determine the precise role of GAD-reactive T cells in the development of diabetes.

Another possibility regarding the prevention of β cell destruction in GAD-suppressed NOD mice is that suppression of GAD in β cells may have rendered the β cells more resistant to destruction by T cells and/or macrophages because normal β cells expressing GAD show expression of tissue transglutaminase, which is known to promote apoptosis of cells, whereas tissue transglutaminase is suppressed in GAD-suppressed β cells. A further possibility is that a diabetes-resistant gene from the strain of origin might have been transmitted to the transgenic offspring, as these antisense GAD transgenic mice were produced using eggs from (SJL \times C57BL/6) F2 mice, which are diabetes resistant. Systemic GAD65 knockout mice backcrossed with NOD mice for 4 generations still developed diabetes and insulinitis similar to wild-type NOD mice.⁵¹ However, it is difficult to draw any definite conclusions from this study, as mouse β cells predominantly express GAD67 and very low levels of GAD65, and these GAD65 knockout mice still express GAD67. Therefore, β cell-specific conditional GAD65/67 knockout NOD mice are essential to find whether the expression of GAD in β cells truly plays

a critical role in the initiation of β cell-specific autoimmune diabetes.

Insulin

Insulin was the first diabetes-related autoantigen to be discovered. Autoantibodies to insulin are found in 50%–70% of type 1 diabetic children⁵² and are the first sign of an ongoing autoimmune process.⁵³ It was also reported that the early appearance of insulin autoantibody predicts early development of diabetes in NOD mice.⁵⁴ Insulin B chain-specific CD4⁺ T cell clones accelerate diabetes in NOD.scid mice.⁵⁵ Transgenic expression of mouse proinsulin II under a MHC class II gene promoter prevented the development of diabetes in NOD mice.⁵⁶ These results suggest that insulin autoantigen plays an important role in the development of type 1 diabetes. However, the pathogenic role of insulin autoantibody and insulin-reactive T cells needs further investigation.

IA-2/IA-2 β

IA-2 and IA-2 β are newly discovered members of the protein tyrosine phosphatase family and are considered to be major autoantigens of type 1 diabetes. Initially, autoantibodies directed against a 37/40-kd tryptic fragment were detected in sera of type 1 diabetes patients.⁵⁷ Later, IA-2 was identified as the precursor of the 40-kd fragment,^{22,58} and IA-2 β (also known as phogrin) was identified as the precursor of the 37-kd fragment.^{23,59} IA-2 and IA-2 β have a high degree of homology and are located in secretory granules, but the role of these molecules in β cells is not clear. Autoantibodies to IA-2 are present in 70%–80% of type 1 diabetic children,⁶⁰ making them useful serological markers for human type 1 diabetes. In addition, T cells from diabetic patients respond to IA-2 antigen.^{11,12} However, the precise role of the IA-2 and IA-2 β antigens in the pathogenesis of type 1 diabetes is unknown.

MACROPHAGES

Macrophages and dendritic cells are among the first cell types to infiltrate the pancreatic islets during the disease process in NOD mice and BB rats.^{61–63} This infiltration precedes invasion of the islets by T lymphocytes, NK cells, and B lymphocytes.⁶⁴ It was reported that B cells also play an important role as antigen-presenting cells (APCs) for β cell antigens in the process of the development of β cell-specific autoimmunity in NOD mice.⁶⁵ The activation of β cell-reactive T cells may be initiated from the processing and presentation of β cell autoantigens by APCs in the pancreatic islets. APCs that present β cell autoantigenic peptides in

conjunction with MHC molecules migrate to the pancreatic lymph nodes where they are recognized by circulating naive β cell–autoreactive T cells. The naive T cells are then activated, migrate to the pancreatic islets, and are further activated by re-encountering the cognate β cell antigens (Fig. 2).

Inactivation of macrophages in NOD mice or BB rats significantly prevented the development of diabetes.^{66–68} Further studies showed that macrophages are required for the development of effector T cells that destroy β cells in NOD mice⁶⁹ (Fig. 3). T cells in macrophage-depleted NOD mouse recipients did not destroy transplanted NOD islets, indicating that T cells in a macrophage-depleted environment lose their ability to differentiate into cytotoxic T cells. The level of IL-4 secreted from T_H2 cells was increased, whereas the level of IFN- γ secreted from T_H1 cells was decreased in macrophage-depleted NOD mice, indicating that down-regulation of the T_H1 immune response and up-regulation of the T_H2 immune response may be factors in the loss of the ability of T cells in a macrophage-depleted environment to kill β cells.⁶⁹ T cell proliferation to islet antigens or GAD was significantly decreased when splenocytes from macrophage-depleted NOD mice were used as APCs, suggesting

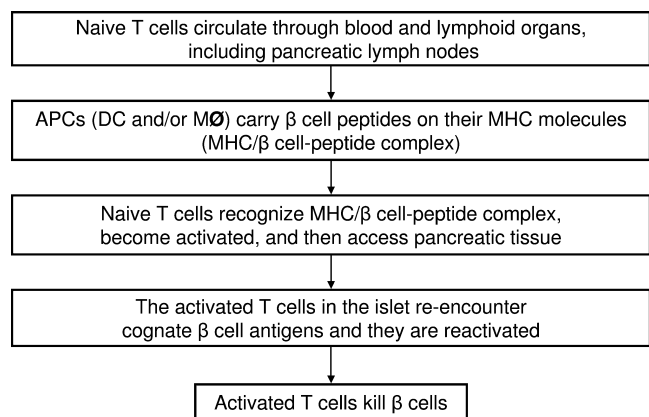


FIGURE 2. Activation of β cell–autoreactive T cells by β cell autoantigens and APCs in autoimmune type 1 diabetes. Naive β cell–autoreactive T cells may circulate through the blood and lymphoid organs. β Cell autoantigens are processed and presented by APCs (dendritic cells [DC], macrophages, and B cells) in the pancreatic islets. Mature APCs, which present β cell autoantigen peptides in conjunction with MHC molecules, migrate to the pancreatic lymph nodes. Naive β cell–autoreactive T cells in the circulation recognize the MHC/ β cell autoantigen peptide complex on APCs and become activated. These activated β cell–reactive T cells access the pancreatic islets and re-encounter cognate β cell antigens and become reactivated. The activated β cell–reactive T cells can then kill β cells.

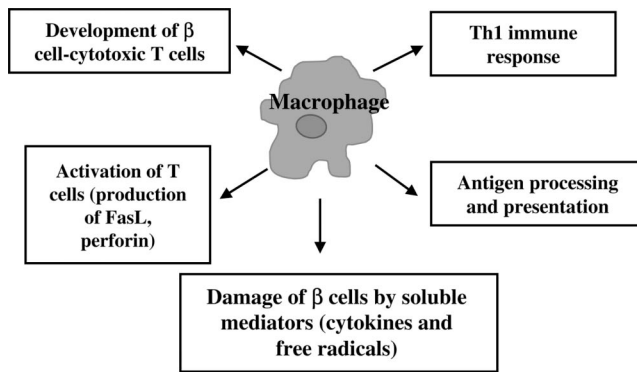


FIGURE 3. Role of macrophages in the development of autoimmune type 1 diabetes. Macrophages are involved in β cell antigen processing and presentation and the induction of the T_H1 immune response, contributing to the creation of microenvironment for the development and activation of β cell cytotoxic T cells. In addition, macrophages produce soluble mediators that are toxic to β cells. This results in the destruction of β cells and subsequent development of autoimmune diabetes.

that depletion of macrophages results in the reduction of APC function and subsequently down-regulation of antigen-specific $CD4^+$ T cell activation (Fig. 3).

Splenic T cells from macrophage-depleted NOD mice showed a significant decrease in the expression of FasL and perforin as compared with macrophage-containing NOD mice.⁶⁹ This result suggests that macrophages are required for the activation of cytotoxic T cells that can destroy pancreatic β cells. Taken together, we suggest that IL-12 secreted by macrophages may activate T_H1 -type $CD4^+$ T cells and subsequently the IL-2 and IFN- γ produced by these activated $CD4^+$ T cells may assist in maximizing the activation of $CD8^+$ T cells. Down-regulation of islet cell-specific T cell activation may be another factor contributing to the impairment of the capability of T cells to kill β cells in macrophage-depleted NOD mice.

Macrophages can also damage β cells by production of soluble mediators such as oxygen free radicals and other cytokines including IL-1 β , TNF- α , and IFN- γ ^{70–73} (Fig. 3). The toxic effect produced by activated macrophages on β cells is thought to be mediated by the superoxide anion and hydrogen peroxide. The expression of IL-1 β , TNF- α , and IFN- γ is detected during early insulinitis in NOD mice and BB rats.^{74,75} It has been reported that IL-1 is selectively cytotoxic to pancreatic β cells *in vitro*,⁷⁶ and IL-1 receptors are present on β cells.⁷⁷ IL-1 and TNF- α inhibit insulin secretion from isolated islets.^{78,79} The destruction of β cells induced by IL-1 is potentiated by TNF- α . *In vitro* studies reveal that the cytotoxic effect of IL-6, TNF- α , lymphotoxin, and IFN- γ on the islets is cumulative. These mediators,

acting alone or synergistically, destroy islets. Thus, treatment with IL-1 receptor antagonist, soluble IL-1 receptor, anti-IL-6 antibody, anti-TNF- α antibody, or anti-IFN- γ antibody decreases the incidence of diabetes.⁸⁰

Activated macrophages produce reactive oxygen species including oxygen radicals, hydroxyl radicals, and NO species (Fig. 3). Studies suggest that IL-1 β - and TNF- α -stimulated endogenous iNOS activity induces NO production in β cells, which contributes to β cell injury.⁸¹ The generation of NO in response to cytokines in β cells⁸² and the generation of peroxynitrite was detected in β cells of NOD mice.⁸³ The β cells are very sensitive to free radicals because β cells exhibit very low free radical scavenging activity.^{84,85} Thus, overexpression of cytosolic superoxide dismutase (Cu/Zn-SOD) in β cells prevents diabetes.^{86,87} In addition, SOD mimetics protect from diabetes induced by the diabetogenic T cell clone, BDC2.5, in NOD.scid mice.⁸⁸ Reactive oxygen species can damage β cells by causing DNA strand breaks, which induce the DNA repair enzyme poly(ADP) ribose polymerase (PARP). PARP uses and depletes nicotinamide adenosine dinucleotide (NAD), leading to necrosis of β cells. In line with this, PARP-deficient mice are resistant to diabetes induced by a low dose of streptozotocin.⁸⁹

T CELLS

It is known that both MHC class II-restricted $CD4^+$ T cells and MHC class I-restricted $CD8^+$ T cells play a critical role in the pathogenesis of type 1 diabetes in NOD mice. Athymic NOD and NOD.scid mice do not develop insulinitis or diabetes.^{90,91} In addition, treatment of NOD mice with anti-CD3 antibodies inhibits the development of diabetes.⁹² However, the precise role of $CD4^+$ and $CD8^+$ T cells in the pathogenesis of autoimmune type 1 diabetes is not clearly understood.

Both $CD4^+$ and $CD8^+$ T cells are required to transfer diabetes, and $CD4^+$ T cells transfer insulinitis, but not diabetes to NOD.scid mice.⁹¹ $CD4^+$ T cells are required for the recruitment of β cell-cytotoxic $CD8^+$ T cells into the islets,⁹³ but some $CD8^+$ T cell clones from diabetic NOD mice can transfer diabetes without the help of $CD4^+$ T cells.^{94,95} However, other results showed that some $CD4^+$ T cell clones can induce diabetes in the absence of $CD8^+$ T cells,⁹⁶ and splenocytes from diabetic donors can transfer disease into recipients lacking MHC class I expression on their islets,⁹⁷ suggesting that $CD4^+$ T cells alone have the ability to induce diabetes.

We have cloned many $CD4^+$ and $CD8^+$ islet-reactive T cells from lymphocytes infiltrating the pancreatic islets of NOD mice⁹⁸ (Table 1). Islet-specific $CD8^+$ T cell

Table 1. CD4⁺ and CD8⁺ T cell clones and their reactivity characteristics.

Clones*	Phenotype	Destruction of β cells [†]	Cytotoxicity to NOD islets (⁵¹ Cr-release assay, T/E = 1/20, %) [‡]
NY1.1	CD4	+	6.3
NY3.1	CD4	+	5.7
NY3.2	CD4	–	1.2
NY4.1	CD4	+	5.9
NY4.	CD4	–	1.1
NY2.3	CD8	+++	26.3
NY5.2	CD8	++	19.5
NY6.3	CD8	++	20.6
NY8.3	CD8	+++	28.7
NY9.3	CD8	++	22.1

*CD4⁺ and CD8⁺ T cells were cloned from the islet infiltrates of acutely diabetic female NOD mice.

[†]Destruction of β cells was evaluated by morphological examination using light and electron microscopy.

[‡]Cytotoxicity to islet cells was evaluated by morphological examination using phase-contrast microscopy and ⁵¹Cr-release cytotoxicity assay.
T/E, target/effector.

clones selectively destroyed β cells in vitro (Figs. 4 and 5), whereas CD4⁺ T cell clones did not destroy β cells but attached closely to them⁸⁴ (Fig. 4). Further studies using cloned CD4⁺ and CD8⁺ T cells revealed that MHC class I-restricted cytotoxic CD8⁺ T lymphocytes play an important role as final effectors in β cell destruction in vivo and that CD4⁺ T cells are required for the activation of CD8⁺ T cells and their recruitment into the pancreatic islets.^{93,98} Among the CD4⁺ and CD8⁺ T cell clones produced, NY8.3 (CD8⁺ T cell clone) and NY4.1 (CD4⁺ T cell clone) were used to establish T cell receptor transgenic mice. TCR- β transgenic NOD mice with TCR- β rearrangements of NY8.3 showed a 10-fold increase in frequency of precursors of β cell-

specific cytotoxic T lymphocytes and an accelerated onset, but not an increased incidence, of diabetes.⁹⁹ TCR- $\alpha\beta$ transgenic mice, with both α - and β -TCR rearrangements of NY8.3, showed a 400-fold increase in the peripheral frequency of β cell-specific cytotoxic lymphocytes and a dramatically accelerated onset of diabetes without an increase in disease incidence.¹⁰⁰ TCR- $\alpha\beta$ transgenic mice with both α - and β -TCR rearrangements of the NY4.1 clone⁹⁸ also showed an accelerated onset of diabetes as a result of a more rapid progression of islet inflammation.¹⁰⁰ However, mice with only the TCR- β rearrangement of NY4.1 became diabetic later than the mice with the TCR- α and - β rearrangement of NY4.1, indicating that the accelerated onset of diabetes in these mice required the coexpression of both the TCR- α and - β transgenes.

Cytokines produced by T cells also play an important role in the pathogenesis of autoimmune type 1 diabetes (Figs. 1 and 6). Many different approaches have been tried to study the role of cytokines in the pathology of type 1 diabetes, including systemic administration of cytokines, neutralization of cytokines, and knockout of cytokine genes.¹⁰¹ In general, T_H1 cytokines (IL-2, IFN- γ , TNF- β) cause the development of the disease, whereas T_H2 or Th3 cytokines (IL-4, IL-10, TGF- β) prevent the disease. However, the role of cytokines in the pathogenesis of autoimmune type 1 diabetes is complex. Treatment of NOD mice with anti-IFN- γ antibody prevented the development of diabetes,¹⁰² and transgenic expression of IFN- γ resulted in the development of diabetes in diabetes-resistant mice.¹⁰³ However, the genetic absence of IFN- γ in NOD mice results in a delay in the development of diabetes but does not prevent its development.¹⁰⁴ Systemic administration of IL-4¹⁰⁵ or IL-10¹⁰⁶ prevented type 1 diabetes in NOD mice, and the transgenic expression of IL-4 in β cells also prevented the disease.¹⁰⁷ However, local expression of IL-10 in the islets accelerated the development of diabetes in NOD mice, and IL-4 knockout NOD mice

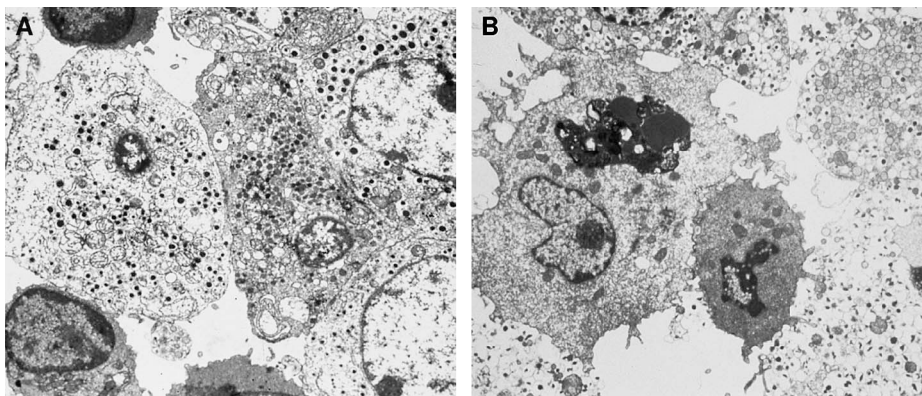


FIGURE 4. Distinct difference in interaction with β cells between CD4⁺ and CD8⁺ T cell clones. Islet-derived T cell clones NY4.1 (CD4⁺) (A) and NY2.3 (CD8⁺) (B) were incubated with islets from silica-treated NOD mice. After 2.5 hours, tissues were examined by electron microscopy. A: CD4⁺ T cells (NY4.1) attached closely to β cells but did not cause cell lysis. B: CD8⁺ T cells (NY2.3) attached closely to β cells and extended pseudopods into them, causing cell damage (loss of plasma membrane and electron-dense cytoplasm).

did not show accelerated disease onset.¹⁰⁸ Therefore, the interactions of the many different cytokines in the immune system are complex and the development of diabetes may depend on which way the finely tuned immune balance of immunoregulatory T cells is tipped (Figs. 1 and 6).

Effector T cells destroy β cells through direct contact with surface ligands of apoptosis-inducing receptors, such as FasL and membrane-bound TNF- α , or through the secretion of perforin molecules, which facilitate the passage of protease granzymes (Figs. 1 and 6). Granzymes activate nucleases in the cells and kill them. In addition, proinflammatory cytokines produced from T cells contribute to the apoptosis of β cells. Systemic Fas-deficient NOD mice did not develop insulinitis or diabetes,¹⁰⁹ and the expression of Fas increases during

the development of diabetes, suggesting that Fas plays an important role in β cell destruction. However, a recent study reported that β cell-specific Fas-deficient mice, in which CD4⁺ T cells with a transgenic TCR specific for influenza hemagglutinin (HA) cause diabetes in mice that express HA under control of the rat insulin promoter, developed autoimmune diabetes with slightly accelerated kinetics, indicating that Fas-dependent apoptosis of β cells is a dispensable mode of β cell death in autoimmune type 1 diabetes.¹¹⁰ TNF receptor 1-deficient NOD mice failed to develop diabetes,¹¹¹ and NOD mice lacking perforin expression developed insulinitis but not diabetes,¹¹² suggesting that the TNF and perforin pathway appears to play an important role for β cell killing.

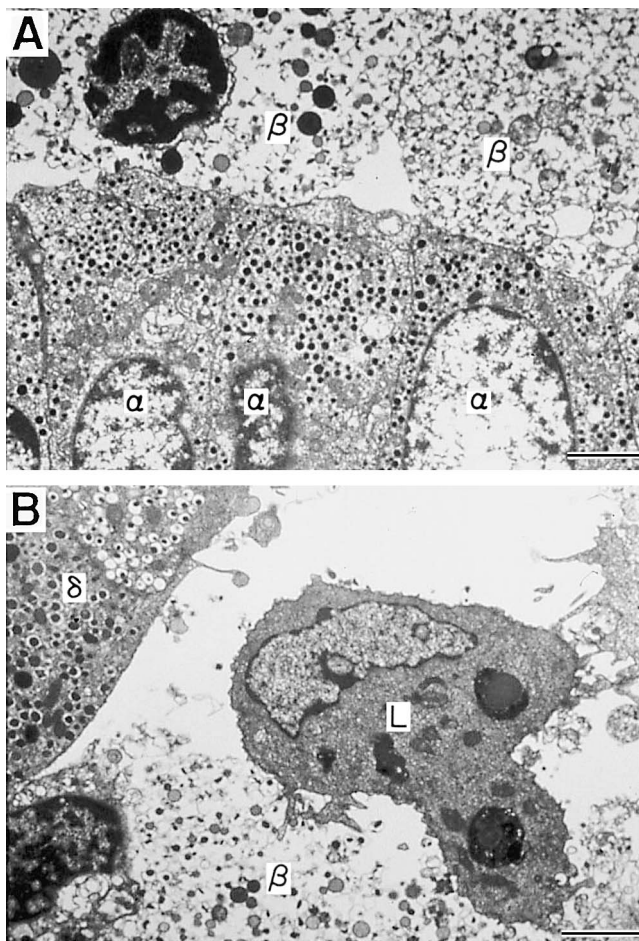


FIGURE 5. Selective destruction of β cells by NY2.3 (CD8⁺) T cells. NY2.3 T cells were incubated with islets from silica-treated NOD mice. After 2.5 hours, tissues were examined under the electron microscope. β cells (β) were extensively damaged, whereas α cells (α) (A) and δ cells (δ) (B) were spared. L, CD8⁺ cytotoxic T lymphocyte. Horizontal bar = 0.1 μ m.

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CONCLUSION

The cause of autoimmune type 1 diabetes is multifactorial, and both genetic and environmental factors are involved in the initiation and progression of β cell destruction. Thus, the elucidation of pathogenic mechanisms involved in the etiology of the disease is difficult. Based on experimental results from studies using NOD mice and BB rats over the past 3 decades, we have reviewed the possible pathogenic mechanisms involved in the autoimmune destruction of pancreatic β cells in type 1 diabetes and summarized them in the model shown in Figure 1. This model shows the possible interactions between β cell autoantigens and immunocytes such as macrophages, dendritic cells, T cells, and their secretory products in connection with MHC class I and II molecules, based on our research and that of others primarily using animal models of type 1 diabetes. This model may not encompass all aspects of the pathogenic mechanisms involved in autoimmune diabetes in humans; nevertheless, it may provide helpful information with respect to the synergistic destruction of pancreatic β cells by immunocytes and their cytokines and a basis for the formation of new hypotheses for further investigation.

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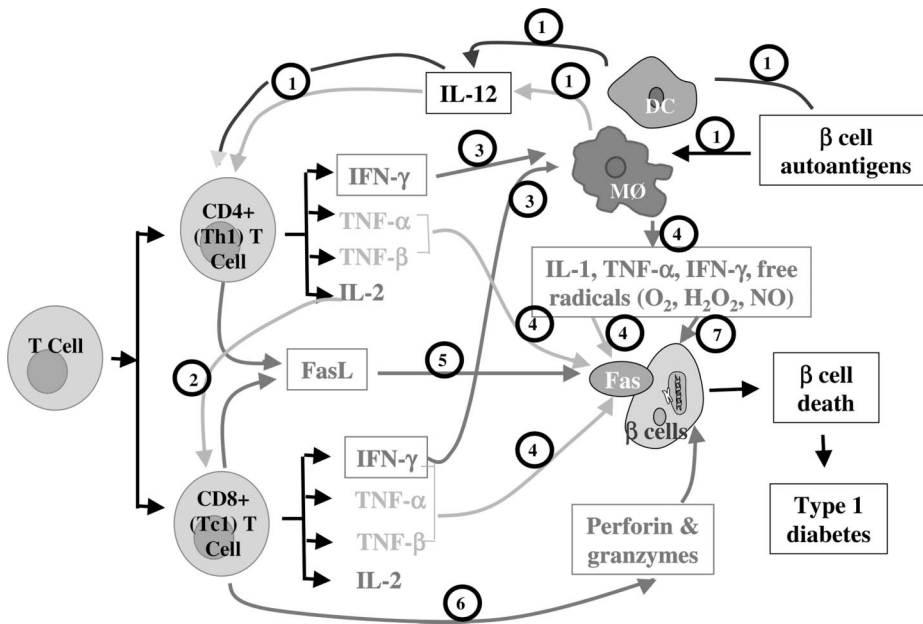


FIGURE 6. Schematic representation of the collaboration between macrophages and T cells in the destruction of pancreatic β cells. β Cell autoantigens may be released from the β cells during spontaneous turnover of β cells. The antigens are then processed by dendritic cells (DC) and/or macrophages (M Φ) and presented to helper T cells (CD4⁺ T_H1 cell) in conjunction with MHC class II molecules. The activated macrophages secrete IL-12, which activates CD4⁺ T_H1-type T cells (1). The CD4⁺ T cells secrete cytokines such as IFN- γ , TNF- α , TNF- β , and IL-2. While this process is taking place, β cell-specific precytotoxic T cells (CD8⁺ Tc1 T cell) may be recruited

into the islets. These precytotoxic T cells may be activated by IL-2 and other cytokines released by CD4⁺ T_H1 cells to differentiate into CD8⁺ effector T cells (2). IFN- γ released by CD4⁺ T_H1 cells and cytotoxic CD8⁺ T cells may cause macrophages to become cytotoxic (3). These cytotoxic macrophages release substantial amounts of β cell-cytotoxic cytokines (including IL-1 β , TNF- α , and IFN- γ). Cytokines released from macrophages and T cells may induce the expression of Fas on pancreatic β cells. β Cells are destroyed by Fas-mediated apoptosis (4, 5) and/or granzymes and perforin (cytolysin), which are toxic to β cells (6). Oxygen free radicals (nitric oxide, H₂O₂) secreted from activated macrophages can also kill β cells (7).

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