Arch Pharm Res Vol 22, No 5, 437-447, 1999 Review

Cellular and Molecular Roles of β Cell Autoantigens, Macrophages and T Cells in the Pathogenesis of Autoimmune Diabetes

Ji-Won Yoon[1,2,3] and Hee-Sook Jun[2]

Dept. of Microbiology and Infectious Disease, [1]Laboratory of Viral and Immunopathogenesis of Diabetes, [2]Julia McFarlane Diabetes Research Centre, Faculty of Medicine, The University of Calgary, 3330 Hospital Drive N. W., Calgary, Alberta T2N 4N1, Canada and The Department of Endocrinology, [3]College of Medicine and The Laboratory of Endocrinology, [3]Institute for Medical Science, Ajou University, 5 Wonchon-Dong, Paldal-Gu, Suwon, Korea

(Received August 30,1999)

Type I diabetes, also known as insulin-dependent diabetes mellitus (IDDM) results from the destruction of insulin-producing pancreatic β cells by a progressive β cell-specific autoimmune process. The pathogenesis of autoimmune IDDM has been extensively studied for the past two decades using animal models such as the non-obese diabetic (NOD) mouse and the BioBreeding (BB) rat. However, the initial events that trigger the immune responses leading to the selective destruction of the β cells are poorly understood. It is thought that β cell autoantigens are involved in the triggering of β cell-specific autoimmunity. Among a dozen putative β cell autoantigens, glutamic acid decarboxylase (GAD) has been proposed as perhaps the strongest candidate in both humans and the NOD mouse. In the NOD mouse, GAD, as compared with other β cell autoantigens, provokes the earliest T cell proliferative response. The suppression of GAD expression in the β cells results in the prevention of autoimmune diabetes in NOD mice. In addition, the major populations of cells infiltrating the islets during the early stage of insulitis in BB rats and NOD mice are macrophages and dendritic cells. The inactivation of macrophages in NOD mice results in the prevention of T cell mediated autoimmune diabetes. Macrophages are primary contributors to the creation of the immune environment conducive to the development and activation of β cell-specific Th1-type $CD4^+$ T cells and $CD8^+$ cytotoxic T cells that cause autoimmune diabetes in NOD mice. $CD4^+$ and $CD8^+$ T cells are both believed to be important for the destruction of β cells. These cells, as final effectors, can kill the insulin-producing β cells by the induction of apoptosis. In addition, $CD8^+$ cytotoxic T cells release granzyme and cytolysin (perforin), which are also toxic to β cells. In this way, macrophages, $CD4^+$ T cells and $CD8^+$ T cells act synergistically to kill the β cells in conjunction with β cell autoantigens and MHC class I and class II antigens, resulting in the onset of autoimmune type I diabetes.

Key words: β cell autoantigens, Macrophages, T cells, Type 1 diabetes.

INTRODUCTION

Type I diabetes is believed to be caused by the progressive loss of insulin-producing pancreatic β cells, by a β cell-specific autoimmune process (Yoon and Jun, 1998; Rossini *et al.*, 1993; Tisch and McDevitt, 1996; Bach, 1995; Nerup *et al.*, 1988; Lernmark *et al.*, 1990; Rabinovich, 1994; Verge and Eisenbarth, 1996; Delovitch and Singh, 1997). Although the pathogenesis of autoimmune IDDM has been extensively studied, the precise mechanisms involved in the initiation and progression of β cell destruction are not clear. Animal models used in the study of IDDM, such as the BioBreeding (BB) rat and the nonobese diabetic (NOD) mouse, have enhanced our understanding of the pathogenic mechanisms of this disease. Extensive studies on the immunopathology of Type I diabetes using these animal models reveal that

Correspondence to: J. W. Yoon, Department of Microbiology and Infectious Disease, Laboratory of Viral and Immunopathogenesis of Diabetes, Julia McFarlane Diabetes Research Centre, Faculty of Medicine, The University of Calgary 3330 Hospital Dr. N.W. Calgary, Alberta, Canada T2N 4N1
E-mail: yoon@ucalgary.ca

β cell autoantigens, macrophages, dendritic cells, B lymphocytes and T lymphocytes are clearly involved in the β cell-specific autoimmune process. In this review article, we will briefly discuss the involvement of β cell autoantigens, macrophages and T cells in the pathogenesis of autoimmune diabetes in animals.

β cell autoantigens

Islet cell autoantigens, which are the targets of autoimmune attack in IDDM, have proven difficult to identify. The specificity of circulating autoantibodies present in the sera of IDDM patients and diabetic animals has been investigated extensively. Over twenty years ago, Bottazzo *et al.* (1974) and MacCuish *et al.* (1974) first detected antibodies directed against the pancreatic islets. Since that time, several studies have revealed that islet cell antibodies are prevalent in patients with IDDM (Bonifacio *et al.*, 1990). It is known that peripheral $CD4^+$ T cells from prediabetic and early diabetic patients proliferate in response to islet autoantigens which reacted with IDDM-associated autoantibodies (Verge and Eisenbarth, 1996). Autoantigens identified in humans, NOD mice, and BB rats include islet cell autoantigens, thought to possess the properties of sialic acid containing glycolipid (Nayak *et al.*, 1985); insulin (Palmer *et al.*, 1983); the insulin receptor (Maron *et al.*, 1984); a 52kD protein (Karounos and Thomas, 1990; Karounos *et al.*, 1990); a 69kD protein (Peitropaolo *et al.*, 1993; Karjalainen *et al.*, 1992); glutamic acid decarboxylase (GAD) (Baekkeskov *et al.*, 1990); IA-2, 37/40kD tryptic fragments of a 64kD antigen (different from GAD) (Bonifacio *et al.*, 1995; Lan *et al.*, 1996); heat shock protein 65 (HSP 65) (Elias *et al.*, 1990; Birk *et al.*, 1996; Jones *et al.*, 1990); carboxypeptidase H (Castano *et al.*, 1991); the glucose transporter (Johnson *et al.*, 1990); and a 38kD autoantigen (Roep *et al.*, 1990; Arden *et al.*, 1996; Ko *et al.*, 1991; Ko *et al.*, 1994). The precise role these auto-antigens play in IDDM is not fully understood. Several of these autoantigens and their involvement in IDDM will be discussed below.

Glutamic acid decarboxylase

It is believed that GAD is a major islet cell autoantigen; thus, GAD has been extensively studied. In 1990 Baekkeskov *et al.* identified this 64kD antigen in the pancreatic β cells of IDDM patients as glutamic acid decarboxylase (GAD), the biosynthetic enzyme of the inhibitory neurotransmitter gamma-amino-butyric acid (GABA) (Baekkeskov *et al.*, 1990). Anti-64kD autoantibodies were detected in over 85% of newly diagnosed diabetic patients (Baekkeskov *et al.*, 1987) and in about 80% of patients at high risk for developing IDDM (Atkinson *et al.*, 1990).

Immunization of NOD mice with purified GAD results in the tolerization of GAD-reactive T cells and blocks the development of T cell responses to other β cell antigens, thus preventing insulitis and diabetes (Kaufman *et al.*, 1993; Elliot *et al.*, 1994). Kaufman *et al.*(1993) found that the initial immune response directed against pancreatic islets in NOD mice was a Th1 response to a confined region of GAD (amino acids 509-528 and 524-543) and that later responses were directed against another region of GAD (amino acids 246-266) and other autoantigens, such as HSP65 and insulin (Kaufman *et al.*,1993). Recently, it was reported that transgenic NOD mice that hyperexpress GAD in their β cells showed a lower incidence of diabetes. However, another line in which the GAD transgene expression was lower did not show a protective effect (Bridgett *et al.*, 1998). GAD-reactive $CD4^+$ Th1 cells isolated from diabetic NOD mice induced diabetes in NOD.severe combined immunodeficiency disease (*scid*) mice (Zekzer *et al.*, 1998) and the overall expression of GAD in NOD mice accelerated the onset and increased the incidence of the disease (Geng *et al.*, 1998). These results suggest that GAD plays an important role in the pathogenesis of autoimmune diabetes. However, controversy surrounds the role GAD plays in the pathogenesis of IDDM. Chen *et al.* have studied the reactivity of T cells to a GAD65-derived peptide, GAD65 residue 524-543, in NOD mice and two congenic NOD strains, B10.H-2^7_g and NOD.B$6^{I12\text{-}Tshb}$ (Chen *et al.*, 1994). They demonstrated that the response to GAD65 524-543 was MHC class II-restricted and that T cell responses to GAD-derived peptides can be elicited in mice resistant to the development of spontaneous IDDM. Thus, Chen *et al.* suggested that peripheral tolerance to GAD is not associated with the prevention of diabetes. Also, another research group found that T cells from H-2 identical nonNOD-H-$2^{g\,7}$ or nondiabetic NOD mice did not proliferate against GAD65 (Bieg *et al.*, 1994). Our recent study showed that β cell-specific suppression of GAD expression in two lines of anti-sense GAD transgenic mice resulted in the prevention of autoimmune diabetes, whereas any level of GAD ex-pression in the β cells in other lines of anti-sense GAD transgenic NOD mice resulted in the development of autoimmune diabetes, similar to that seen in transgene-negative NOD mice (Lim *et al.*, 1998; Yoon *et al.*,1999). This result indicates that GAD may be a triggering autoantigen in the development of autoimmune IDDM in NOD mice.

Insulin

Insulin is a logical candidate for an autoantigen of IDDM because insulin is the only known β cell-specific antigen related to IDDM. It has been reported that oral intake of insulin retards disease progression in the NOD mouse as a result of the induction of immunoregulatory T cells (Zhang *et al.*, 1991). One study identified insulin-

reactive T cells in NOD mice. Also, insulin B chain-specific $CD4^+$ T cell clones accelerate diabetes in young NOD mice and these cell clones adoptively transfer disease in NOD-*scid* mice (Daniel *et al.*, 1994). Transgenic expression of mouse proinsulin II under a MHC class II gene promotor prevented the development of diabetes in NOD mice (French *et al.*, 1997). These results suggest that the insulin autoantigen plays an important role in the development of IDDM. Anti-insulin antibodies (IAAs) have been detected in more than 59% of the patients diagnosed with late preclinical/recent onset IDDM (Palmer *et al.*, 1983; Wilkin and Nicholson, 1984). However, the pathogenic role of IAAs and insulin-reactive T cells needs further investigation. There is an interesting report which examines cross-reactivity between insulin and the islet-expressed retroviral antigen p73 (Serreze *et al.*, 1988). However, the role of this cross-reactivity in the pathogenesis of autoimmune IDDM is not known.

38kD antigen

Anti-38kD autoantibodies were originally identified in human diabetic sera (Baekkeskov *et al.*, 1982). Roep *et al.* identified a 38kD antigen, which was recognized by a T cell clone established from newly diagnosed IDDM patients, from the insulin secretory granule (Roep *et al.*, 1990). Recently these researchers cloned and sequenced a novel murine cDNA encoding this antigen named imogen 38 (Arden *et al.*, 1996). We found that the 38kD antigen in BB rats is the only delayed-expressed islet cell autoantigen whose antibody is consistently found in acutely diabetic DP-BB rats (Ko *et al.*, 1991; Ko *et al.*, 1994). As a result of its delayed expression, this 38kD autoantigen may be considered nonself, which may trigger β cell-specific autoimmunity. Whether there are any molecular similarities between imogen 38 and our delayed-expressed 38kD islet cell autoantigen remains to be determined. Interestingly, one study reports that cytomegalovirus induces antibodies directed against the 38kD antigen in humans (Pak *et al.*, 1990), but, in this instance, the role of the 38kD autoantigen remains to be determined.

IA-2 Autoantigen (37/40kD tryptic fragment)

IA-2 is a newly discovered member of the protein tyrosine phosphatase (PTP) family (Bonifacio *et al.*, 1995; Lan *et al.*, 1996) and is considered to be one of the major autoantigens of IDDM. The IA-2 protein is the precursor to the 37 and/or 40kD islet tryptic fragment (Bonifacio *et al.*, 1995; Lan *et al.*, 1996). Autoantibodies directed against IA-2 have been detected in 70% of IDDM patients. But these autoantibodies are not detected in NOD mice or BB rats (DeSilva *et al.*, 1996). The IA-2 autoantigen from a rat β cell line (RIN5AH) reacts with sera from IDDM patients (Bonifacio *et al.*, 1995). Antibodies to the IA-2 autoantigen, but not anti-GAD antibodies, were detected in patients who developed acute onset IDDM (Christie *et al.*, 1994), suggesting that antibodies to IA2 are useful serological markers for human IDDM. However, the precise role of the IA-2 antigen in the pathogenesis of IDDM is unknown.

ICA 69

ICA 69 has been identified as a β cell autoantigen. This autoantigen reacts with sera from IDDM patients. ICA 69 also cross-reacts with bovine serum albumin (BSA) (Peitropaolo *et al.*, 1993). Some epidemiological studies revealed that cow's milk may be associated with the development of IDDM, since IDDM patients have an increased level of BSA antibodies (Martin *et al.*, 1991). Since this 69kD β cell autoantigen can be induced by IFN-γ, which may be produced by environmental factors, it has been speculated that there is an association between environmental factors and the induction of the 69kD autoantigen, resulting in the development of IDDM (Karjalainen *et al.*, 1992). Neonatal injection of NOD mice with T cell epitope of ICA69 reduces the incidence of diabetes (Karges *et al.*, 1997). In contrast, some studies do not support the association of BSA with the development of IDDM (Atkinson *et al.*, 1993). Whether this 69kD β cell autoantigen is associated with autoimmune IDDM remains to be determined.

Macrophages

The major populations of cells infiltrating the islets during the early stage of insulitis in BB rats and NOD mice have been shown to be macrophages and dendritic cells (Kolb *et al.*, 1986; Voorbij *et al.*, 1989; Walker *et al.*, 1988; Jasen *et al.*, 1994). This infiltration precedes invasion of the islets by T lymphocytes, natural killer (NK) cells and B lymphocytes (Amano and Yoon, 1990). In addition, electron microscopy has revealed that most of the single cells present at an early stage of insulitis in BB rats are macrophages (Kolb *et al.*, 1986). Intraperitoneal injections of silica, a substance known to be toxic to macrophages, into cyclophosphamide (CY)-treated NOD mice or BB rats nearly completely prevents the development of diabetes and insulitis (Lee *et al.*, 1988a; Oschilewski *et al.*, 1985; Lee *et al.*, 1988b). This result suggests that macrophages play an important role in the development of insulitis and diabetes in NOD mice. However, the precise role of macrophages in T cell-mediated autoimmune diabetes in NOD mice remains unknown.

We first examined whether macrophages are required for the development of the effector T cells that destroy β cells. Splenocytes from macrophage-depleted NOD

mice did not induce diabetes in NOD.*scid* mice, while those from control NOD mice in which macrophages were present did, indicating that macrophages are required for the development of β cell-cytotoxic effector T cells NOD mice. Our further study showed that T cells in the macrophage-depleted NOD mouse recipients did not destroy the transplanted NOD islets, indicating that T cells in a macrophage-depleted environment lose their ability to differentiate into cytotoxic T cells that can destroy pancreatic β cells (Jun *et al.*, 1999).

We next asked why the T cells in a macrophage-depleted environment lose the ability to destroy β cells. The depletion of macrophages would be expected to result in the impairment of IL-12 production. A substantial decrease in the production of IL-12 could suppress the Th1 immune response. Th1-type T cells are believed to play a pathogenic role in the autoimmune destruction of β cells. Therefore, a decrease in the Th1 immune response in a macrophage-depleted immune environment may result in the loss of the β cell destructive capacity of the Th1 T cells. On the basis of this hypothesis, we examined the Th1 and Th2 immune responses in macrophage-depleted NOD mice by the measurement of IFN-γ and IL-4 gene expression. We found that the level of IL-4, secreted from Th2-type T cells, was increased, while the level of IFN-γ, secreted from Th1-type T cells, was decreased, indicating that down-regulation of the Th1 immune response and up-regulation of the Th2 immune response may be factors in the loss of the ability of T cells in a macrophage-depleted environment to kill β cells. Our further study showed that the expression of the IL-12Rβ2 subunit (preferentially expressed in Th1-type T cells) was significantly decreased in T cells from macrophage-depleted NOD mice, as compared with NOD mice in which macrophages were present. This result supports the supposition that a decrease in the Th1 immune response in macrophage-depleted NOD mice may be an important factor contributing to the impairment of the capability of T cells to kill β cells (Jun *et al.*, 1999).

Among the key roles played by macrophages, along with dendritic cells and B lymphocytes, is the processing and presentation of antigens to $CD4^+$ helper T cells in association with MHC-II molecules (Unuaue, 1984). Thus, we asked whether the depletion of macrophages would affect the antigen-presenting function in NOD mice. We measured the T cell proliferation response to islet antigens and GAD in the presence of splenocytes, as antigen presenting cells (APC), from macrophage-depleted or macrophage-containing NOD mice. The T cell proliferative response was significantly decreased when splenocytes from macrophage-depleted NOD mice were used as APC, suggesting that the depletion of macrophages results in the down-regulation of antigen-specific $CD4^+$ T cell activation (Jun *et al.*, 1999).

Finally, we examined whether the depletion of macrophages would influence the level of T cell activation. We measured the level of expression of FasL and perforin in splenic T cells from macrophage-depleted NOD mice, and found a significant decrease in the expression of FasL and perforin in splenic T cells from macrophage-depleted NOD mice as compared with macrophage-containing NOD mice (Jun *et al.*, 1999). These results suggest that macrophages are required for the activation of the cytotoxic T cells that can destroy pancreatic β cells.

Although further studies to elucidate the precise mechanism of the involvement of macrophages in T cell activation remain to be performed, our studies have shown that IL-12 secreted by macrophages may activate Th1-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. The down-regulation of islet cell-specific T cell activation may be another major factor contributing to the impairment of the capability of T cells to kill β cell in macrophage-depleted NOD mice.

In addition to the role of macrophages in the T cell-mediated destruction of β cells, we also examined other factors which may be involved in the destruction of these cells. These include macrophage-derived soluble mediators such as oxygen free radicals and other cytokines including IL-1β, TNF-α and IFN-γ. We found that the expression of the cytokines IL-1β, TNF-α, and IFN-γ was significantly decreased in macrophage-depleted NOD mice as compared with PBS-treated control NOD mice. These cytokines, which are released from activated macrophages, are believed to be toxic to β cells (Pankewycz *et al.*, 1995; Mandrup-Poulsen *et al.*, 1987; Appels *et al.*, 1989; Pukel *et al.*, 1988). The toxic effect produced by activated macrophages on β cells is thought to be mediated by the superoxide anion and hydrogen peroxide. The β cell is very sensitive to the production of free radicals because islet cells exhibit very low free radical scavenging activity (Faust *et al.*, 1996; Asayama *et al.*, 1986). Cytokines produced by islet-infiltrating macrophages may contribute to β cell damage by inducing the production of oxygen free radicals in the islets (Malaisse *et al.*, 1982; Corbett and McDaniel, 1992). (Fig. 1)

T cells

Cumulative evidence indicates that T cells play a critical role in the pathogenesis of autoimmune Type I diabetes in NOD mice and BB rats. It has been shown that the development of diabetes was prevented by neonatal thymectomy in BB rats (Like *et al.*, 1982), and BB rats treated with monoclonal antibodies (OX-19) directed against the antigens expressed on the surface

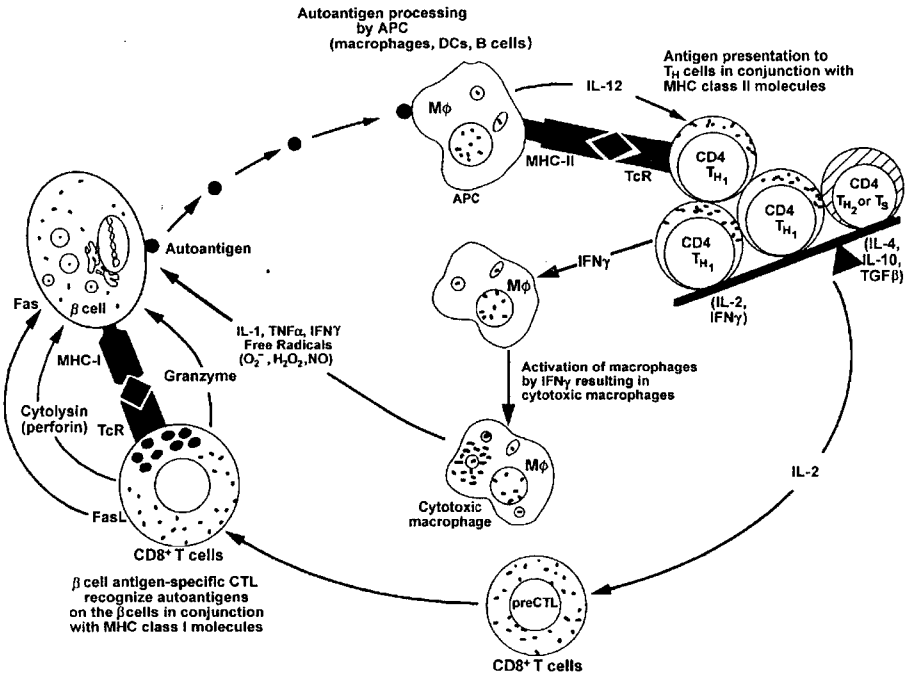

Fig. 1. Schematic representation of the development of β cell-specific autoimmune IDDM. β cell autoantigens released from the β cells during spontaneous turnover or insult by viral infection are processed by macrophages and presented to helper T cells in association with MHC class II molecules. Macrophages release IL-12, which activates T_H1-type $CD4^+$ T cells. While this process is taking place, β cell-specific precytotoxic T cells may be recruited to the islets. These precytotoxic T cells may be induced by IL-2 and other cytokines released by $CD4^+$ helper T cells to differentiate into effector T cells. IFNγ released by helper T cells may cause macrophages to become cytotoxic. These cytotoxic macrophages release substantial amounts of β cell-toxic cytokines (including IL-1β, TNFα, and INFγ) and free radicals. In addition, the helper T cells secrete interleukins that activate other helper T cells, B lymphocytes, and cytotoxic T cells. The autoantigen-specific $CD8^+$ cytotoxic T cells, as final effectors, may recognize the autoantigens expressed on many unaffected β cells, in association with MHC class I molecules. These CLTs release granzyme and cytolysin (perforin), which are toxic to β cells. In addition, Fas-mediated apoptosis is involved in β cell destruction. In this way, macrophages, T cells, and cytokines synergistically destroy β cells, resulting in the development of autoimmune IDDM.

of all T cells do not develop diabetes, indicating that T cells play an important role in the destruction of β cells (Like *et al.*, 1986). In addition, lymphocytes from diabetic BB rats transfer the disease to young diabetes-prone BB rats (Koevary *et al.*, 1983; Edouard *et al.*, 1993).

In the NOD mouse model, it is clear that both $CD4^+$ and $CD8^+$ T cells are involved in the development of the disease. Athymic NOD mice and NOD.*scid* mice do not develop insulitis or diabetes (Ogawa *et al.*, 1985; Dardenne *et al.*, 1989; Makino *et al.*, 1986). In addition, the treatment of NOD mice with anti-CD3 antibodies inhibits the development of diabetes (Christianson *et al.*, 1993). Most transfer studies of NOD splenic T cells into NOD mice show that the transfer of diabetes requires both $CD4^+$ and $CD8^+$ T cells (Miller *et al.*, 1988; Yagi *et al.*, 1992; Thivolet *et al.*, 1991). However, the specific roles that these T cells play in diabetogenesis remain unclear. Diabetogenic $CD4^+$ T cells may undergo activation and differentiation into effector cells upon the release of β cell autoantigens as a result of $CD8^+$ T cell-mediated destruction of β cells. This hypothesis is supported by the observation that splenic $CD4^+$ T cells from prediabetic NOD mice transfer insulitis, but not diabetes, to NOD.*scid* mice (Makino *et al.*, 1986), and that MHC class I/$CD8^+$ T cell-deficient NOD mice do not develop diabetes (Katz *et al.*, 1993; Wicker *et al.*, 1994). On the other hand, there is evidence suggesting that the generation of β cell-cytotoxic $CD8^+$ T effector cells requires the assistance of $CD4^+$ T cells (Nagata *et al.*, 1994). However, some islet-reactive $CD4^+$ and $CD8^+$ T cell clones transfer diabetes without any help from other cells (Peter-son and Haskins, 1996; Wong *et al.*, 1996). Recently, it was reported that MHC class I-restricted $CD8^+$ T cells are required for all but the end stages of diabetes development in NOD mice and use a prevalent T cell receptor a chain gene rearrangement in the initial phase of β cell destruction (Dilorenzo *et al.*, 1998).

We have cloned many $CD4^+$ and $CD8^+$ islet-reactive T cells from lymphocytes that had infiltrated the pan-

creatic islets of NOD mice (Nagata and Yoon, 1992). We have shown that islet cell-specific $CD8^+$ T cell clones selectively destroy β cells *in vitro,* whereas $CD4^+$ T cell clones do not destroy β cells but attach closely to them (Nagata and Yoon, 1992). Our further studies using cloned $CD4^+$ and $CD8^+$ T cells reveal that MHC class I-restricted cytotoxic $CD8^+$ T lymphocytes (CTLs) 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 for their recruitment into the pancreatic islets (Nagata *et al.*, 1994; Nagata and Yoon, 1992). We established T cell receptor (TCR) transgenic NOD mice using our two T cell clones NY8.3 ($CD8^+$ T cell clone) and NY4.1 ($CD4^+$ T cell cone). The TCRβ transgenic NOD mice (8.3-TCRβ-NOD) with TCRβ rearrangements of a β cell-specific $CD8^+$ clone (NY8.3) showed a tenfold increase of precursor frequency of β cell-specific CTLs (β-CTLs) and an accelerated onset of diabetes, but not an increased incidence of diabetes (Verdaguer *et al.*, 1996). The TCRαβ transgenic mice (8.3-NOD) with TCRα and TCRβ rearrangements of NY 8.3 showed a 400-fold increase in the peripheral frequency of β-CTLs and a dramatically accelerated onset of diabetes but not an increased incidence of the disease. The transgenic mice (4.1 NOD mice) with both TCRα and TCRβ rearrangements of a β cell-specific $CD4^+$ T cell clone (NY4.1) (Nagata and Yoon, 1992) also showed an accelerated onset of diabetes as a result of a more rapid progression of islet inflammation (Schmidt *et al.*, 1997). In addition, the 4.1-TCRβ-NOD mice, with only the TCRβ rearrangement, became diabetic later than did the 4.1-NOD mice (103 ± 20 vs 46 ± 19 days), indicating that the accelerated onset of the disease in these mice required the coexpression of the TCR-α and TCR-β transgenes.

Cytokines produced by T cells also play an important role in the pathogenesis of autoimmune IDDM. In general, Th1 cytokines (IL-2, IFN-γ) cause the development of the disease, while Th2 or Th3 cytokines (IL-4, IL-10, TGF-β) prevent the disease. However, the role of cytokines in the pathogenesis of autoimmune IDDM is complex. Forexample, treatment of NOD mice of BB rats with anti-IFN-γ prevents the development of diabetes (Debray-Sachs *et al.*, 1991) and the transgene expression of IFN-γ in β cells of normal mice results in the development of IDDM (Sarvetnick *et al.*, 1998). However, the genetic absence of IFN-γ in NOD mice results in a delay of the development of diabetes, but does not prevent it (Hultgren *et al.*, 1996). Systemic administration of IL-4 (Cameron *et al.*, 1997) or IL-10 (Pennline *et al.*, 1994) prevents IDDM in NOD mice and the transgenic expression of IL-4 on β cells prevents the development of diabetes (Mueller *et al.*, 1996). However, local expression of IL-10 in the islets accelerates the development of diabetes in NOD mice, and IL-4 knockout NOD mice did not show accelerated disease onset (Wang *et al.*, 1998). Therefore, the interactions of the many different cytokines in the immune system are complicated and the development of diabetes may depend upon which way the finely tuned balance of immunoregulatory T cells is tipped.

Recently, possible mechanisms for T cell-mediated β cell destruction have been elucidated. $CD8^+$ cytotoxic T cells destroy β cells through the perforin and granzyme pathway as well as through the Fas-Fas ligand (Fas-L) interaction (Fig. 1). NOD mice lacking perforin expression were found to develop insulitis, but not diabetes (Kagi *et al.*, 1997). Fas-deficient NOD mice were found to be free of diabetes and insulitis, and Fas-mediated apoptosis of the β cells was suggested to be the major mechanism for β cell damage (Chervonsky *et al.*, 1997; Itoh *et al.*, 1997). On the other hand, TNFα-TNFαR-mediated apoptosis may play a greater role in the destruction of β cells by $CD4^+$ T cells (Kurrer *et al.*, 1997).

CONCLUSION

β cell autoantigens, particularly glutamic acid decarboxylase (GAD), macrophages and T cells play critical roles in the pathogenesis of autoimmune diabetes in NOD mice and BB rats, animal models of human diabetes. NOD mice in which GAD expression is suppressed specifically in the β cells do not develop diabetes, indicating that β cell autoantigens are required for the onset of the disease. Macrophages, as well as dendritic cells, are one of the first cell types to infiltrate the pancreatic islets. The presentation of β cell-specific autoantigens to $CD4^+$ helper T cells by macrophages, dendritic cells and/or B lymphocytes, in association with MHC class II molecules, may be the initial step in the development of β cell-specific autoimmunity. In addition, macrophages are required for the activation of both $CD4^+$ T cells and $CD8^+$ cytotoxic effector T cells (Fig. 1). This is evidenced by the loss of the ability of T cells in a macrophage-depleted environment to differentiate into β cell-cytotoxic T cells, resulting in the prevention of autoimmune diabetes.

In unmanipulated NOD mice, both $CD4^+$ and $CD8^+$ T cells are required for the development of diabetes. Although some uncertainty remains with regard to the role of $CD4^+$ and $CD8^+$ T cells in the pathogenesis of autoimmune diabetes, it appears that $CD8^+$ T cells are the major final effectors of β cell damage in spontaneous autoimmune diabetes. In humans, most of the immunocytes infiltrating the pancreatic β cells at the time of diagnosis of IDDM are $CD8^+$ T cells, suggesting that these cells play a major role in the destruction of β cells at a later stage of the autoimmune process as final effectors.

Cytokines secreted by immunocytes, including macrophages, $CD4^+$ T cells and $CD8^+$ T cells may regulate the direction of the immune response toward Th1 or Th2 dominance as well as cytotoxic effector cell dominance or suppressor cell dominance. Pancreatic β cells in the islets may be killed by Fas-mediated apoptosis through a Fas/Fas ligand interaction and by perforin and granzymes released by the activated $CD8^+$ T cells, as well as by TNF-α-TNFαR-mediated apoptosis in the case of $CD4^+$ T cell involvement.

Therefore, the activated macrophages, Th1-type $CD4^+$ T cells and β cell-specific $CD8^+$ T cells, as well as the cytokines secreted by these cells, act synergistically to destroy the β cells in association with β cell-specific autoantigens and MHC class I and II molecules, resulting in the development of autoimmune diabetes (Fig. 1).

ACKNOWLEDGEMENTS

Supported by grants from the Canadian Diabetes Association, Medical Research Council of Canada, the Juvenile Diabetes Foundation International and the Alberta Heritage Foundation for Medical Research. JWY is a Heritage Medical Scientist Awardee of the Alberta Heritage Foundation for Medical Research. HSJ is a senior research fellow at the Julia McFarlane Diabetes Research Centre. The authors gratefully acknowledge the editorial assistance of K. Clarke and A. Kyle.

REFERENCES CITED

Amano, K. and Yoon, J. W., Studies on autoimmunity for initiation of beta-cell destruction: V: Decrease of macrophage dependent T-effector cells and natural killer cytotoxicity in silica-treated BB rats. *Diabetes,* 39, 590-596 (1990).

Appels, B., Burkart, V., Kantwerk-Funke, M., Funda, J., Kolb-Bachofen, V. and Kolb, H., Spontaneous cytotoxicity of macrophages against pancreatic islet cells. *J Immunol.,* 142, 803-808 (1989).

Arden, S. D., Roep, B. O., Neophytou, P. I., Usac, E. F., Duinkerken, G., de Vries, R. R. P. and Hutton, J. D., Imogen 38: a novel 38-kD islet mitochondrial autoantigen recognized by T cells from a newly diagnosed type I diabetic patient. *J Clin Invest.,* 97, 551-561 (1996).

Asayama, K., Kooy, N. W. and Burr, I. M., Effect of vitamin E deficiency and selenium deficiency on insulin secretory reserve and free radical scavenging systems in islets; decrease of islet manganosuperoxide dismutase. *J Lab Clin Med.,* 107, 4559-4564 (1986).

Atkinson, M. A., Bowman, M. A., Kao, K. J., Campbell, L., Dush, P. J., Shah, S. C., Simell, O. and Maclaren, N. K., Lack of immune responsiveness to bovine serum albumin in insulin-dependent diabetes. *New Engl J Med.,* 329, 1853-1858 (1993).

Atkinson, M. A., McLaren, N. K., Scharp, D., Lacy, P. E. and Reiley, W. J., 64.000 Mr autoantibodies as predictors of insulin-dependent diabetes. *Lancet,* 335, 1357-1360 (1990).

Bach, J. F. Insulin-dependent diabetes mellitus as a β cell targeted disease of immunoregulation. *J Autoimmun.,* 8, 439-463 (1995).

Baekkeskov, S., Jan-Aanstoot, H., Christgau, S., Reetz, A., Solimena, M., Cascalho, F., Folli, F., Richter-Olesen, H. and DeCamilli, P., Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. *Nature,* (Lond.), 347, 151-156 (1990).

Baekkeskov, S., Landin, M., Kristensen, J. K., Srikanta, S., Bruining, G. J., Mandrup-Poulsen, T., deBeaufort, C., Sveldner, J. S., Einsenbarth, G. S., Lindgren, F., Sundquist, G. and Lernmark, A., Antibodies to a 64,000 Mr human islet cell antigen precede the clinical onset of insulin-dependent diabetes. *J Clin Invest.,* 79, 926-934 (1987).

Baekkeskov, S., Nielsen, J. H., Marner, B., Bilde, T., Ludvigsson, J. and Lernmark, A., Autoantibodies in newly diagnosed diabetic children immunoprecipitate human pancreatic islet cell proteins. *Nature,* (Lond.), 298, 167-169 (1982)

Bieg, S., Seissler, J., Herberg, L., Northemann, W. and Scherbaum, W. A., GAD_{65} is recognized by T-cells, but not by antibodies from NOD mice. *Autoimmunity,* 17, 189-194 (1994).

Birk, O. S., Elias, D., Weiss, A.S., Rosen, A., Van der Zee, R., Walker, M. D. and Cohen, I. R., NOD mouse diabetes: The ubiquitous mouse HSP60 is a β cell target antigen of autoimmune T cells. *J Autoimmun.,* 9, 159-166 (1996).

Bonifacio, E., Bingley, P. J., Shattock, M., Dean, B. M., Dunger, D., Gale, E. A. and Bottazzo, G. F., Quantification of islet-cell antibodies and prediction of insulin-dependent diabetes. *Lancet,* 335, 147-149 (1990).

Bonifacio, E., Lampasona, V., Genovese, S., Ferrari, M. and Bosi, E., Identification of protein tyrosine phosphatase-like IA-2 (islet cell antigen 512) as the insulin dependent diabetes-related 37/40K autoantigen and a target of islet-cell antibodies. *J Immunol.,* 155, 5419-5426 (1995).

Bottazzo, G. F., Florin-Christensen, A. and Doniach, D., Islet cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiency. *Lancet,* 2, 1279-1283 (1974).

Bridgett, M., Cetkovic-Cvrlje, M., ORourke, R., Shi, Y., Narayanswami, S., Lambert, J., Ramiya, V., Baekkeskov, S. and Leiter, EH., Differential protection in two transgenic lines of NOD/Lt mice hyperexpressing the autoantigen GAD65 in pancreatic beta-cells. *Diabetes,* 47,

1848-1856. (1998).
Cameron, M. J., Arreaza, G. A., Zucker, P., Chensue, S. W., Strieter, R. M., Chakrabarti, S. and Delovitch, T. L., IL-4 prevents insulitis and insulin-dependent diabetes mellitus in nonobese diabetic mice by potentiation of regulatory T helper-2 cell function. *J Immunol.*, 159, 4686-4692 (1997).
Castano, L., Russo, E., Zhou, L., Lipes, M. A. and Eisenbarth, G., Identification and cloning of a granule autoantigen (Carboxypeptidase-H) associated with type I diabetes. *J Clin Endocrinol & Metab.*, 73, 1197-1201 (1991).
Chen, S. L., Whiteley, P. J., Freed, D. C., Rothbard, J. B., Peterson, L. B. and Wicker, L. S., Responses of NOD congenic mice to a glutamic acid decarboxylase-derived peptide. *J Autoimmun.*, 7, 635-641 (1994).
Chervonsky, A. V., Wang, Y., Wong, F. S., Visinitin, I., Flavell, R. A., Janeway Jr, C. A. and Matis, L. A., The role of Fas in autoimmune diabetes. *Cell*, 89,17-24 (1997).
Christianson, S. W., Shultz, L. D. and Leiter, E. H., Adoptive transfer of diabetes into immunodeficient NOD-scid/scid mice. Relative contributions of CD4+ and CD8+ T cells from diabetic versus prediabetic NOD. NON-Thy-1a donors. *Diabetes*, 42, 44-55 (1993).
Christie, M. R., Genovese, S., Cassidy, D., Bosi, E., Brown, T. J., Lai, M., Bonifacio, E. and Bottazzo, G. F., Antibodies to islet 37k antigen, but not to glutamate decarboxylase, discriminate rapid progression to IDDM in endocrine autoimmunity. *Diabetes*, 43, 1254-1259 (1994).
Corbett, J. A. and McDaniel, M. L., Does nitric oxide mediate autoimmune destruction of β cells? Possible therapeutic interventions in IDDM. *Diabetes*, 41, 897-903 (1992).
Daniel, D., Gill, R. G., Schloot, N. and Wegmann, D., Epitope specificity cytokine production profile and diabetogenic activity of insulin-specific T cell clones isolated from the NOD mouse. *Eur J Immunol.*, 25, 1056-1062 (1994).
Dardenne, M., Lepault, F., Bendelac, A. and Bach, J. F., Acceleration of the onset of diabetes in NOD mice by thymectomy at weaning. *Eur J Immunol.*, 19, 889-895 (1989).
DeSilva, M. G., Jun, H. S., Yoon, J. W., Notkins, A. L. and Lan, M. S., Autoantibodies to IA-2 not detected in NOD mice or BB rats. *Diabetologia*, 39, 1237-1238 (1996).
Debray-Sachs, M., Carnaud, C., Biotard, C., Cohen, H., Gresser, I., Bedossa, P. and Bach, J. F., Prevention of diabetes in NOD mice treated with antibody to murine IFN-γ. *J Autoimmunity.*, 4, 237-248 (1991).
Delovitch, T. L. and Singh, B., The nonobese diabetic mouse as a model of autoimmune diabetes: Immune dysregulation gets the NOD. *Immunity*, 7, 727-738 (1997).
Dilorenzo, T. P., Graser, R. T., Ono, T., Christianson, G. J., Chapman, H. D., Roopenian, D. C., Nathenson, S. G. and Serreze, D. V., Major histocompatibility complex class I-restricted T cells are required for all but the end stages of diabetes development in nonobese diabetic mice and use a prevalent T cell receptor a chain gene rearrangement. *Proc Natl Acad Sci.*, 95, 12538-12543 (1998).
Edouard, P., Hiserodt, J. C., Plamondon, C. and Poussier, P., $CD8^+$ T-cells are required for adoptive transfer of the BB rat diabetic syndrome. *Diabetes*, 42, 390-397 (1993).
Elias, D., Markovits, D., Reshef, T., Van der Zee, R. and Cohen, I. R., Induction and therapy of autoimmune diabetes in the non-obese diabetic mouse by a 65-kDa heat shock protein. *Proc Natl Acad Sci., USA*, 87, 1576-1580 (1990).
Elliot, J. F., Qin, H. Y., Bhatti, S., Smith, D. K., Singh, B. K., Dillon, T., Lauzon, J. and Singh, B., Immunization with the larger isoform of mouse glutamic acid decarboxylase (GAD67) prevents autoimmune diabetes in NOD mice. *Diabetesm*, 43, 1494-1499 (1994).
Faust, A., Kleemann, R., Rothe, H. and Kolb, H., Role of macrophages and cytokines in β-cell death. In Shafrir E, editor. *Lessons from Animal Diabetes VI*, Birkhauser, Boston, Cambridge, pp. 47-56 (1996).
French, M. B., Allison, J., Cram, D. S., Thomas, H. E., Dempsey-Collier, M., Silva, A., Georgiou, H. M., Kay, T. W., Harrison, L.C. and Lew, A. M., Transgenic expression of mouse proinsulin II prevents diabetes in nonobese diabetic mice. Diabetes, 46, 34-39 (1997).
Geng, L., Solimena, M., Flavell, R. A., Sherwin, R. S. and Hayday, A. C., Widespread expression of an autoantigen GAD65 transgene does not tolerize non-obese diabetic mice and can exacerbate disease. *Proc Natl Acad Sci., USA*, 95, 10055-10060 (1998).
Hultgren, B., Huang, X., Dybdal, N. and Stewart, T. A., Genetic absence of γ-interferon delays but does not prevent diabetes in NOD mice. *Diabetes*, 45, 812-817 (1996).
Itoh, N., Imagawa, A., Hanafusa, T., Waguri, M., Yamamoto, K., Iwahashi, H., Moriwaki, M., Nakajima, H., Miyagawa, J., Namba, M., Makino, S., Nagata, S., Kono, N. and Matsuzawa, Y., Requirement of Fas for the development of autoimmune diabetes in nonobese diabetic mice. *J Exp Med.*, 186, 613-618 (1997).
Jasen, A., Homo-Delarche, R., Hooijkaas, H., Leenen, P. J., Dardenne, M. and Drexhage, H. A., Immunohistochemical characterization of monocyte-macrophages and dendritic cells involved in the initiation of insulitis and beta-cell destruction in NOD mice. *Diabetes*, 43, 667-675 (1994).
Johnson, T. H., Crider, B. P., McCorkle, K., Alford, M. and Unger, R. H., Inhibition of glucose transport into rat

islet cells by immunoglobulins from patients with new-onset insulin-dependent diabetes mellitus. *New Engl J Med.*, 322, 653-659 (1990).

Jones, D. B., Hunter, N. R.and Duff, G. W., Heat shock protein 65 as a β cell antigen of insulin-dependent diabetes. *Lancet*, 335, 583-585 (1990).

Jun, H. S., Yoon, C. S., Zbytnuik, L., van Rooijen, N. and Yoon, J. W., The role of macrophages in T cell-mediated autoimmune type 1 diabetes in NOD mice. *J Exp Med.*, 189, 347-358 (1999).

Kagi, D., Odermatt, B., Seiler, P., Zinkernagel, R. M., Mak, T.W. and Hengartner, H., Reduced incidence and delayed onset of diabetes in perforin-deficient nonobese diabetic mice. *J Exp Med.*, 186, 989-997 (1997).

Karges, W., Hammond-McKibben, D., Gaedigk, R., Shibuya, N., Cheung, R. and Dosch, H. M., Loss of self-tolerance to ICA69 in nonobese diabetic mice. *Diabetes*, 46, 1548-1556 (1997).

Karjalainen, J., Martin, J. M., Knip, M., Ilonen, J., Robinson, B. H., Savilahti, E., Akerblom, H. and Dosch, H. M., A bovine albumin peptide as a possible trigger of insulin-dependent diabetes mellitus. *N Engl J Med.*, 327, 302-307 (1992).

Karounos, D. G. and Thomas, J. W., Recognition of common islet antigen by autoantibodies from NOD mice and humans with IDDM. *Diabetes*, 39, 1085-1090 (1990).

Karounos, D. G., Wolinsky, J. S., Gillard, B. K. and Thomas, J. W., Molecular mimicry in type I diabetes: An antigenic determinant on a rubella virus protein is shared with a 52kD beta cell autoantigen. *Diabetes*, 39, 96a (Abstr.) (1990).

Katz, J., Benoist, C. and Mathis, D., Major histocompatibility complex class I molecules are required for the development of insulitis in non-obese diabetic mice. *Eur J Immunol.*, 23, 3358-3360 (1993).

Kaufman, D., Clare-Salzler, M., Tian, J., Forsthuber, T., Ting, G., Robinson, P., Atkinson, M. A., Sercarz, E. E., Tobin, A. J. and Lehmann, P. V., Spontaneous loss of T-cell tolerance to glutamic acid decarboxylase in murine insulin-dependent diabetes. *Nature*, 366, 69-72 (1993).

Ko, I. Y., Ihm, S.H. and Yoon, J. W., Studies on autoimmunity for initiation of beta-cell destruction VIII. Pancreatic beta cell dependent autoantibody to a 38 kilodalton protein precedes the clinical onset of diabetes in BB rats. *Diabetologia*, 34, 548-554 (1991).

Ko, I. Y., Jun, H. S., Kim, G. S. and Yoon, J. W., Studies on autoimmunity for initiation of beta-cell destruction X. Delayed expression of a membrane-bound islet cell-specific 38 kDa autoantigen that precedes insulitis and diabetes in the BB rat. *Diabetologia*, 37, 460-465 (1994).

Koevary, S., Rossini, A. A., Stroller, W. and Chick, W., Passive transfer of diabetes in the BB/W rat. *Science*, 220, 727-728 (1983).

Kolb, H. G., Kantwerk, U., Treichel, T., Kurner, U., Kiesel, T. and Hoppe, V., Kolb-Bachofen., Prospective analysis of islet lesions in BB rats. *Diabetologia*, 29(Suppl. 1), A559 (1986).

Kurrer, M. O., Pakala, S. V., Hanson, H. L. and Katz, J. D., β cell apoptosis in T cell-mediated autoimmune diabetes. *Proc Natl Acad Sci USA.*, 94, 213-218 (1997).

Lan, M. S., Wasserfall, C., Maclaren, N. K. and Notkins, A. L., IA-2, a transmembrane protein of the protein tyrosine phosphatase family, is a major autoantigen in IDDM. *Proc Natl Acad Sci USA.*, 93, 6367-6370 (1996).

Lee, K. U., Amano, K. and Yoon, J. W., Evidence for initial involvement of macrophage in development of insulitis in NOD mice. *Diabetes*, 37, 989-991 (1988a).

Lee, K. U., Pak, C. Y., Amano, K. and Yoon, J. W., Prevention of lymphocytic thyroiditis and insulitis in diabetes-prone BB rats by the depletion of macrophages. *Diabetologia*, 31, 400-402 (1988b).

Lernmark, A., Barmeier, H., Dube, S., Hagopian, W., Karlsen, A. E., Markholst, H., Wilson, C. and Wassmuth, R., Molecular analysis of the pathogenesis of beta cell destruction in insulin-dependent diabetes mellitus. *Western J Med.*, 153, 499-502 (1990).

Like, A. A., Biron, C. A., Weringer, E. J., Byman, K., Sroczynsaki, E. and Guberski, D.L., Prevention of diabetes in Biobreeding/Worcester rats with monoclonal antibodies that recognize T-lymphocytes or natural killer cells. *J Exp Med.*, 164, 1145-1159 (1986).

Like, A. A., Kislauskis, E., Williams, R. M. and Rossini, A. A., Neonatal thymectomy prevents spontaneous diabetes mellitus in the BB/W rat. *Science*, 216, 644-646 (1982).

Lim, H. W., Huang, Q. Q., Yoon, C. S., Jun, H. S., Hirasawa, K. and Yoon, J. W., Complete prevention of autoimmune IDDM by β-cell-specific suppression of GAD expression in transgenic NOD mice. *Diabetes*, 47(Suppl. 1), A211 (1998).

MacCuish, A. C., Irvine, W. J., Barnes, E.W. and Duncan, L. J., Antibodies to pancreatic islet cells in insulin-dependent diabetes with coexistent autoimmune disease. *Lancet*, 2, 1529-1531 (1974).

Makino, S., Harada, M., Kishimoto, Y. and Hayashi, Y., Absence of insulitis and overt diabetes in athymic nude mice with NOD genetic background. *Exp Anim.*, 35, 495-499 (1986).

Malaisse, W. J., Malaisse-Lagae, F., Sener, A. and Pipeleers, D. G., Determinants of the selective toxicity of alloxan to the pancreatic β cell. *Proc Natl Acad Sci., USA*, 79, 927-930 (1982).

Mandrup-Poulsen, T., Bendtzen, K., Dinarello, C. and Nerup, J., Human tumor-necrosis factor potentiates human interleukin 1-mediated rat pancreatic beta cell-cytotoxicity. *J Immunol.*, 139, 4077-4082 (1987).

Maron, R., Elias, D., deJong, H., Bruining, J., van Rood, J. J., Schechter, Y. and Cohen, I. R., Autoantibodies to the insulin receptor in juvenile onset diabetes. *Nature*, 303, 817-819 (1984).

Martin, J. M., Trink, B., Daneman, D., Dosch, H. M. and Robinson, B. H., Milk proteins in the etiology of insulin-dependent diabetes mellitus (IDDM). *Ann Med.*, 23, 447-452 (1991).

Miller, B. J., Appel, M. C., O'Neill, J. J. and Wicker, L. S., Both the Lyt-2+ and L3T4$^+$ T cell subsets are required for the transfer of diabetes in nonobese diabetic mice. *J Immunol.*, 140, 52-58 (1988).

Mueller, R., Krahl, T. and Sarvetnick, N., Pancreatic expression of interleukin-4 abrogates insulitis and autoimmune diabetes in nonobese diabetic (NOD) mice. *J Exp Med.*, 1093-1099 (1996).

Nagata, M., Santamaria, P., Kawamura, T., Utsugi, T. and Yoon, J. W., Evidence for the role of CD8$^+$ cytotoxic T cells in the destruction of pancreatic beta cells in NOD mice. *J Immunol.*, 152, 2042-2050 (1994).

Nagata, M. and Yoon, J. W., Studies on autoimmunity for initiation of beta cell destruction: IX. Electron microscopic evidence on a distinct difference in the destruction of beta cells between CD4$^+$ and CD8$^+$ T cell clones derived from lymphocytes infiltrating the islets of NOD mice. *Diabetes*, 41, 998-1008 (1992).

Nayak, R. C., Omar, M. A. K., Rabizadeh, A., Srikanta, S. and Eisenbarth, G. S., Cytoplasmic islet cell antibodies: Evidence that the target antigen is a sialoglycoconjugate. *Diabetes*, 34, 617-619 (1985).

Nerup, J., Mandrup-Poulsen, T., Molvig, J., Helqvist, S., Wogensen, L., Egeberg, J., Mechanisms of pancreatic β cell destruction in type 1 diabetes. *Diabetes Care*, 11(suppl 1), 16-23 (1988).

Ogawa, M., Maruyama, T., Hasegawa, T., Kanaya, T., Kobayashi, F., Tochino, Y. and Uda, H., The inhibitory effect of neonatal thymectomy on the incidence of insulitis in non-obese diabetes (NOD) mice. *Biomed Res*, 6, 103-106 (1985).

Oschilewski, U., Kiesel, U. and Kolb, H., Administration of silica prevents diabetes in BB rats. *Diabetes*, 34, 197-199 (1985).

Pak, C. Y., Cha, C. Y., Rajotte, R. V., McArthur, R. G. and Yoon, J. W., Human pancreatic islet cell-specific 38kD autoantigen identified by cytomegalovirus-induced monoclonal islet cell autoantibody. *Diabetologia*, 33, 569-572 (1990).

Palmer, J. P., Asplin, C. M., Clemons, P., Lyen, K., Tapati, O., Raghu, P. and Pauquett, T. L., Insulin autoantibodies in insulin-dependent diabetes before insulin treatment. *Science*, 222, 1337-1338 (1983).

Pankewycz, O. G., Guan, J. X. and Benedict, J. F., Cytokines as mediators of autoimmune diabetes and diabetic complications. *Endocrin Rev.*, 16(2), 164-176 (1995).

Peitropaolo, M., Castano, L., Babu, S., Buelow, R., Kuo, Y. L., Martin, S., Martin, A., Powers, A. C., Prochazka, M., Naggert, J., Leither, E. H. and Eisenbarth, G. S., Islet cell autoantigen 69kD (ICA 69). Molecular cloning and characterization of a novel diabetes-associated autoantigen. *J Clin Invest.*, 92, 359-371 (1993).

Pennline, K. J., Roque-Gaffney, E. and Monahan, M., Recombinant human IL-10 prevents the onset of diabetes in the nonobese diabetic mouse. *Clin Immunol Immunopathol*, 71, 169-175 (1994).

Peterson, J. D. and Haskins, K., Transfer of diabetes in the NOD-scid mouse by CD4 T-cell clones. Differential requirement for CD8 T-cells. *Diabetes*, 45, 328-336 (1996).

Pukel, C., Baquerizo, H. and Rabinovitch, A., Destruction of rat islet cell monolayers by cytokines. Synergistic interactions of interferon-gamma, tumor necrosis factor, lymphotoxin, and interleukin-1. *Diabetes*, 37,133-136 (1988).

Rabinovitch, A., Immunoregulatory and cytokine imbalances in the pathogenesis of IDDM. Therapeutic intervention by immunostimulation? *Diabetes*, 43, 613-621 (1994).

Roep, B. O., Arden, S., de Vries, R. R. P. and Hutton, J., T-cell clones from a type I diabetes patient respond to insulin secretory proteins. *Nature*, (Lond.), 345, 632-634 (1990).

Rossini, A. A., Greiner, D. L., Friedman, H. P. and Mordes, J. P., Immunopathogenesis of diabetes mellitus. *Diabetes Reviews*, 1, 43-75 (1993).

Sarvetnick, N., Liggitt, D., Pitts, S., Hansen, S. and Stewart, T., Insulin-dependent diabetes mellitus induced in transgenic mice by ectopic expression of class II MHC and interferon-gamma. *Cell*, 52, 773-782 (1998).

Schmidt, D., Verdaguer, J., Averill, N. and Santamari, P., A mechanism for the major histocompativility complex-linked resistance to autoimmunity. *J Exp Med.*, 186, 1059-1075 (1997).

Serreze, D. V., Leiter, E., Kuff, E. L., Jardieu, P. and Ishizaka, K., Molecular mimicry between insulin and retroviral antigen p73. Development of cross-reactive autoantibodies in sera of NOD and C57BL/KsJ db/db mice. *Diabetes*, 37, 351-358 (1988).

Thivolet, C., Bendelac, A., Bedossa, P., Bach, J. F. and Carnaud, C., CD8$^+$ T cell homing to the pancreas in the nonobese diabetic mouse is CD4$^+$ T cell-dependent. *J Immunol.*, 146, 85-88 (1991).

Tisch, R. and McDevitt, H., Insulin-dependent diabetes mellitus. *Cell*, 85, 291-297 (1996).

Unuaue, E. R., Antigen-presenting function of the macrophage. *Ann Rev Immunol.*, 2, 395-428 (1984).

Verdaguer, J., Yoon, J. W., Averill, N., Utsugi, T., Park, B. J. and Santamaria, P., Acceleration of diabetes in NOD mice expressing beta cell-cytotoxic T cell-derived TcRβ transgene. *J Immunol*, 157, 4726-4735 (1996).

Verge, C. F. and Eisenbarth, G. S., Prediction of type I diabetes: the natural history of the prediabetic period. In: Eisenbarth, G. S., Lafferty, K. J., editors. *Type I Diabetes: Molecular, Cellular and Clinical Immunology,* Oxford University Press, New York, pp. 230-258 (1996).

Voorbij, H. A. M., Jeucken, P. H. M., Kabel, P. J., DeHaan, M. and Drexhage, H. A., Dendritic cells and scavenger macrophages in pancreatic islets of prediabetic BB rats. *Diabetes*, 38,1623-1629 (1989).

Walker, R., Bone, A. J., Cooke, A. and Baird, J. D., Distinct macrophage subpopulations in pancreas of prediabetic BB/E rats: possible role for macrophages in pathogenesis of IDDM. *Diabetes*, 37,1301-1304 (1988).

Wang, B., Gonzales, A., Hglund, P., Katz, J. D., Benoist, C. and Mathis, D., Interleukin-4 deficiency does not exacerbate disease in NOD mice. *Diabetes*, 47, 1207-1211 (1998).

Wicker, L.S., Leiter, E. H., Todd, J. A., Renjilian, R. J., Peterson, E., Fischer, P. A., Podolin, P. L., Zijlstra, M., Jaenisch, R. and Peterson, L. B., Beta2-microglobulin-deficient NOD mice do not develop insulitis or diabetes. *Diabetes*, 43, 500-504 (1994).

Wilkin, T. J. and Nicholson, S., Autoantibodies against human insulin. *Br Med J.*, 288, 349-352 (1984).

Wong, F. S., Wen, L., Visintin, I., Flavell, R. A. and Janeway Jr, C. A., CD8 T cell clones from young nonobese diabetic (NOD) islets can transfer rapid onset of diabetes in the absence of CD4 cells. *J Exp Med.*, 183, 67-76 (1996).

Yagi, H., Matsumoto, M., Kunimoto, K., Kawaguchi, J., Makino, S. and Harada, M., Analysis of the roles of $CD4^+$ and $CD8^+$ T cells in autoimmune diabetes of NOD mice using transfer to NOD athymic nude mice. *Eur J Immunol.*, 22, 2387-2393 (1992).

Yoon, J. W. and Jun, H. S., Insulin-dependent diabetes mellitus. In: Roitt IM and Delves PJ, editors. *Encyclopedia of Immunology,* Second Edition. London, UK: Academic Press Ltd., pp. 1390-1398 (1998).

Yoon, J. W., Yoon, C. S., Lim, H. W., Huang, Q. Kang, Y., Pyun, K. H., Hirasawa, K., Sherwin, R. S. and Jun, H. S., Control of autoimmune diabetes in NOD mice by GAD expression or suppression in β cells. Science, 284, 1183-1187(1998).

Zekzer, D., Wong, F. S., Ayalon, O., Millet, I., Altieri, M., Shintani, S., Solimena, M. and Sherwin, R. S., GAD-reactive CD4+ Th1 cells induce diabetes in NOD/SCID mice. *J Clin Invest.*, 101, 68-73 (1998).

Zhang, Z. J., Davidsen, L., Eisenbarth, G. S., Weiner, H. L. and Weiner, R. S., Suppression of diabetes in nonobese diabetic mice by oral administration of porcine insulin. *Proc Natl Acad Sci., USA.*, 88, 10252-10256 (1991).