Ajou Medical Journal
Vol. 2, No. 1, 1997

Cellular and Molecular Pathogenic Mechanisms of Type I Diabetes

Ji-Won Yoon[1,2], Yup Kang[1], Hyeon-Man Kim[3], Kwan-Woo Lee[3]
Yoon-Sok Chung[3], Millina Lee[4] and Hee-Sook Jun[2]

[1]Laboratory of Endocrinology, Institute for Medical Sciences, Ajou University
[2]Laboratory of Viral and Immuno-Pathogenesis of Diabetes,
Julia McFarlane Diabetes Research Center, Faculty of Medicine,
The University of Calgary, Calgary, Alberta, Canada
[3]Department of Endocrinology, School of Medicine, Ajou University
[4]Department of Microbiology, School of Medicine, Ajou University

Cumulative evidence suggests that Type I Diabetes (Insulin-dependent diabetes mellitus: IDDM) results from progressive destruction of insulin producing pancreatic β-cells. Genetic susceptibility appears to be a prerequisite for the development of IDDM but, as the concordance rate for IDDM between identical twins approaches only 40%, environmental factors must also be involved in the clinical expression of the disease. Humoral autoimmunity precedes the onset of IDDM; however, it appears that the islet cell autoantibodies detected in the sera of patients with IDDM are a secondary phenomenon, generated by autoantigens released from damaged β-cells. The presence of these autoantibodies is valuable for the prediction and diagonosis of IDDM. Indications of the involvement of cell-mediated autoimmune responses in the pathogenesis of IDDM have come from autopsies performed on patients who have died from acute onset diabetes. Their pancreata show CD8+-rich lymphocytic infiltrates, which also contain macrophages, CD4+ T-cells, and natural killer cells. In animal models for IDDM, such as the BioBreeding (BB) rat and the non-obese diabetic (NOD) mouse, macrophages and CD4+ and CD8+ T-cells appear to be involved in the destruction of pancreatic β-cells. Macrophages play an important role in the initiation of insulitis, as evidenced by the prevention of further autoimmune precesses when they are inactivated.

Several β-cell autoantigens, including a 64 kD protein (glutamic acid decarboxylase (GAD)), 37 kD/40 kD tryptic fragments of 64 kD protein, a 38 kD protein, a 52 kD protein, and a 69 kD heat shock protein, have been identified, although the precise roles of these β-cell autoantigens in the pathogenesis of IDDM have not been elucidated. It has been hypothesized that presentation of β-cell autoantigens, released during spontaneous turnover of β-cells, through antigen presenting cells, such as macrophages and dendritic cells, to CD4+ T helper cells, in conjuntion with MHC class II molecules, might be the initial step in the development of autoimmune IDDM. Activated CD4+ helper T-cells can secrete cytokines, such as interleudin-2. While this process is taking place, β-cell autoantigen-specific CD8+ precytotoxic T-cells may be recruited to the islets and differentiated into effector cells by the interleukin-2 and other cytokines released by CD4+ T helper cells. The autoantigen-specific CD8+ cytotoxic T-cells, as final effectors, may recognize the antigens expressed on the many unaffected β-cells, in conjuntion with MHC class I molecules. Free radicals and cytokines, such as interleukin-1, released by macrophages may act synergistically with the CD8+ effector T-cells, leading to the destruction of pancreatic β-cells. Development of autoimmune IDDM can be prevented by either induction of immunological tolerance using β-cell autoantigens or by induction of suppressor T-cells by microbial agents or bacterial products. In addition, T-cell receptor diversity in the pathogenesis of IDDM, use of transgenic mice for studies on diabetes, and induction of immunological tolerance will also be discussed in this chapter.

Key Words: Autoimmunity, Genetic susceptibility, Environmental factor, Autoantigen, Suppressor T-cell

Reprint requests to: Dr Ji-Won Yoon, Laboratory of Endocrinology, Institute for Medical Science, Ajou University, Suwon 442-749, Korea. Tel) 0331-219-5710, Fax) 0331-219-5708

INTRODUCTION

Type I diabetes, also known as insulin-dependent diabetes mellitus (IDDM), results from the destruction of insulin-producing pancreatic β-cells, which leads to hypoinsulinaemia and hyperglycaemia. During the past few decades, genetic factors, environmental factors (viral infections, diet, toxins), and autoimmunity have been extensively studied as the possible causes of pancreatic β-cell destruction (Fig. 1)[1~2]. Evidence indicates that autoimunity is involved in the destruction of β-cells in the majority of patients with IDDM[3~6]. Cumulative data supporting an aetiological role for autoimmunity include mononuclear cell infiltration of pancreatic islets[7], the presence of circulating islet cell autoantibodies (ICAs)[8], recurrence of diabetes in pancreatic transplants from non-affected monozygotic twins[9], and delay of the disease or reduction of its incidence by cyclosporin treatment[10~11]. The infiltrating immunocytes in the pancreatic islets of individuals affected by IDDM are composed of a variety of T- and B-lymphocytes, monocytes, and macrophages[12]. These cells surround and infiltrate the pancreatic islets during the acute phase of IDDM and disappear in the chronic phase of the disease, leaving in their wake an islet in which β-cells, but not other endocrine cells (α, δ, and pancreatic polypeptide producing cells), have been destroyed indicating a selective destruction β-cells. Although the autoimmune pathogenesis of IDDM has been extensively studied, precise mechanisms for the initiation and the process of β-cell destruction are poorly understood.

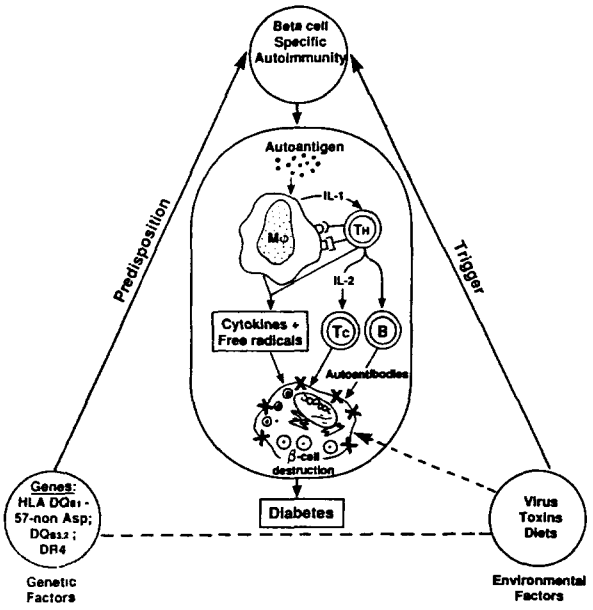

Fig. 1. Aetiology of IDDM. Genetic susceptibility (e.g. major histocompatibility complex genes (including DQB1*0302) and/or other genes) appears to be a prerequisite in most, if not all, cases of IDDM. However, as the concordance rate for the development of IDDM by identical twins appears to be around only 35%, non-genetic, environmental factors such as viruses, diet, and β-cell toxins must influence the clinical expression of genetic susceptibility. Certain envronmental factors, such as viruses and toxins, may act as primary injurious agents to pancreatic β-cells or as triggering agents for autoimmunity in humans and animals. Once β-celll-specific autoimmunity has developed, autoimmune-mediated destruction of β-cells results in IDDM. β-cell-specific autoantigens, macrophages, helper T-cells, and autoantigen- specific cytotoxic T-lymphocytes (CTLs) are involved in the autoimmune process. In addition, free radicals and cytokines released from marophages and T-cells act synergistically in β-cell destruction with the autoantigen-specific T-effector cells.

Studies using animal models for IDDM, such as the Bio-Breeding (BB) rat and non-obese diabetic (NOD) mouse, have enhanced our understanding of the pathogenic mechanisms for the disease. The BB rat was first described in 1974 among Wistar-derived rats in Canada[13]. IDDM develops in 60~80% of BB rats within 110 days after birth. The pattern of the disease in the BB rat is similar to human IDDM, exept for the association with T-cell lymphopenia in the BB rat[14] (Table 1). Mononuclear cell infiltration of BB rat pancreatic islets can be seen prior to the onset of hyperglycaemia and ketoacidosis[15]. Islet cell autoantibodies, such as islet cell surface antibodies (ICSAs) can also be detected as early as 40 days of age in the BB rat[15], Concanavalin A (Con A) treated splenocytes from acutely diabetic BB rats can induce diabetes when injected into young, non-diabetic rats. In addition, recurrence of diabetes in the BB rat occurs when it is transplanted with non-diabetic pancreatic islets. Furthermore, diabetes in the BB rat can be prevented by several forms of immunotherapy[16~17]. Another animal model for spontaneously developed IDDM is the NOD mouse, which was originally developed in Japan from a CTS-subline of outbred ICR mice[18]. The diabetic syndrome in NOD mice is similar to that of humans and BB rats, except for sex differences (Table 1). In the NOD mouse,

Table 1. Comparison among human, BB rat and NOD mouse

Characteristic	Human	BB rat	NOD mouse
Clinical features			
Insulin dependence	+	+	+
Equal sex distribution	+	+	−
Insulitis	+	+	+
Humoral immunity			
ICA	+	+	+
ICSA	+	−	+
Insulin autoantibody	+	+	+
Cellular immunity Lymphopenia	−	+	−
Passive transfer	ND*	+	+
MHC association	+	+	+
Prevention by immunosuppression	ND*	+	+
Associated with other autoimmune diseases	+	+	+

*ND: not determined

there is a high prevalence of the disease in females (70~80%), as compared with males (10~20%).

Establishment of these animal models and development of powerful molecular biological tools, such as transgenic technology[19~22] and the polymerase chain reaction (PCR)[23], have fostered enormous progress in diabetes research over the last decade. In spite of this progress, the precise pathogenic mechanisms for the initiation and process of β-cell destruction remain still shrouded. In this paper, we will briefly discuss the role of autoimmunity in the pathogenesis of IDDM in conjuntion with genetic and environmental factors, and the possible prevention of the disease.

Genetics

Genetic susceptibility to human IDDM appears to be a prerequisite for the development of the disease. If one parent has IDDM, the risk of the offspring developing the disease is about 8~10% and this risk increases to over 23% if both parents have IDDM[24]. That susceptibility to IDDM has a genetic basis has been further supported by results from molecular and cellular analyses of human leukocyte antigen (HLA) genes[25~26]. A strong association has been observed between susceptibility to IDDM and specific alleles of HLA genes. Certain HLA class II alleles (HLA-DR3 and HLA-DR4) were found to be more strongly associated with IDDM than class I alleles (HLA-B8, HLA-B15) in linkage disequilibrium with HLA-DR3 and HLA-DR4[27]. As definition of HLA alleles became more precise, it became clear that HLA-DQ was more strongly associated with the disease than HLA-DR[28] and that the shifting association could be explained by linkage disequilibrium. In Caucasians, HLA-DQβ chains with an amino acid different from aspartic acid at position 57 are associated with susceptibility to IDDM[29~30]. However, later studies found that the HLA association with IDDM did not depend exclusively on DQβ position 57, but on its combination with allelic variants encoded by other polymorphic loci[31~32]. While the actual significance of the association of the 57th amino acid residue with susceptibility to IDDM and its function are unknown, the association does suggest that the HLA-DQB1 gene is, by itself, a susceptibility factor. The DQB1*0302 (DQ3.2) gene, especially, appears to be the most likely susceptibility gene for IDDM in the Caucasian population[33~34]. This gene (DQB1*0302) is one specific allele, or polymorphic variant, encoded by the DQB1 locus, which encodes upwards of 20 different alleles. The HLA class II region contains several linked genetic loci and particular genes such as the DQB1*0302 (DQ3.2) at the DQB1 locus are linked to other HLA genes at the HLA-DR locus. The DQB1A*0302 gene is linked specifically DR genes which encode the HLA-DR4 type.

The DQB1*0302 (DQ3.2) gene encodes a polypetide component of the class II molecule antigen-binding cell surface glycoprotein. The DQB1*0302 gene is structurally related to another DQB1 allele DQB1*0301 (HLA-DQ3.1) which is not associated with IDDM. Both DQB1*0301 and DQB1*0302 are associated with HLA-DR4; however, they are found on distinct DR4-positive haplotypes. Their gene products (DQB1*0301 and DQB1*0302) differ from each other by six amino acid residues, four of which lie in the NH_2-terminal domain of the HLA class II molecule. This molecule appears to be responsible for critical antigen and T-cell interactions. Each of these four codons appears to play an important role in immune recognition[35~37]. The presence of glycine at codon 45 is key DQB1*0302 serological specificity and influences T-cell recognition of the molecule. The predominant T-cell recognition epitopes on the DQB1*0302 molecule are com-

plex. Most T-cell recognition parameters appear to be influenced by α-chain polymorphism. Single residue substitutions within the DQB1*0302 β-chain at codon 26 or 57 were sufficient to abolish or restore recognition by specific T-cell clones[35]. These effects were dependent on the specific DQ α-chain present[33].

In contrast to the Caucasian population, amino acid residue 57 of the HLA-DQ β chain (DQB1) is not correlated with IDDM susceptibility in oriental populations, including the Japanese[38], Koreans and Chinese. Thus the hypothesis that the presence of aspartic acid at amino acid residue 57 of the HLA-DQ β chain protects against the development of IDDM is not tenable for oriental IDDM patients.

There is also a familial association of IDDM with other autoimmune diseases. In particular, there is a strong association of IDDM with autoimmune thyroid disease (ATD). A recent study of families with both IDDM and ATD has indicated that HLA polymorphism, especially at the DQ β1 locus, modulates the susceptibility to each disease in a complex fashion, with each chromosome playing a role[39].

In the BB rat, a number of investigations have correlated the inheritance of phenotypic markers, such as the BB rat's severe T-lymphopenia, and genetic markers for the major histocompatibility complex (MHC) with the development of diabetes[40~43]. Results from these studies indicate that F1 animals are not lymphopenic and do not develop diabetes. When a series of different normal rat strains (which are not $RT1^u$ class II) are used to produce F2 crosses, only approximately 2% of the F2 animals develop overt diabetes. Development of diabetes correlates with the inheritance of both severe lymphopenia and the BB rat's MHC[42]. While development of diabetes in non-lymphopenic BB-drived rats has been observed in rare instances[44], no permanently non-lymphopenic strain of rat which spontaneously develops diabetes has been created. It is therefore likely that the severe lymphopenic phenotype of the BB rat, inherited in an autosomal recessive fashion, is not essential for the development of diabetes but increases the probability of its development. Recently, a lymphopenic gene was mapped to chromosome 14[45].

A series of breeding studies indicates that the development of diabetes by BB rats is strongly linked to a gene within the MHC[41~42,46]. Using rats bearing recombinant MHC[47~48], found that the MHC-associated diabetes susceptibility genes mapped to the class II region on chromosome 20 in the BB rat[41]. It appears that all RT1 haplotypes expressing class II genes, which are *u*, are diabetogenic, independent of class I alleles. The development of diabetes has been attributed to a lack of T-lymphocytes expressing the RT6 cell susrface alloantigen[49]. These studies are the strongest evidence to date that a class II gene is essential for diabetes susceptibility in the BB rat. It is noteworthy that Lewis rats, whose class II region is not diabetogenic, and BB rats, whose class II region is diabetogenic, both lack aspartic acid at position 57 of their DQ β chain[50]. This suggests that the allelic amino acid at this position in the BB rat probably does not serve as a disease marker and that there may be other unidentified class II allelic differences involved in susceptibility to IDDM in the BB rat. T-cell receptor polymorphisms have not been linked to diabetes in the BB rat.

Early genetic studies in mice revealed that the susuceptibility of NOD mice to IDDM is also linked to the major histocompatibility complex (H-2 of mice) and is inherited as a recessive trait[51~52]. Later studies in H-2 congenic strains of NOD mice revealed that the susceptibility provided by H-2 (idd-1) is not recessive, but rather a dominant trait with incomplete penetrance[53~54]. The NOD mouse is unique in that its H-2 complex encodes for novel I-A molecules (A α^d and unique A β) in which aspartic acid at position 57 of the β-chain is replaced by serine[55,30] and that it lacks I-E expression[51]. Studies in NOD transgenic mice expressing normal I-E or other I-A molecules, and which did not develop diabetes, indicated that the unique H-2 complex of the NOD mouse plays a direct role in its susceptibility to IDDM[56~58]. However, expression of I-E in NOD mice itself is not sufficient to prevent insulitis or diabetes. Although non-NOD MHC products confer protection from diabetes, they cannot do so if only expressed in the thymus and not in bone-marrow-derived cells[60].

IDDM susceptibility in the NOD mouse is also determined by at least eight other unlinked loci (idd-2 through idd-9)[51~52,54,61~62]. The idd-2 gene(s) is weakly linked to the Thy1/Alp-1 cluster on chromosome 9. Idd-3 has been associated with a defective Fc receptor for IgG, encoded on chromosome 3[63] and is associated with both insulitis and diabetes. This mutation has been associated with increased antibody-dependent cellular cytotoxicity (ADCC) by monocytes, which are an essential component in the onset of insulitis and diabetes in

NOD mice. Idd-4, which maps to chromosome 11, is especially associated with early onset of IDDM and may influence the frequency of insulitis and control the progression of severe insulitis to overt diabetes. Idd-5 is linked to the interleukin-1 (IL-1) receptor and the Lsh/Ity/Bcg genes on chromosome 1[64]. IL-1, an antigen-presenting-cell-derived cofactor in T-cell activation, is β-cell cytotoxic[65]. The Lsh/Ity/Bcg genes, which control susceptibility or resistance to infection by *Leishmania donovani, Salmonella typhimurium*, and *Mycobacterium bovis*[66~68], are also expressed in macrophages and could thus play a role in insulitis and diabetes.

Another non-MHC-linked gene associated with peri-insulitis and sialitis is linked to the Bc1-2 locus on chromosome 1[69]. Interestingly, the Bc1-2 gene increases cellular resistance to signals that induce apoptosis[70~71]. Expression of a Bc1-2 transgene in T-cells inhibits T-cell death and perturbs thymic negative selection[71] and, when expressed on mouse B cells, can cause a lupus-like syndrome[72]. The Bcl-2 gene may play an important role in the early events that lead to IDDM. It thus appears likely that a series of alleles at different loci, each necessary but not sufficient for the development of diabetes by NOD mice, underlie its susceptibility to diabetes.

Environmental factors

As described above, susceptibility to IDDM is determined by genetic factors; however, the concordance for IDDM between monozygotic twins approcahes only 35~36%[73], suggesting that, along with genetic factors, non-genetic, environmental factors influence the clinical expression of the disease (Fig. 2). Environmental factors such as viruses, toxins, diet and stress have all been implicated as possible environmental determinants of IDDM[74] (Fig. 1).

1) Viruses: Regarding the induction of diabetes by viruses, there are at least two different mechanisms by which viruses can cuase IDDM. One mechanism involves the direct infection of β-cells and viral replication within them. The replication of virus in β-cells results in their lysis and the infected animal or human subsequently becomes hypoinsulinaemic and hyperglycaemic. The second mechanism involves the triggering of β-cell-specific autoimmunity by the virus, leadng to autoimmune destruction of the β-cells. Several ways in which viruses might trigger β-cell-specific autoimmunity have been put forward. If the virus incorporates host antigens into its envelope or modifies host antigens, the virus may render the infected cell or some component of it foreign to the host[75~77]. Alternatively, certain viruses trigger autoimmunity by des-

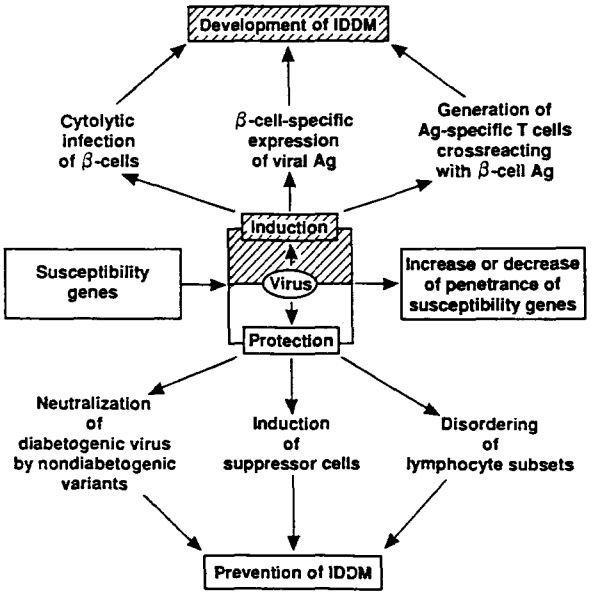

Fig. 2. Role of viruses in the destruction of pancreatic β-cells leading to an increase or decrease in the penetrance of susceptibility genes. Genetic susceptibility appears to be a prerequisite for the development of IDDM; however, environmental factors such as viruses may act as inducible agents or preventative agents. With regard to inducible agents, some viruses including EMC-D virus and Coxsackie B4 virus can induce diabetes by infecting and destroying β-cells in a genetically susceptible host (cytolytic infection of β-cells). In addition, certain viruses (e.g. retrovirus in NOD mice and rubella virus in hamsters and humans) may alter a normally existing β-cell antigen into an immunogenic form or might induce a new antigen which may trigger β-cell-specific autoimmune IDDM (β-cell-specific expression of viral antigens). Furthermore, other viruses (e.g. Kilham's rat virus in DRBB rats) may generate antigen-specific effector T-cells which may cross-react with β-cell-specific autoantigens, leading to autoimmune IDDM (generation of antigen-specific T-cells cross-reacting with β-cell antigens). With regard to preventative agents, diabetogenic EMC-D virus-induced diabetes in mice can be prevented by vaccination with a non-diabetogenic EMC-B virus (neutralization of infectious virus). Certain viruses may induce suppressor T-cells which can prevent T-cell-mediated autoimmue IDDM (induction of suppressor T-cells). Some other viruses such as lymphocytic choriomeningitis virus (LCMV) may prevent IDDM in NOD mice by selectively depleting the CD4+ T-cell subset (disordering of lymphocyte subsets). Thus, viruses as environmental factors may affect the penetrance of susceptibility genes.

troying or stimulating subpopulations of lymphocytes (e.g. helper or suppressor T-cells), resulting in a problem with immunoregulation. A third possible mechanism involves viral generation of viral-antigen-specific T effector cells which recognize β-cell-specific antigens on the β-cells by molecular mimicry[78~79].

(1) Encephalomyocarditis virus; The best-studied model of virus-induced IDDM is the encephalomyocarditis (EMC) virus, which acts as a primary injurious agent in damaging β- cells[80~82]. Susceptibility to EMC- virus-induced diabetes is controlled by genetically determined host factors[81,83~85]. A role for T-cells in β-cell destruction in EMC-M-infected mice was originally suggested in early studies[86~88]; however, later studies with EMC-D virus did not support a T-cell-mediated mechanism[89~90]. More recent studies in our laboratory have revealed that macrophages play a critical role in the process of β-cell destruction at the early stage of EMC-D virus infection[91]. Mice infected with pure diabetogenic EMC-D virus developed long-term complications, such as glomerulosclerosis, changes in the cornea and retinal vessels and decreases in bone formation and mineralization, similar to those seen in humans with IDDM[92~93]. It has also been learned that development of EMC-induced diabetes is controlled by a single gene of the host and a single amino acid of the polyprotein of the virus[94].

(2) Coxsackie B4 virus; Coxsackie B4 virus has been shown to induce diabetes in SJL/J mice and in Patas monkeys by infecting and destroying beta cells[95~97]. Early epidemiological studies showed that patients with IDDM of recent onset had higher neutralizing antibody titres to Coxsackie B4 virus than either normal non-diabetic subjects, or patients who had had IDDM for longer than three months. These studies suggested that human IDDM may sometimes be associated with Coxsackie B4 virus infection[98~99]. Further support for the concept that Coxsackie B4 virus might trigger some cases of human IDDM came 15 years ago when a variant of Coxsackie B4 virus isolated from the pancreas of a diabetic patient was found to induce diabetes in mice[100]. Several later epidemiological studies also described an association between IDDM and Coxsackie B4 viral infection[101~105]. It is thought that Coxsackie B4 viral infection enhances the expression of a 64 kD autoantigen, glutamic acid decarboxylase (GAD)[106~107]. In humans, there is homology between GAD and a Coxsackie B4 viral protein, suggesting that the viral protein may trigger β-cell-specific autoimmunity[108].

(3) Rubella virus; The congenital rubella syndrome (CRS) provides additional evidence that viral infections may sometimes play a role in human diabetes[109~116]. These reports, as well as a large prospective study of 242 patients[117], have shown that approximately 12~20% of individuals with CRS develop diabetes in 5 to 20 years. Because islet cell and anti-insulin antibodies were found in more than 20% of non-diabetic patients with CRS and in 50~80% of diabetic patients with CRS, it is thought that CRS might have an autoimmune basis. Patients with CRS and diabetes have a significantly increased frequency of HLA-DR3 and a significantly decreased frequency of HLA-DR2[118]. A majority of patients with CRS also have an abnormal carbohydrate metabolism[117~118].

We have developed a diabetic animal model that closely corresponds to the diabetes observed in human with CRS[114]. After infection with β-cell-passaged rubella virus, neonatal Golden Syrian hamsters develop diabetes. In these animals there is evidence of an autoimmune process taking place. This evidence includes the detection of mononuclear cell infiltration and measurable circulating islet cell antibodies.

(4) Cytomegalovirus; A case report of a 13 month old child with congenital cytomegalovirus (CMV) infection who developed IDDM has implicated CMV in the development of IDDM[120]. CMV is known to infect pancreatic islet cells[103] and in combination with more lethal betatropic agents may also be a causal factor in diabetes[121].

It has been shown, using both dot and *in situ* hybridization techniques, that 20% of IDDM patients appear to have cytomegaloviral genome in their pancreatic islets[122]. Furthermore, 80% of patients who had both anti-CMV antibodies and cytomegaloviral genome also had islet cell autoantibodies, suggesting that CMV is associated with IDDM. Our recent study showed that human CMV can induce an islet cell autoantibody that reacts with a 38 kD islet cell autoantigen[123]. This reaction probably results from similar epitopes being shared by islet-cell-specific proteins and antigenic determinants of CMV. In addition, insulitis and CMV-like particles have been observed in the pancreas of a spontaneously diabetic rodent, *Octodon degus*[124].

(5) Mumps virus; Gamble[125] demonstrated that mumps infec-

tion apparently precedes the development of diabetes in some newly diagnosed diabetic children, implicating this virus in the development of IDDM. Since some children appear to develop islet cell autoantibodies during parotiditis, it has been hypothesized that an infection with mumps virus may also induce autoimmunity[126].

(6) Retrovirus; β-cell-specific expression of endogenous retroviruses has been associated with the development of insulitis and diabetes in NOD mice[127~128]. Studies conducted during the mid-1980s found that C-type-like retrovirus particles were present in pancreatic β-cells from both C3H-db/db[129] and NOD[130] mice. As well, intracisternal A-type particles (IAPs) were also found in β-cells from genetically diabetic mice including C57BL/KSJ (db/db), DBA/2J (DB/DB) and CheB/FeJ (db/db) mice[131]. The presence of retrovirus particles in β- cells clearly correlated with the presence of insulitis lesions[130]. Two separate studies using NOD mice, each employing different methods, showed that retroviral gag protein was present exclusively in β-cells, and not other pancreatic cells[132~133]. The precise pathogenic role of retroviral gene expression and/or gene product in β-cells in autoimmune IDDM in NOD mice is not known. Possibly, presentation of retrovirus antigen on the β-cells by antigen-presenting cells may be the initial step in the autoimmune destruction of β-cells. An immune response to a specific antigen on the target cell involves the activation of CD4+ T-cells, which are only activated when they interact with antigens presented on the surface of a macrophage or other antigen presenting cell. Our previous experimental results support this possibility, since elimination of antigen-presenting macrophages resulted in the prevention of β-cell-specific autoimmune processes in NOD mice[134]. Alternatively, retroviral genomes (e.g. IAPs) in NOD β- cells may alter the expression of cellular genes possibly resulting in a β-cell-specific altered antigen(s). This altered antigen might be perceived as foreign by immunocytes, thus leading to β-cell-specific autoimmunity. Either possibility, β-cell-specific expression of retrovirus-group- specific antigens or retrovirus-induced β-cell-specific altered antigens, could result in the eventual generation of cytotoxic cross-reactive effector T-lymphocytes which recognize specific determinants of 'self-proteins' on β-cells. This would lead to the development of β-cell-specific autoimmune IDDM in NOD mice. Alternatively, retroviral gene expression may be a phenomenon secondary to the disease process.

(7) Kilham's rat virus; Another virus that has recently been implicated in animal diabetes is Kilham's rat virus (KRV), which has been shown to cause autoimmune diabetes in diabetes-resistant BB (DRBB) rats, without distinct infection of β-cells[135]. A further study has suggested that wide spread infection of peripancreatic and other lymphoid tissue, but not pancreatic β-cell, by KRV triggers autoimmune diabetes by perturbing the immune system of genetically predisposed DRBB/Wor rats[136]. It has also been speculated that KRV infection may generate antigen-specific cytotoxic T-cells and that the KRV-specific effector T-cells may then attack β-cells[137].

2) Toxins: A number of chemically dissimilar agents, such as alloxan, streptozotocin, chlorozotocin, vacor and cyproheptadine, can cause diabetes in rodents[139]. Vacor has also been shown to be diabetogenic in humans. Diabetogenic chemicals may destroy β-cells by several different mechanisms. They may generate oxygen free radicals and alter endogenous scavengers of these reactive species. Through breakage of DNA, they may also increase the activity in β-cells of the enzyme depleting nicotinamide adenine dinucleotide, poly-ADP ribose synthetase. A third possible mechanism by which diabetogenic chemicals may induce diabetes is through the inhibition of active calcium transport and calmodulinactivated protein kinase activity. The effect of each of the chemicals listed above has been previously reviewed in detail elsewhere[74].

3) Diet: Food diabetogens have been shown to affect the expression of the diabetic syndrome in BB rats and NOD mice. The source of dietary protein seems to be the most important factor[53,139~144], and intact protein appears to be required for full expression of genetic susceptibility for developing diabetes in BB rats. Wheat gluten, soybean meal, alfalfa, and skim milk powder have been identified as the most likely sources of dietary diabetogens in rodents[139,143,145~146]. There is some circumstantial evidence suggesting that diet plays a role in the development of human autoimmune IDDM[141,147~148].

Autoimmunity

Several immunologic abnormalities precede the clinical onset of IDDM. The involvement of humoral autoimmunity is indicated by the detection of various circulating autoanti-

bodies, while cell-mediated autoimmune responses against islet antigens have also been observed. In this section humoral and cell-mediated autoimmunity in IDDM will be discussed.

1) Humoral autoimmunity: Over twenty years ago, Bottazzo et al[8], and MacCuish et al[149] first reported the detection of antibodies directed against pancreatic islets. Since that time it has been shown in several studies that antibodies are prevalent in patients with IDDM. In addition to islet cell antibodies, the sera of patients with IDDM may also contain antibodies directed against the islet cell surface[150~151], thyroid and adrenal cortex[152], the adrenal medulla[153], thymic hormones and HLA-DQ molecules[154], pancreatic cytokeratin[155] and lymphocyte surfaces[156]. Sera from diabetic patients containing islet cell surface antibodies (ICSAs) may be specifically cytotoxic to β-cells in the presence of complement[150,157], but it has not, however, been proven that *in vivo* administration of such antibodies can induce diabetes. Moreover, diabetes has not been observed in the infants of mothers who developed IDDM while pregnant. Transplacental passage of anti-islet antibodies can be demonstrated in the umbilical blood of newborn infants of mothers positive for islet cell antibodies[158]. Recurrence of insulitis in transplanted pancreatic islets obtained from an identical twin or other sibling occurs in the absence of islet cell antibodies[159], suggesting that autoantibodies may not play a critical role in β- cell destruction; rather, a large body of accumulated data from studies in NOD mice, BB rats, and humans indicate that IDDM is a cell-mediated disease. It is generally accepted that autoantibodies detected in the sera of patients with IDDM are a secondaty phenomenon, generated by autoantigens released from damaged β-cells. Despite this, the presence of some of the above autoantibodies are valuable for the prediction and diagnosis of IDDM.

(1) Islet cell antibodies; ICAs can be detected in over 60% of newly diabetic IDDM patients[160~161] and in their first-degree relatives who develop diabetes[162]. Of first-degree relatives of an IDDM patient, 1 in 50 express high titres of ICA ($\rangle$ 80 JDF units), and approximately 8% per year develop diabetes[163]. Enzymatic digestion and blocking experiments using islet extracts suggest that the target antigens had the properties of a glycolipid with sialic acid[164]. Additional studies have revealed that the possible candidates for these target autoantigens are GT3[165] and a ganglioside on thin layer chromatography (TLC) migrating between GM2 and GM1[166].

(2) Anti-insulin antibodies; Anti-insulin antibodies (IAAs), which bind insulin molecules have been found in over 59% of patients with late preclinical/recent onset IDDM[167~168]. Interestingly, cross-reactivity exists between insulin and an islet- expressed retroviral antigen p73[169]. Insulin is the only known β-cell-specific IDDM antigen and oral intake of insulin is reported to retard disease progression in the NOD mouse[170]. The pathological role of IAAs and insulin-reactive T-cells deserves further study.

(3) Antibodies against glutamic acid decarboxylase (64 kD protein); Autoantibodies precipitating a 64 kD protein from human islets have been detected in the sera from the majority of patients with preclinical/recent onset IDDM[171~172]. This 64 kD pretein has been identified as the autoantigen, glutamic acid decarboxylase (GAD)[173]. Antibodies against 37 or 40 kD tryptic fragments of GAD are distinct from those against GAD and are highly sensitive markers for the development of IDDM[174~175]. Sequence homology between isoforms of GAD65 and GAD67, and a Coxsackie B virus protein has been found[108] and antibodies against GAD have also been detected in NOD mice[176~177].

(4) Antibodies against a 38 kD protein; In the above reports where sera from patients with IDDM immunoprecipitated GAD, a protein band of 38 kD was also immunoprecipitated. Autoantibodies against this 38 kD protein are also found in sera from BB rats[178]. The 38 kD membrane-bound islet-cell- specific autoantigen was not detected early in the life of DPBB rats, but later appeared to be correlated with ongoing insulitis[176]. This phenomenon may account for the decreased antigenicity of islet grafts obtained from neonatal BB rats, which are preserved in diabetic BB rats into whom they are transplanted[179]. The delayed expression of the 38 kD autoantigen in BB rats may trigger the development of autoimmune IDDM. Interestingly, CMV can also induce antibodies against the 38 kD autoantigen in humans[123].

(5) Antibodies against carboxypeptidase H; Carboxypetidase H (CPH) is probably involved in the cleavage of proinsulin. The autoantibody against CPH was detected in sera from 40% of preclinical subjects[180].

(6) Antibodies against bovine serum albumin; Antibodies against bovine serum albumin (BSA) have been found in patients with IDDM[181], NOD mice[182] and BB rats[183]. A recent

report suggested that ABBOS (a region of the BSA molecule extending from position 152 to position 168) was responsible for the binding site of this antibody, which cross-reacts with γ-interferon-induced 69 kD heat shock proteins on β-cells[148]. It is proposed that BSA protein in milk may be a trigger molecule during infancy and later leads to the development of diabetes through islet cross-reactive autoimmunity.

(7) Antibodies against glucose transport protein (GLUT-2); IgG from subjects with recent onset IDDM specifically inhibits high K_m glucose uptake by dispersed rat islets[184]. This effect is abolished by the adsorption of IgG against GLUT-2 expressing cells, indicating the presence of anti-GLUT-2 autoantibodies. However, there is no direct evidence of these antibodies in patients with IDDM.

(8) Other aspects of humoral autoimmunity; Patients with IDDM who have ICSAs also have increased numbers of B-lymphocytes, and the proportion of these lymphocytes secreting immunoglobulin is also increased[185~187]. Increases in CD5+ B-lymphocytes, which are normally only seen in foetal and perinatal individuals, have been reported in patients with IDDM[188~191] as well as with other autoimmune diseases. Autoantibodies that bind to self and exogenous antigens are produced by CD5+ B-lymphocytes, although this autoantibody production could also be a function of alterations in the role of T-cells[192].

2) Cell-mediated autoimmunity: Indications of the involvement of cell-mediated autoimmune responses in the pathogenesis of IDDM have been provided by autopsies performed on acute-onset patients whose pancreata show lymphocytic infiltration. As well, activated T-cells expressing MHC class II antigens are found in the insulitic islets of recent-onset patients[193~195] and there are increased numbers of activated helper/inducer T-cells circulating in the sera of patients with recent-onset IDDM[193,195~198]. T-cells expressing interleukin-2 (IL-2) receptors are also increased[193] in some patients with IDDM, indicating the involvement of the immune system.

Functional lymphocyte abnormalities may also be present in patients with IDDM, in addition to changes in subpopulations. Using the leukocyte migration test, it has been shown that leukocytes in IDDM patients are sensitized to pancreatic antigens and inhibit cell migration[199~201]. Lymphocytes from an acutely diabetic child were found to be cytotoxic in vitro to a human insulinoma line[202]. Most recent studies have shown that pancreatic islets from acutely diabetic patients contain macrophages, CD4+ and CD8+ T-cells, as well as natural killer (NK) cells[203~204] and there is evidence that T-cells as well as macrophages are involved in the destruction of β-cells. The role of cell-mediated autoimmunity in the pathogenesis of IDDM has been extensively studied in animal models such as the BB rat and NOD mouse. In this section the role of immunocytes and islet cell autoantigens in the pathogenesis of IDDM will be discussed.

(1) Role of macrophages and autoantigens in the initiation of β-cell-specific autoimmunity; Despite clear evidence for the involvement of cell-mediated autoimmunity in the development of diabetes in BB rats and NOD mice, the initial event which triggers β-cell destruction remains unidentified[205]. Examination of immunocytes infiltration the islets and their correlation with the time course of insulitis gives some insight into this initial event. Several studies have found that the major population of infiltrating cells during the early stages of insulitis are macrophages/dendritic cells[206~209]. These cells precede invasion by T-lymphocytes, NK cells, and B-lymphocytes[210]. Electron microscopy has found that most of the single cells present at an early stage of insulitis in BB rats are macrophages[206]. Silica, known to be toxic to macrophages, when administered intraperitoneally to young BB rats and NOD mice, completely prevents the development of insulitis and diabetes. This suggests that macrophages play an important role in the initiation of insulitis[134,211]. Our further experimental results showed that early, long-term silica treatment can deplete or inactivate macrophages, which in turn may prevent the development of β-cell-specific effectors. Late, short-term silica treatment, however, does not affect the destruction of β-cells by pre-existing effectors in NOD mice[212]. In BB rats, ConA-activated spleen cells prepared from silica-treated BB rats did not induce insulitis and diabetes in BB neonates, whereas ConA-activated spleen cells from untreated BB rats did, indicating that administration of silica particles to BB rats results in the loss of ability by splenocytes to transfer adoptively insulitis and diabetes[213]. Short-term silica administration to BB rats did not, however, alter the ability of splenocytes to adoptively transfer insulitis and diabetes after ConA activation.

Further investigation of splenocytes from silica-treated BB

rats revealed that silica treatment results in a significant reduction in the percentage and number of immunocytes, including CD4+ T-lymphocytes, CD8+ T-lymphocytes and NK cells[213]. The prevention of insulitis and diabetes in silica-treated BB rats probably results from a decrease in the number of macrophage-dependent T-lymphocytes and a reduction in NK cell cytotoxicity.

Macrophages are antigen-presenting cells (APCs) and present processed antigens to helper T-cells, in the context of MHC class II molecules present on the cell surface[179,214~215]. It is hypothesized that, prior to the development of insulitis, there might be antigenic changes on the β-cells which can be recognized as non-self, and which are processed and presented by APCs. The presentation of β-cell autoantigens by macrophages to CD4+ helper T-cells might be the initial step in the development of autoimmune diabetes[179,216].

Islet cell autoantigens which are the targets of autoimmune attack in IDDM have proven difficult to identify and have been studied largely by investigating the specificities of circulating autoantibodies present in the sera of most IDDM patients and also in animals. It is known that peripheral CD4+ T-cells from prediabetic and early diabetic patients proliferate in response to autoantigens recognized by IDDM-associated autoantibodies. They include islet cell autoantigens (which are thought to be glycolipids conjugated with sialic acid[163]), insulin, a 52 kD protein (carboxypeptidase H), a 69 kD heat shock protein, a 64 kD protein (GAD), 37 kD and 40 kD tryptic fragments of tyrosine phosphatase, and a 38 kD autoantigen. Among these autoantigens, the 64 kD protein, GAD, and the 38 kD protein have been implicated as playing a role in the initiation and/or process of autoimmune IDDM.

The precise role of GAD in the pathogenesis of autoimmune IDDM is not known. GAD is not β-cell specific, but is expressed in the testes, ovaries, thymus and stomach[217~218], as well as in human pancreatic α, δ and pancreatic polypeptide producing cells[219] and in Escherichia coli strains present in the gut[220]. Some investigators had speculated in earlier studies that GAD may act as an immuno-modulator in the pathogenesis of IDDM, as some prokaryotic products, such as lipopolysaccharides and staphylococcal enterotoxins (superantigens), can act as immunomodulators[221~222]; however, two recent studies in NOD mice have suggested that GAD is a key target antigen in the induction of murine IDDM[176~177]. These studies suggest that autoimmunity to GAD triggers T-cell responses to other β-cell antigens and that spontaneous autoimmune disease can be prevented in NOD mice by tolerization to GAD. Whether GAD is a target antigen in the initiation of murine autoimmune IDDM remains to be confirmed.

One recent study showed that a 38 kD autoantigen is expressed at about 30 days of age, the same time at which circulating antibodies against the 38 kD antigen are first detected[223]. Similar autoantibodies have also been reported in humans[171]. In this study, autoantibodies in the sera from all four tested new-onset IDDM patients immunoprecipitated a 38 kD islet cell protein prepared from HLA-DR3-positive donor islets. To date, the 38 kD protein the only delayed-expressed islet cell autoantigen whose antibody is consistently found in acutely diabetic BB rats[223]. Adams et al[224] showed that delayed expression of a transgene in β-cells resulted in a failure to establish self-tolerance and consequently produced autoimmune lesions in the pancreatic islets of several lines of mice. In contrast, mice that expressed this transgene early in life were tolerant. In non-diabetic WF rats, the 38 kD islet-cell-specific protein is expressed in both neonatal and adult islets. This early expression of the 38 kD protein is comparable with early expression of the transgene seen in the transgenic mice studied by Adams et al[224]. Buschard et al[225] reported that diabetes in BB rats could be prevented by neonatal stimulation of β-cells. This procedure is thought to induce early antigen expression on β-cells and subsequent tolerance. It is thus speculated that the presentation of 38 kD autoantigens, released during spontaneous turnover of β-cells, in conjunction with MHC class II molecules, might be the initial step in the development of autoimmune IDDM in BB rats. This hypothesis is supported by earlier reports that inactivation of macrophages by silica treatment stops further immune processes and leads to the prevention of insulitis and diabetes.

Roep et al[226] identified a 38 kD antigen that is recognized by a T-cell clone established from a newly diagnosed patient with IDDM. Subcellular fractionation studies using rat insulinoma cells indicated that the antigenic determinant recognized by this T-cell clone is an integral membrane component of the insulin secretory granule[226]. Granular membrane proteins are transiently exposed to the cell surface during

exocytosis and their accessibility to components of the immune system is thought to be a function of the secretory activity of β-cells. At the present time, it is not known whether there are any molecular similarities between this 38 kD protein isolated from insulinoma cells and the membrane-bound, delayed-expressed 38 kD islet cell autoantigen.

On the basis of the above studies, we suggest that the delayed expression of a gene encoding for a β-cell-specific 38 kD autoantigen may result in a breakdown in self-tolerance, leading to the initiation of β-cell-specific autoimmunity in the BB rat.

(2) The role of T-cells in the destruction of pancreatic β- cells; Time course studies have shown that β-cell infiltration by macrophages is followed by that of T-lymphocytes leading to insulitis and diabetes[12,203,227~228]. In both transplanted[9,229~232] and non-transplanted[12,203] diabetic patients most of the infiltrating lymphocytes at the time of diagnosis are CD8+ T-cells. This finding suggests a role for these cells late in the disease process.

Two phenomena have been observed that indicate that T-cell-mediated autoimmunity is clearly involved in the destruction of β-cells in the BB rat. First, BB rats treated with monoclonal antibodies (OX19) against antigens expressed on the surface of all T-cells do not develop insulitis or thyroiditis, indicating the important role played by T-cells in diabetogenesis in the BB rat[17]. The development of diabetes in BB rats could also be prevented by performing neonatal thymectomies or by immune suppression[16,233~237]. Second, when non-diabetic BB or Wistar-Furth rats were injected with conA-stimulated spleen cells derived from acutely diabetic BB rats, diabetes was adoptively transferred[238].

As with human IDDM, insulitis is seen in the NOD mouse both at the time of and prior to hyperglycaemia. Both CD4+ and CD8+ islet-cell-specific T-cell lines and clones have been isolated from NOD islets[216,239~245], but the role of each T-cell subset in the diabetogenic process is poorly understood. Although certain adoptive transfer studies using islet-specific CD4+ T-cell clones have questioned whether CD8+ T cells have a role in autoimmune murine IDDM[240,242], other studies using cloned and uncloned T-cells have indicated that both CD8+ and CD4+ T-cell subsets are required for the transfer of diabetes in NOD mice[246~249], NOD athymic nude mice[250] and NOD-scid/scid mice[251]. We have recently shown that a single β-cell-specific CD8+ cytotoxic T-cell clone (CTL) can transfer severe insulitis and disease to irradiated NOD mice, but only if co-injected with splenocytes depleted of CD8+ T-cells[252]. When recipient mice were treated with and anti- CD4 monoclonal antibody, neither insulitis nor diabetes occurred[252]. These studies indicate that, even when activated in vitro, CTL may require the assistance of CD4+ T-cells to effect β-cells damage. When considered together, these studies suggest that MHC class I restricted CD8+ cytotoxic T-cells may play an important role as final effectors of β-cell destruction, whereas CD4+ T-cells may be required for insulitis.

How CD4+ and CD8+ islet-reactive T-cells interact to destroy β-cells is not known and deserves further study. Recently, it has been suggested that linkage of helper and killer epitopes on the surface of one antigen-presenting cell is a prerequisite for productive interaction between the two cells[253]. In that case, the APC must process external antigens for MHC class I restricted presentation. It has been postulated that dendritic cells, which appear to be the primary APCs in spontaneous IDDM[254], could perform this activity[255]. Furthermore, it has been shown that the CD4+ and CD8+ epitopes do not have to be part of the same molecule and that neither the MHC restriction specificities of the interacting T-cells nor the MHC molecules they express have an influence on their capacity to collaborate. Direct T-T cell contact does not appear to be required and interleukin-2 and γ-interferon play a crucial role in this process[253]. Because, even when fully differentiated, β-cell-specific CD8+ cytotoxic T-cells cannot home into islets in the absence of T-cell help[252], we postulate that CD4+ T-cells recruited to the site may induce expression of CD8+ specific homing receptors on pancreatic endothelial cells which, in turn, may promote influx of precytotoxic precursors into the islets. The latter, if bearing β-cell-specific T-cell receptors, may differentiate into effector CTLs and destroy β-cells. This hypothesis is represented in Fig. 3.

These studies do not exclude other effector mechanisms for the destruction of pancreatic β-cells in NOD mice, such as non-MHC-restricted β-cell cytotoxicity[256~257]. For example, islet-reactive CD4+ T-cell clones can accelerate the development of diabetes in young NOD mice[240], transfer insulitis to irradiated I-E-transgenic mice[242] and destroy grafted islets in

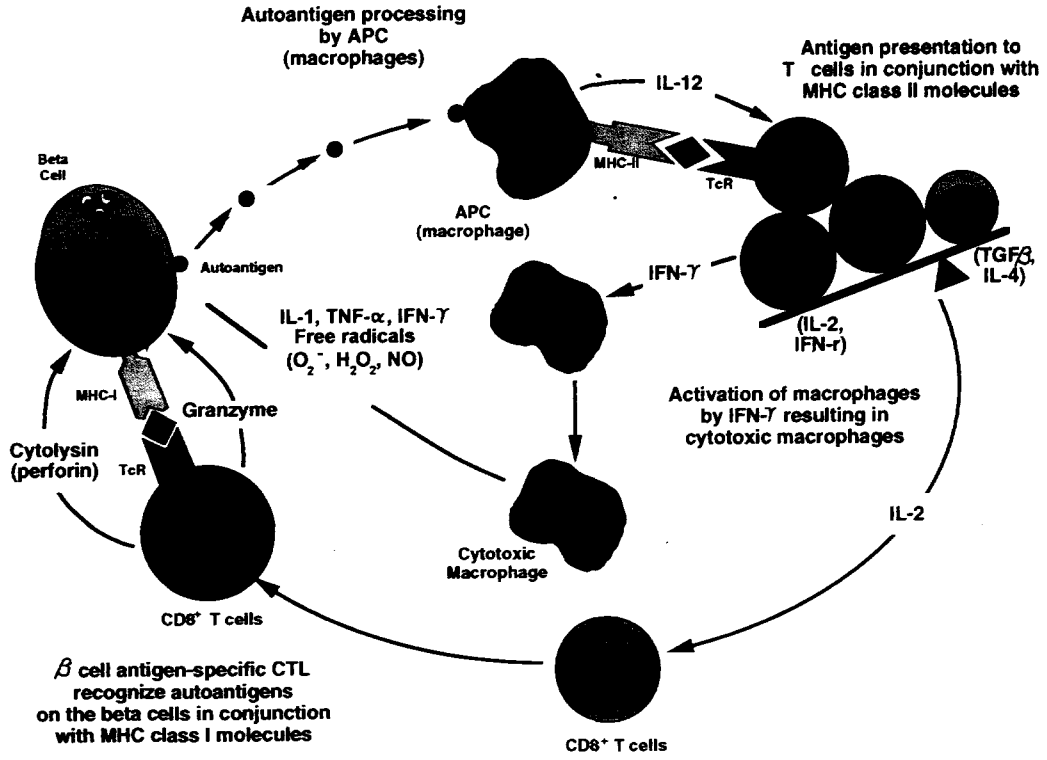

Fig. 3. Schematic diagram of hypothesis for the possible collaboration of macrophages, CD4+ T cells, and CD8+ T cells in the destruction of beta cells in conjunction with MHC-class I and II molecules. β-cell autoantigens may be released from β-cells during spontaneous turnover. The antigens are then processed by macrophages and presented to helper T-cells in association with MHC class II molecules. While this process is taking pace, β-cell-specific pre-cytotoxic T cells may be recruited to the islets and differentiated into effector T-cells by IL-2 and other cytokines released by CD4+ helper T-cells. IFN-γ released by helper T-cells (T_H1) can cause macrophages to become cytotoxic macrophages. The cytoxic macrophages release substantial amounts of β-cell-toxic cytokins (including IL-1, TNF-α, and IFN-γ) 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 the many unaffected β-cells, in conjunction with MHC class I molecules. These CTL also release granzyme and cytolysin (perforin) which are toxic to β-cells. In this way, free radicals, cytokins, macrophages, helper T-cells and cytotoxic T-cells may act synergistically in the destruction of beta cells, leading to the clinical onset of IDDM.

diabetes-resistant mice[258]. Since diabetes and insulitis can occur even when responding T-cells are unable to recognize islet-specific antigen directly on β-cells[254], autoreactive CD4+ T-cells may be able to effect β-cell damage by non-specific inflammatory mechanisms, such as by releasing β-cell-toxic cytokines. It has been shown that IL-1 and tumour necrosis factor have a cytotoxic effect on BB rat pancreatic β-cells in vitro[65,259]. However, this may be only one of several mechanisms that cause β-cell loss in IDDM, especially since the well-established HL-associations with IDDM do not correlate with the monokine secretory phenotype (high in subjects with IDDM-associated HLA genotypes and low in subjects with HLA genotypes providing resistance to IDDM)[260].

It is thought that regulatory T-cells are present in the islet tissue of NOD mice[244]. The islets of NOD mice may contain both effector cells and cells capable of inhibiting these effector cells. It is believed that the immuno-regulatory balance can be shifted in favour of either suppression of the effector cells, impairing β-cell destruction, or the enhancement of the effector cells, leading to β-cell destrction[249].

(3) T-cell receptor diversity and the pathogenesis of T-cell- mediated autoimmunity; Most CD4+CD8- and CD4-CD8+ T-cells express α-β T-cell receptors (TcRs) and recognize antigenic epitopes presented by MHC class II and

class I molecules respectively. CD4-CD8-peripheral T-cells constitute approximately 0.5-10% of the total T-cell population, and the great majority of CD4-CD8-T-cells express a TcR composed of γ-and δ-chains[254]. Each TcR chain is encoded by several genes (D and/or J and V) which rearrange during T- cell ontogeny to generate functional TcR rearrangements. In the germline, these genes are organized into multiple families, each composed of several members sharing > 75% sequence homology; 14Vγ (4 families), 7Vδ (6 families), over 50Vα (22 families) and over 50Vβ (20 familes) genes have been described in humans[261~264]. The high degree of variability already determined by differential J-(D)-V usage is increased by the enormous diversity of the junctional region, where all 20 amino acids may be found at each residue. Studies using specific antigenic systems have indicated that although conservation of the predominant α- and β-chain gene sequences is not required for antigen/MHC specificity, there is a significant correlation between selective usage of certain TcR sequences and recognition of certain antigen/MHC complexes[265].

Studies on several animal models of autoimmunity have suggested an association between autoimmune responses and selective T-cell receptor usage; the most striking results have been reported in the autoimmune disease experimental allergic encephalomyelitis (EAE), an animal model for multiple sclerosis (MS)[266]. In contrast, studies of NOD mouse insulitis T-cells[267~268] and islet-reactive T-cell clones[242~244] have suggested a diverse TcR repertoire. Of special note is the observation that islet-infiltrating T-cells from young NOD mice (4-6 weeks of age) use a limited TcR repertoire[267,269~270]. Since the predominant Vβ elements were strikingly different in each case, such restricted TcR usage may not play a major role in the disease process. Studies of insulitis CD8+ and $\gamma\delta$ + T-cells isolated from the pancreatic grafts of transplanted diabetic patients undergoing recurrence of IDDM have also revealed restricted TcR usge and oligoclonality. However, as in mice, the predominant clonotypes in each patient were different and junctional sequences (VDJ) showed considerable heterogeneity within and between patients. Altogether, these findings suggest that these islet-infiltrating T-cells may recognize multiple MHC-peptide complexes on β-cells or may be recruited to the site by superantigen-like antigen[271]. Therefore, any hypothesis attempting to explain the immunopathogenesis of IDDM must take this diversity into consideration (Fig. 3).

(4) Involvement of cytokines and free radicals in β-cell destruction;

① **Cytokines;** So far we have discussed the possibility that β-cells are targeted by autoreactive immunocytes, such as T-cells, macrophages and natural killer cells. Biochemical mediators of cytotoxicity, including cytokines, perforins, and proteases, may also selectively destroy β-cells[272]. Interleukin-1 (IL-1) has been extensively studied as one candidate cytokine for this selective destruction[273~275] and has been reported to be selectively cytotoxic in vitro[276]. In the same report, IL-1 was also shown to induce transient diabetes in normal rats[276], and it has been suggested that IL-1 receptors are present on β-cells[277]. While arguments about whether IL-1 selectively affects only β-cells and not other endocrine cells, or not have been ongoing, it has been shown that seven days of exposure to IL-1β did produce ultrastructural degenerative changes only in β-cells[278]. Several further studies have shown that IL-1β cytoxicity in a certain time-dose window is clearly restricted to β-cells[279~281]. The effects of IL-1 are potentiated by tumour necrosis factor (TNF)[259]. In vitro studies have shown that the effects of cytokines, interleukin-6 (IL-6), TNF, lymphotoxin, and γ-interferon are additive[282] and that these mediators, acting alone or synergistically, may destroy isloted islets or islets in cell cultrues[283~286]. On the basis of these observations, it is likely that cytokines may play an important role in the destruction of β-cells. The precise mechanisms whereby cytokines effect the destruction β-cells remain to be determined. One study on TNF showed that it enhances IL-1 cytotoxicity in vitro, but that it protects NOD mice from developing diabetes[287]. Regarding BB rats, Kolb and his colleagues have shown that human recombinant IL-2 when administered to BB rats increased the incidence of diabetes and that an earlier age of onset was observed[288]. In contrast, higher doses of IL-2 were not seen to have any effect on the incidence of diabetes in other studies[289]. Expression of interferon-γ in transgenic mice was seen to cerrelate with their development of an immune form of diabetes, suggesting that interferon-γ may play a role in the pathogenesis of autoimmune IDDM[290]. One recent paper has demonstrated that the expression of interferon-α in β-cells, of transgenic mice can induce diabetes. In these animals, the

selective loss of β-cells, but the relative sparing of pancreatic α-cells and δ-cells, was associated with a mixed inflammation centred on the islets[291]. These results suggest that activation of local macrophages and natural killer cells by interferon-α may be the primary cause of the β-cell loss.

② **Free radicals;** Several studies have implicated the role of oxygen-derived radicals and their metabolites in mcarophage/ neutrophil-mediated tissue injury. Macrophages and neutrophils undergo a respiratory burst in response to their activation by particulate and/or specific soluble inflammation mediators[292~294]. This is associated with a 2-to 20-fold increase in oxygen consumption and increased glucose metabolism via the hexose monophosphate shunt, depending on the cell and nature of the stimulus[295]. In conjunction with an increase in oxygen consumption, neutrophils and macrophages have been shown to generate both the superoxide anion (O^-_2) and hydrogen peroxide (H_2O_2). The superoxide anion may act as either an oxidant or a reductant, depending on the substance with which it reacts. It may act as an oxidant, gaining an electron to form H_2O_2. When two superoxide anions react with each other to form H_2O_2 and O_2 in a dismutation reaction, one anion acts as an oxidant and the other as a reductant. H_2O_2 may also be formed by phagocytic cells, which involves the direct double reduction of oxygen without the intermediate formation of the superoxide anion. An example of this reaction is the formation of H_2O_2 by the interaction of glucose and glucose oxidase. It appears that the toxic effect of activated macrophages on eukaryotic cells is mediated through the superoxide anion and hydrogen peroxide, although the mechanisms of cell injury are not clearly understood.

As discussed above, the cellular and molecullar mechanisms for mediating the effect of cytokines on β-cells are unknown. Among the various mechanism proposed, generation of oxygen free radicals in the targets cells has been implicated[296] as islet cells contain very low oxygen free radical scavenging activities[297~298] and are very vulnerable to free radicals[298]. It has been suggested that the cytokine products of infiltrating macrophages and lymphocytes may contribute to β-cell damage by inducing the production of oxygen free radicals in the islet cell[282].

③ **Transgenic mice, immunological tolerance and insulindependent diabetes mellitus;** Thymic tolerance has been well established as the primary mechanism for inducing tolerance to self-antigens. T-cell tolerance to antigens expressed in the thymus results in clonal deletion[299~309] or functional inactivation (anergy)[310~313] of self-reactive T-cells. Anergic T-cells express self-reactive T-cell receptors but cannot be activated by self antigen either in vivo or in vitro or by anti-TcR antibodies in the absence of interleukin-2[314~317]. T-cells can also undergo tolerance induction in the periphery. Peripheral clonal deletion has been demonstrated for antigen-specific and MIs-specific T-cells[318~320]. Down-regulation of TcR or co-receptors by self-reactive T-cells in the periphery can result in peripheral unresponsiveness, which may or may not be restored in vitro by polyclonal stimulation or stimulation with antigen[321~322]. Unresponsiveness to antigens expressed in the periphery has also been demonstrated in studies of transgenic mice expressing selected proteins (viral proteins, allogenic MHC molecules, and cytokines) in various organs during the development of the immune system[224,291,323~328]. Some of these models, including those expressing interferon-γ and interferon-α in β-cells, developed lymphocytic infiltration of the islets of Langerhans and diabetes, although the effector cell population and the mechanisms which triggered the infiltration were not characterized in detail[224,291,327~328]. Other models developed a non-immunologically-mediated form of hyperglycaemia, possibly as a result of over- expression of the transgene leading to perturbation of protein synthesis or transport[290,325,329]. With the exception of the model of Morahan et al[326], neither thymocytes nor peripheral T-cells from transgenic mice could mount a transgenic antigen-proliferative response, sugesting the involvement of thymic tolerance. Recently, Health et al[330] have suggested that thymic tolerance may have also been involved in the model of Morahan et al[326], owing to intrathymic expression of the allogenic MHC molecules. Other groups have found that the potential transgene-reactive T-cells were present and able to proliferate in response to the transgenic antigen in vitro, but an autoimmune response in the transgenic animal was not seen[331~333]. In the model of Oldstone et al[334], lymphocytic choriomeningitis virus (LCMV)-trangenic mice (expressing LCMV in β-cells) were able to generate virus-specific CTL response upon restimulation with the infecting virus. This response, however, was retarded in their development in several sublines, suggesting a temporary state of T-cell hyporesponsiveness. Simi-

lar studies were carried out by Ohashi et al[335] in mice trangenic for both a LCMV-specific TcR and LCMV. In this model, self-reactive T-cells remained functionally silent, apparently because of the inability of the β-cells to activate an immune response properly. Despite the enormous progress that has been made in our understanding of how tolerance is imposed upon self-reactive T-cells, the precise mechanism responsible for the breakdown of tolerance to β-cells in IDDM remains to be determined.

CONCLUSION

The development of IDDM is multifactorial and it is therefore not easy to simplify the pathogenic mechanisms involved in the aetiology of the disease. Both genetic and environmental factors are involved in the autoimmune process leading to pancreatic β-cell desctruction (Fig. 1). Macrophages are clearly involved early in this process since inactivation of macrophages results in the prevention of autoimmune IDDM. Accordingly, it is possible that the presentation of β-cell-specific autoantigens by macrophages to CD4+ T helper cells in conjunction with MHC class II molecules might be the initial step in the development of autoimmune diabetes. Most of the infiltrating lymphocytes at the time of diagnosis in diabetic patients are CD8+ T-cells, suggesting that CD8+ T-cells play a major role in the destruction of pancreatic β-cells at a later stage of the autoimmune process(Fig. 3). In animal models, CD4+ T-cells are required for the destruction of pancreatic β-cells by CD8+ T-cells, which may act as final effectors. Free radicals and cytokines, such as interleukin-1, released by macrophages and monocytes may work synergistically with the action of the CD8+ effector T-cells, to destroy pancreatic β-cells, leading to autoimmune IDDM.

ACKNOWLEDGEMENTS

The authors gratefuly acknowledge the secretarial assistance of Hye Sun Lee.

REFERENCES

1. Rossini AA, Greiner DL, Friedman HP and Mordes JP: Immunopathogenesis of diabetes mellitus. Diabetes Rev 1: 43-75, 1993
2. Yoon JW: Viral pathogenesis of insulin-dependent diabetes mellitus. In autoimmunity and the pathogenesis of diabetes (eds. F Ginsberg-Fellner and RC McEvoy) New York, Springer, pp206-255, 1990
3. Gorusch AN, Spencer KM, Lister J, McNally JM, Dean BM, Bottazzo GR and J Ludworth AG: Evidence for a long prediabetic period in type 1 (insulin-dependent) diabetes mellitus. Lancet 2: 1363-1365, 1981
4. Eisenbarth GS: Type I diabetes mellitus: a chronic autoimmune disease. New Eng J Med 314: 1360-1368, 1986
5. Maclaren NK, Schatz DA, Drash A and Grave G: Initial pathogenic events in IDDM. Diabetes 38: 534-538, 1989
6. Lernmark A, Bärmeier H, Dube S, Hagopian W, Karlsen A and Wassmuth R: Autoimmunity of diabetes. Endocrinol Metab Clin North AM 20: 589-617, 1991
7. Gepts W and De Mey J: Pathological anatomy of the pancreas in juvenile diabetes mellitus. Diabetes 14: 619-633, 1965
8. Bottazzo G, Florin-Christensen A and Doniach D: Islet cell antibodies in diabetes mellitus with polyendocrine deficiency. Lancet 2: 1279-1283, 1974
9. Sibley RK, Sutherland DE, Goetz FC and Micheal AF: Recurrent diabetes mellitus in the pancreas iso-and allograft. A light and electron microscopic and immunohistochemical analysis of four cases. Lab Invest 53: 132-144, 1985
10. Bourgnères PF, Carel JC, Castano L, Boitard C, Gardin JP, Landais P, Hors J, Mihatsch MJ, Paillard M, Chaussain JL and Bach JF: Factors associated with early remission of type I diabetes in children treated with cyclosporine. New Engl J Med 318: 663-670, 1988
11. Bourgnères PF, Landais P, Boisson C, Carel JC, Frament N, Boitard C, Chaussain JL and Bach JF: Limited duration of remission of insulin dependency in children with recent overt type I diabetes treated with low-dose cyclosporin. Diabetes 39: 1264-1272, 1990
12. Bottazzo G, Dean B, McNally J, MacKay E, Swift P and Gamble D: In situ characterization of autoimmune phenomena and expression of HLA molecules in the pancreas of diabetic insulitis. New Engl J Med 313: 353-360, 1985
13. Marliss E, Nakhooda A, Poussier P and Simma A: The diabetic syndrome of the 'BB' Wistar rat: possible relevance to type 1 (insulin-dependent) diabetes in man. Diabetologia 22: 225-232, 1982
14. Rossini AA, Mordes JP and Handler ES: Speculations on etiology of diabetes mellitus: tumbler hypothesis. Diabetes 37: 257-261, 1988
15. Marliss EB: The Juvenile Diabetes Foundation workshop on spontaneously diabetic BB rat as potential for insight into human juvenile diabetes. Metab Clin Exp 32(suppl 1): 1-166, 1983
16. Like AA, Rossini AA, Appel MC, Guberski DL and Williams

RM: Spontaneous diabetes mellitus: reversal and prevention in the BB/W rat with antiserum to rat lymphocytes. Science 206: 1421-1423, 1979

17. Like AA, Biron CA, Weringer EJ, Byman K, Sroczynsaki E and Guberski DL: 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
18. Makino S, Kunimoto K, Munaoko Y, Mizushima Y, Katagiri K and Tochino Y: Breeding of a non-obese diabetic strain of mice. Exp Anirn 29: 1-13, 1980
19. Brinster R, Chen H, Trumbauer M, Senear A, Warren R and Palmiter R: Somatic expression of herpes thymidine kinase in mice following injection of a fusion gene into eggs. Cell 27: 223-231, 1981
20. Costantini F and Lacy E: Introduction of a rabbit β-globulin gene into the mouse germline. Nature 294: 92-94, 1981
21. Wagner E, Stewart T and Mintz B: The human β-globulin gene and a functional viral thymidine kinase gene in developing mice. Proc Natl Acad Sci USA 78: 5016- 5020, 1981
22. Wagner T, Hoppe P, Jollick J, Scholl D, Hodinka R and Gault J: Microinjection of a rabbit beta globulin gene into zygotes and its subsequent expression in adult mice and their offspring. Proc Natl Acad Sci USA 78: 6376- 6380, 1981
23. Mullis K and Faloona F: Specific synthesis of DNA in vitro via a polymerase-catalysed chain reaction. Methods Enzymol 155: 335-350, 1987
24. Soeldner JS: The etiology of diabetes. In Clinical Diabetes Mellitus(ed. GP Kozak), Philadelphia, PA, Saunders, 1982, pp21-35
25. Nerup J, Platz P, Orted-Anderson O, Christy M, Lyngose J, Poulsen J, Ryder L, Thomson M, Milsen L and Svergaard A: HLA antigens and diabetes mellitus. Lancet 2: 133-135, 1974
26. Owerbach D and Gabbay KH: The search for IDDM susceptibility Genes. Diabetes 45: 544-551, 1996
27. Thomsen M, Platz P, Andersen O, Christy M, Lyngose J, Nerup J, Kasmussen K, Ryder LP, Melsen L and Svergaard A: MLC typing in juvenile diabetes mellitus and idiopathic Addison's disease. Transplant Rev 22: 125-147, 1975
28. Schreuder G, Tilanus M, Bontrop R, Bruining G, Giphart M, vanRood JJ and deVries R: HLA-DQ polymorphism associated with resistance to type I diabetes detected with monoclonal antibodies, isoelectric point difference and restriction fragment length polymorphism. J Exp Med 164: 938-943, 1986
29. Horn G, Bugawan T, Long C and Erlich H: Allelic sequence of the HLA-DQ loci: relationship to serology and to insulin-dependent diabetes susceptibility. Proc Natl Acad Sci USA 85: 6012-6016, 1988
30. Todd J, Bell J and McDevitt H: HLA-DQb gene contribute to susceptibility and resistance to insulin-dependent diabetes mellitus. Nature 329: 599-604, 1987
31. Segall M: HLA and the genetics of IDDM: holism versus reductionisms. Diabetes 37: 1005-1008, 1988
32. Sheehy M, Scharf S, Rowe J, Neme de Gimenez M, Meske L, Erlich H and Nepom BS: A diabetes-susceptible HLA haplotype is best defined by a combination of HLA-DR and DQ alleles. J Clin Invest 83: 830-835, 1989
33. Nepom GT: Immunogenetics and IDDM. Diabetes Rev 1: 93-103, 1993
34. Winter WE, Obata M and Maclaren NK: Clinical and molecular aspects of autoimmune endocrine disease. In Clinical and Molecular Aspects of Autoimmune Diseases: Concepts Immunopathol(eds. JM Cruse and RE Lewis Jr) Basel, Karger, 1992, pp189-221
35. Kwok WW, Mickelson E, Masewicz S, Milner EC, Hansen J and Nepom GT: Polymorphic DQ α and DQ β interactions dictate HLA class II determinants of allo-recognition. J Exp Med 171: 85-95, 1990
36. Kwok WW and Nepom GT: Mutagenesis and expression studies of the HLA-DQ3.2 IDDM susceptibility gene. In Genes and Gene Products in the Development of Diabetes Mellitus(eds. J Nerup, T Mandrup-Poulsen and B Hokfelt) Amsterdam, Elsevier, 1989, pp41-53
37. Kwok WW and Nepom GT: Structural and functional constraints on HLA class II dimers implicated in susceptibility to IDDM. In the Genetics of Diabetes(eds. B Tait and L Harrison) London, Saunders, 1991, pp375-394
38. Awata T, Iwamoto Y, Matsuda A, Kuzuya T, Kanazawa Y and Juji T: High frequency of aspartic acid at position 57 of HLA-DQ beta chain in Japanese IDDM and nondiabetic subjects. Diabetes 38(suppl 2): 90a, 1989
39. Santamaria P, Barbosa JJ, Lindstrom AL, Lemke TA, Goetz FC, and Rich SS: HLA-DQ β 1-associated susceptibility that distinguishes Hashimoto's thyroiditis from Graves' disease in type I diabetic patients. J Clin Endocrinol Metab 78: 878-883, 1994
40. Buse J, Rifai-Haddad R, Lees S, Taniguchi H, Chaplin D, Milford EM, Seidman JG, Eisenbarth GS and Jackson RA: Major histocompatibility complex restriction fragment length polymorphism defines three diabetogenic haplotypes in BB and BBN rats. J Exp Med 162: 444-458, 1985
41. Colle E, Guttmann RD, Seemayer T and Michel F: Spontaneous diabetes mellitus syndrome in the rat IV. Immunogenetic interaction of the MHC and non-MHC components of the syndrome. Metabolism 32: 54-61, 1983
42. Jackson RA, Buse JB, Rifai R, Pelletier D, Milford EL, Carpenter CB, Eisenbarth GS, and Williams RM: Two genes required for diabetes in BB rats. Evidence from cyclical intercrosses and backcrosses. J Exp Med 159: 1629-1636,

1984

43. Poussier P, Nakhooda AF, Falk JK, Lee C and Marliss EB: Lymphopenia and abnormal lymphocyte subsets in the 'BB' rat: relationship to the diabetic syndrome. Endocrinology 110: 1825-1827, 1982
44. Like AA, Guberski DL and Butler L: Diabetic Bio Breeding/ Worcester (BB/Wor) rats need not be lymphopenic. J Immunol 136: 3254-3258, 1986
45. Jacob H, Pettersson A, Wilson D, Mao Y, Lernmark A and Lander E: Genetic dissection of autoimmune type I diabetes in the BB rat. Nature Genet 2: 56-60, 1992
46. Colle E, Guttmann RD and Seemayer TA: Spontaneous diabetes mellitus in the rat I. Association with the major histocompatibility complex. J Exp Med 154: 1237-1242, 1981
47. Colle E, Guttmann RD and Fuks A: Insulin-dependent diabetes mellitus is associated with genes that map to the right of the class I RT1: A locus of the major histocompatibility complex of the rat. Diabetes 35: 454-458, 1986
48. Colle E, Ono SJ, Fuks A, Guttmann RD and Seemayer TA: Association of susceptibility to spontaneous diabetes in rats with genes of major histocompatibility complex. Daibetes 37: 1438-1443, 1988
49. Greiner D, Handler E, Nakano K, Mordes J and Rossini A: Absence of the RT-6 cell subset in diabetes-prone BB/W rats. J Immunol 136: 148-151, 1986
50. Holowachuk EW and Greer MK: Unaltered class II histocompatibility antigens and pathogenesis of IDDM in BB rats. Diabetes 38: 267-271, 1989
51. Hattori M, Buse J, Jackson R, Glimcher L, Dorf M, Minami M, Makins S, Moriwaki K, Kuzuya H, Imura H, Strauss WM, Seidman D and Eisenbarth GS: The NOD mouse: recessive diabetogenic gene in the major histocompatibility complex. Science 231: 733-735, 1986
52. Prochazda M, Leither E, Serreze D and Coleman D: Three recessive loci required for insulin-dependent diabetes in non-obese diabetic mice. Science 237: 286-289, 1987
53. Leither E: The role of environmental factors in modulating insulin-dependent diabetes. In the Role of Microorganisms in Non-infectious Diseases(eds. R DeVries, I Cohen and JJ Van Rood) London, Springer, 1990, pp39-55
54. Wicker L, DeLarato N, Pressey A and Peterson L: Speculation on the genetic control of diabetes and insulitis in the NOD mouse. In Frontiers in Diabetes Research Vol 3. Lessons from Animal Diabetes (ed. E Shafrir) London, Smith-Gordon, 1990, pp67-72
55. Acha-Orbea H and McDevitt HO: The first external domain of the nonobese diabetic mouse class II-A beta chain is unique. Proc Natl Acad Sci USA 84: 2435-2439, 1987
56. Lund T, O'Reilly L, Hutchings P, Kanagawa O, Simpson E, Gravely R, Chandler P, Dyson J, Picard JK, Edwards A, Kioussis D and Cooke A: Prevention of insulin dependent diabetes in nonobese diabetic mice by transgenes encoding a modified I-A β chain or normal I-E β chain. Nature 345: 727-729, 1990
57. Miyazaki JM and Yawamura K: Direct evidence for the contribution of the unique I-A Nod to the development of insulitis in non-obese diabetic mice. Nature 345: 722-724, 1990
58. Slattery R, Kjer-Nielsen L, Allison J, Charlton B, Mandel T and Miller J: Prevention of diabetes in nonobese diabetic I-Ak transgenic mice. Nature 345: 724-726, 1990
59. Uehira M, Uno M, Kurner T, Kikutani H, Mori T, Inomoto T, Uede T, Miyazaki J, Kishimoto T and Yamamura K: Development of autoimmune insulitis is prevented in Ealphad but not in Abetak NOD transgenic mice. Int Immunol 1: 209-213, 1989
60. Podolin P, Pressey A, DeLarato N, Fischer P, Peterson L and Wicker L: I-E+ nonobese diabetic mice develop insulitis and diabetes. J Exp Med 178: 793-803, 1993
61. Leither E: The NOD mouse meets the 'Nerup hypothesis': is diabetogenesis the result of a collection of common alleles present in unfavourable combinations? In Frontiers in Animal Diabetes Vol 3. Lessons from Animal Diabetes(ed. E Shafrir) New York, Smith-Gordon, 1990, pp54-58
62. Todd J, Aitman T, Cornall R, Ghosh S, Hall J, Hearne C, Knight AM, Love JM, McAleer MA, Prins JB, Rodrigues N, Lathrop M, Pressey A, DeLarato NH, Peterson LB and Wicker LS: Genetic analysis of autoimmune type I diabetes mellitus in mice. Nature 351: 542-547, 1991
63. Prins J, Todd J, Rodrigues N, Ghosh S, Hogarth P, Wicker L, Gaffney E, Podolin PL, Fischer PA, Sirotina A and Peterson LB: Linkage on chromosome 3 of autoimmune diabetes and defective Fc receptor for IgG in NOD mice. Science 260: 695-698, 1993
64. Cornall R, Prins J, Todd J, Pressey A, DeLarato N, Wicker L and Peterson LB: Type I diabetes in mice is linked to the interleukin-1 receptor and Lsh/Ity/Beg genes on chromosome 1. Nature 353: 262-265, 1991.
65. Mandrup-Poulsen T, Bendtzen K, Nerup J, Dinarello C, Svenson M and Nielsen J: Affinity-purified human interleukin-1 is cytotoxic to isolated islets of Langerhans. Diabetologia 29: 63-67, 1986
66. Blackwell J, Roach T, Atkinson S, Ajioka J, Barton C and Shaw M: Genetic regulation of macrophage priming/activation: The Lsh gene story. Immunol Lett 30: 241-248, 1991
67. Malo D, Schurr E, Epstein D, Vekemans M, Skamene E and Gros P: The host resistance locus Bcg is tightly linked to a group of cytoskeleton-associated protein genes that include villin and desmin. Genomics 10: 356-364, 1991

68. Schurr E, Malo D, Radzioch D, Buschman E, Morgan K, Gros P and Skamene E: Genetic control of innate resistance to mycobacterial infections. Immunol Today 12: 42-45, 1991
69. Garchon H, Bedossa P, Eloy L and Bach J: Identification and mapping to chromosome 1 of a susceptibility locus for periinsulitis in non-obese diabetic mice. Nature 353: 260-262, 1991
70. Sentman C, Shutter J, Hockenbery D, Kanagawa O and Korsmeyer S: Bcl-2 inhibits multiple forms of apoptosis but not negative selection in thymocytes. Cell 67: 879- 888, 1991
71. Strasser A, Harris A and Cory S: Bcl-2 transgene inhibits T cell death and perturbs thymic self-censorship. Cell 67: 889-899, 1991
72. Strasser A, Whittingham S, Vaux D, Bath M, Adams J, Cory S and Harris AW: Enforced Bcl-2 expression in B-lymphoid cells prolongs antibody responses and elicits autoimmune disease. Proc Natl Acad Sci USA 88: 8661-8665, 1991
73. Barnett A, Eff C, Leslie R and Pyke D: Diabetes identical twins. A study of 200 pairs. Diabetologia 20: 87-93, 1981
74. Yoon JW: Role of viruses and environmental factors in induction of diabetes. In Human Diabetes: Genetic, Environmental and Autoimmune Etiology: Current Top in Microbiol Immunol 164(eds. S Baekkeskov and B Hansen) Springer, New York, 1990, pp95-123
75. Bosi E, Todd I, Pujol-Borrell R and Bottazzo G: Mechanisms of autoimmunity: relevance to the pathogenesis of type 1 (insulin-dependent) diabetes mellitus. Diabetes Metab Rev 3: 893-923, 1987
76. Eaten MD: Autoimmunity induced by syngeneic membranes carrying irreversibly absorbed paramyxovirus. Infect Immun 27: 855-861, 1980
77. Massa PT, Dorries R and ter Meulen V: Viral particles induce Ia antigen expression on astrocytes. Nature 320: 543-546, 1986
78. Gianani-R and Sarvetnick N: Virus, cytokines, antigens, and autoimmunity. Proc Natl Acad Aci USA 93: 2257-2259, 1996
79. Oldstone MBA and Notkins AL: Molecular mimicry. In Concepts in Viral Pathogenesis II(eds. A Notkins and MBA Oldstone) New York, Springer, 1991, pp195-201
80. Craighead J and McLane M: Diabetes mellitus: Introduction in mice by encephalomyocarditis virus. Science 162: 913-915, 1968
81. Yoon JW and Notkins AL: Virus-induced diabetes mellitus VI. Genetic determined host differences in the replication of encephalomyocarditis virus in pancreatic beta cells. J Exp Med 143: 170-185, 1976
82. Yoon JW, Lesniak M, Fussganger R and Notkins A: Genetic differences in susceptibility of pancreatic β cells to virus-induced diabetes mellitus. Nature 264: 178-180. 1976
83. Onodera T, Yoon JW, Brown KS and Notkins AL: Evidence for a single locus controlling susceptibility to virus-induced diabetes mellitus. Nature 274: 693-695, 1978
84. Yoon JW, McClintock P, Onodera T and Notkins A: Virus-induced diabetes mellitus: Inhibition by a non-diabetogenic variant of encephalomyocarditis virus. J Exp Med 152: 878-892, 1980
85. Yoon JW and Bae YS: Development of insulin-dependent diabetes mellitus in animals: control by a single gene of the host and a single amino acid of the virus. In Lessons from Animal Diabetes IV(ed. E Shafrir) London, Smith-Gordon, 1993, pp51-60
86. Buschard K, Rygaard J and Lund E: The inability of a diabetogenic virus to induce diabetes mellitus in athymic (nude) mice. Acta Pathol Microbiol Scand C 84: 299-303, 1976
87. Buschard D, Hastrup N and Rygaard J: Virus-induced diabetes mellitus in mice and the thymus-dependent immune system. Diabetologia 24: 42-46, 1983
88. Jansen F, Muterfering H and Schmidt W: Virus-induced diabetes and the immune system: suggestion that appearance of diabetes depends on immune reactions. Diabetologia 13: 545-549, 1977
89. Vialettes B, Baume D, Charpin C, DeMaeyer-Guignard J and Vague P: Assessment of viral and immune factors in EMC virus-induced diabetes: effects of cyclosporin A and interferon. J Clin Lab Immunol 10: 35-40, 1983
90. Yoon JW, McClintock P, Bachurski C, Longstreth J and Notkins A: Virus-induced diabetes mellitus. No evidence for immune mechanisms in the destruction of beta cells by encephalomyocarditis virus. Diabetes 34: 922-925, 1985
91. Baek H and Yoon JW: Direct involvement of macrophages in destruction of β cells leading to the development of diabetes in virus-infected mice. Diabetes 40: 1586-1597, 1991
92. Yoon JW, Rodrigues MM, Currier C and Notkins AL: Long term complications of virus-induced diabetes in mice. Nature 296: 567-569, 1982
93. Yoon JW and Reddi AH: Decreased bone formation and mineralization in virus induced diabetes mellitus. Am J Physiol 246: C177-C179, 1984
94. Bae YS and Yoon JW: Determination of diabetogenicity attributable to a single amino acid Ala-776, on the polyprotein of encephalomyocarditis virus. Diabetes 42: 435-443, 1993
95. Toniolo A, Onodera T, Jordan G, Yoon J and Notkins A: Virus-induced diabetes mellitus: glucose abnormalities produced in mice by all six members of the Coxsackie B virus group. Diabetes 31: 496-499, 1982
96. Yoon JW, Onodera T and Notkins A: Virus-induced diabetes mellitus: beta cell damage and insulin-dependent hypergly-

cemia in mice infected with Coxsackie virus B4. J Exp Med 148: 1068-1080, 1978

97. Yoon JW, London W, Curfman B, Brown R and Notkins A: Coxsackie virus B4 produces transient diabetes in nonhuman primates. Diabetes 35: 712-716, 1986
98. Gamble DR, Kinsley ML, Fitzgerald MG, Bolton R and Taylor KW: Viral antibodies in diabetes mellitus. Br Med J 3: 627-630, 1969
99. Wilson C, Connolly JM and Thomson D: Coxszckie B2 virus infection and acute-onset diabetes in a child. Br Med J 1: 1008, 1977
100. Yoon JW, Austin M, Onodera T and Notkins A: Virus-induced diabetes mellitus: isolation of a virus from the pancreas of a child with diabetic ketoacidosis. New Engl J Med 300: 1173-1179, 1979
101. Banatvala J, Schernthaner G, Schober E, DeSilva L, Bryant J, Borkenstein M, Brown D and Menser MA: Coxsackie B, mumps, rubella and cytomegalovirus specific IgM responses in patients with juvenile-onset insulin-dependent diabetes mellitus in Britain, Austria and Australia. Lancet 1: 1409-1412, 1985
102. Friman G, Fohlman J, Frisk G, Diderholm H, Ewald U, Kobbah M and Tuvemo T: An incidence peak of juvenile diabetes. Relation to Coxsackie B virus immune response. Acta Pediatr Scand 320(suppl): 14-19, 1985
103. Jenson AB, Rosenberg HS and Notkins AL: Pancreatic islet cell damage in children with fatal viral infections. Lancet 2: 354-358, 1980
104. King M, Shaikh A, Bidwell D, Voller A and Banatvala J: Coxsackie B-virus specific IgM responses in children with insulin-dependent diabetes mellitus. Lancet 1: 1397-1399, 1983
105. Wagenknecht L, Roseman J and Herman W: Increased incidence of insulin-dependent diabetes mellitus following an epidemic of Coxackie virus B5. Am J Epidemiol 133: 1024 -1031, 1991
106. Gerling I, Nejman C and Chatterjee N: Effect of coxsackievirus B4 infection in mice on expression of 64,000-Mr autoantigen and glucose sensitivity of islets before development of hyperglycemia. Diabetes 37: 1419-1425, 1988
107. Gerling I and Charrerjee N: Autoantigen (64,000-Mr) expression in Coxsackievirus B4-induced experimental diabetes. Curr Top Microbiol Immunol 156: 55-62, 1990
108. Kaufman M, Erlander M, Clare-Salzler M, Atkinson M, Maclaren N and Tobin A: Autoimmunity to two forms of glutamate decarboxylase in insulin-dependent diabetes mellitus. J Clin Invest 89: 283-292, 1992
109. Forrest J, Menser M and Burgess J: High frequency of diabetes mellitus in young adults with congenital rubella. Lancet 1: 332-334, 1971
110. McEvoy R, Cooper L, Rubinstein P, Fedun B and Ginsberg-Fellner F: Type I diabetes mellitus (IDDM) and autoimmunity in patients with congenital rubella syndrome (CRS): increased incidence of insulin autoantibodies. Diabetes 35 (suppl 1): 187A, 1986
111. Menser M, Forrest J and Bransby R: Rubella infection and diabetes mellitus. Lancet 1: 57-60, 1978
112. Patterson K, Chandra R and Jenson A: Congenital rubella, insulitis and diabetes mellitus in an infant. Lancet 1: 1048-1049, 1981
113. Rabinowe S, George K, Loughin R and Soeldner J: Predisposition to type I diabetes and organ-specific autoimmunity in congenital rubella: T cell abnormalities in young adults. Diabetes 35(suppl 1): 187A, 1986
114. Rayfield E, Kelly K and Yoon JW: Rubella virus-induced diabetes in hamsters. Diabetes 35: 1278-1285, 1986
115. Schopfer K, Matter L, Flueler U and Werder E: Diabetes mellitus endocrine autoantibodies and prenatal rubella infection. Lancet 2: 159, 1982
116. Smithells R, Sheppard S, Marshall W and Peckham C: Congenital rubella and diabetes mellitus. Lancet 1: 439, 1978
117. Ginsberg-Fellner F, Witt ME and Fedun B: Diabetes mellitus and autoimmunity in patients with congenital rubella symdrome. Rev Infect Dis 7(suppl 1): S170-S175, 1985
118. Ginsberg-Fellner F, Fedun B, Cooper LA, Witt ME, Franklin BH, Roman SH, Rubenstein P and McEvoy RC: Interrelationships of congenital rubella and type 1 insulin-dependent diabetes mellitus. In the Immunology of Diabetes Mellitus(eds. MA Jaworski, GD Molnar, RV Rajotte and B Singh) Amsterdam, Elsevier, 1986, pp279-286
119. Ginsberg-Fellner F, Witt ME, Yagihashi S, Dobersen MJ, Taub F, Fedun B, McEvoy RC, Roman SH, Davies TF, Cooper LZ, Rubinstein P and Notkins AL: Congenital rubella-syndrome as a model for type 1 (insulin-dependent) diabetes mellitus: increased prevalence of islet cll surface antibodies. Diabetologia 27(suppl 1): 87-89, 1984
120. Ward KP, Galloway WH and Auchterlonie IA: Congenital cytomegalovirus infection and diabetes. Lancet 1: 497, 1979
121. Onodera T, Suzuki H, Toniolo A and Notkins AL: Virus-induced diabetes: cytomegalo-virus and multiple environmental insults. Diabetologia 24: 218-219, 1983
122. Pak CY, Eun HM, McArthur RG and Yoon JW: Association of cytomegalovirus infection with autoimmune type I diabetes. Lancet 2: 1-4, 1988
123. Pak CY, Cha CY, Rajotte RV, McArthur RG and Yoon JW: Human pancreatic islet cell-specific 38 kD autoantigen identified by cytomegalovirus-induced monoclonal islet cell autoantibody. Diabetologia 33: 569-572, 1990
124. Fox GJ and Murphy JC: Cytomegalic virus-associated insulitis in diabetic. Octodon degus Vet Pathol 16: 625-628,

1979
125. Gamble DR: Relation of antecedent illness to development of diabetes in children. Br Med J 2: 99-101, 1980
126. Helmke K, Otten A and Willems W: Islet cell antibodies in children with mumps infecton. Lancet 2: 211-212, 1980
127. Gaskins H, Prochazka M, Hamaguchi K, Serreze D and Leither E: Beta cell expression of endogenous xenotropic retrovirus distinguishes diabetes-susceptible NOD/Lt from resistant NON/Lt mice. J Clin Invest 90: 2220-2227, 1992
128. Suenaga K and Yoon JW: Association of beta cell-specific expression of endogenous retrovirus with the development of insulitis and diabetes in NOD mice. Diabetes 37: 1722-1726, 1988
129. Leither EH: Type C retrovirus production by pancreatic beta cells: association with accelerated pathogenesis in C3H-db/db ('diabetes') mice. Am J Pathol 119: 22-32, 1985
130. Fukino-Kurihara H, Fujita H, Hakura A, Nonaka K and Tarui S: Morphological aspects on pancreatic islets of non-obese diabetic (NOD) mice. Virchows Arch 10: 107-120, 1985
131. Leither EH and Kuff EL: Intracisternal type A particles in murine pancreatic β cells: immunocytochemical demonstration of increased antigen p73 in genetically diabetic mice. Am J Pathol 114: 46-55, 1984
132. Nakagawa C, Hanafusa T, Miyagawa J. Yutsudo M, Nakajima H, Yakamoto K, Tomita K, Kono N, Hakura A and Tarui S: Retrovirus gag protein p30 in the islets of nonobese diabetic mice: relevance for pathogenesis of diabetes mellitus. Diabetologia 35: 614-618, 1992
133. Pak CY, Lee M and Yoon JW: Possible prevention of IDDM by vaccination with viral specific proteins. In Pediatric and Adolescent Endocrinology: Prediabetes Vol 22(ed. Z Laron), 1993, pp105-112
134. Lee K, Amano K and Yoon JW: Evidence for initial involvement of macrophages in development of insulitis in NOD mice. Diabetes 37: 989-991, 1988
135. Guberski DL, Thomas VA, Shek WR, Like AA, Handler ES, Rossini AA, Wallace JE and Welsh RM: Induction of type 1 diabetes by Kilham's rat virus in diabetes-resistant BB/Wor rats. Science 254: 1010-1013, 1991
136. Brown DW, Welsh RM and Like AA: Infection of peripancreatic lymph nodes but not islets precedes Kilham's rat virus-induced diabetes in BB/Wor rats. J Virol 67: 5873 -5878, 1993
137. Ellerman KE, Richards CA, Guberski DL, Shek WR and Like AA: Kilham Rat virus triggers T-cell-dependent autoimmune diabetes in multiple strains of rat. Diabetes 45: 557-562, 1996
138. Yoon JW, Kim CJ, Pak CY and McArthur RG: Environmental factors and IDDM. Clin Invest Med 10: 457-469, 1987
139. Elliot R and Martin J: Dietary protein: a trigger of insulin-dependent diabetes in the BB rat? Diabetologia 26: 297-299, 1984
140. Issa-Chergui D, Guttmann R, Seemayer T, Kelley V and Colle E: The effect of diet on the spontaneous insulindependent diabetic syndrome in the rat. Diabetes Res 9: 81-86, 1988
141. Scott F: Cow milk and insulin-dependent diabetes mellitus: Is there a relationship? Am J Clin Nutr 51: 489-491, 1990
142. Scott F: Food, diabetes and immunology. In Nutrition and Clinical Immunology(eds. L Kabbash, G Blackburn and S Bell) Boca Raton, FL, CRC Press, 1994, pp332-361
143. Scott F, Sarwar G and Cloutier H: Diabetogenecity of various protein sources in the diet of the BB rat. In Prediabetes(eds. RA Camerini-Davalos and HS Cole), New York, Plenum, 1988, pp277-285
144. Scott F, Elliott R and Kolb H: Diet and autoimmunity: prospect of prevention of type 1 diabetes. Diabetes Nutr Metab 2: 61-69, 1989
145. Hoorfar J, Scott F and Cloutier H: Dietary plant materials and development of diabetes in the BB rat. J Nutr 121: 908-916, 1991
146. Scott F and Marliss E: Conference summary: diet as an environmental factor in development of IDDM. Can J Physiol Pharmacol 69: 311-319, 1991
147. Borch-Johnsen K, Mandrup-Poulsen T, Zachau-Christiansen B, Jones G, Christy M, Kastrup K and Nerup J: Relation between breast-feeding and incidence rates of insulin-dependent diabetes mellitus. Lancet 2: 1083-1086, 1984
148. Karjalainen J, Martin JM, Knip M, Ilonen J, Robinson BH, Savilanti E, Akerblom H and Dosch H: A bovine albumin peptide as a possible trigger of diabetes mellitus. New Engl J Med 327: 302-307, 1992
149. MacCuish AC, Irvine WJ, Baines EW and Duncan LJ: Antibodies to pancreatic islet cells in insulin-dependent diabetes with coexistent autoimmune disease. Lancet 2: 1529-1531, 1974
150. Dobersen MJ, Scharff JE, Ginsberg-Fellner F and Notkins AL: Cytotoxic autoiantibodies to beta cells in the serum of patients with insulin-dependent diabetes mellitus. New Eng J Med 303: 1493-1498, 1980
151. Lernmark A, Freedman ZR, Hofmann C, Rubenstein AH, Steiner DF, Jackson RL, Winter RJ and Traisman HS: Islet cell surface antibodies in juvenile diabetes mellitus. New Engl J Med 229: 375-382, 1978
152. Scherbaum WA, Mogel H, Boehm BO, Hedderich U, Gluck M, Schernthaner G, Bottazzo GF and Pfeiffer EF: Autoantibodies to adrenal medullary and thyroid calcitonin cells in type I diabetes mellitus-a prospective study. J Autoimmun 1: 219-230, 1988
153. Brown FM, Kamalesh M, Adri MNS and Rabinowe SL:

Anti-adrennal medullary antibodies in IDDM subjects and subjects at high risk of developing IDDM. Diabetes Care 11: 30-33, 1988

154. Kolb H and Shinzato M: Antibodies to thymic hormone and HLA-DQ in type I diabetes. Lancet 336: 570-571, 1990
155. Kobayashi T, Nakanishi K, Kajio H, Morinaga S, Sugimoto T, Murase T and Kosaka K: Pancreatic cytokeratin: an antigen of pancreatic exocrine cell autoantibodies in type I (insulin-dependent) diabetes mellitus. Diabetologia 33: 363-370, 1990
156. Segers O, Gorus F, Somers G, van de Winkel M, Vercammen M and Pipeleers D: Cell surface antibodies in type I (insulin-dependent) diabetic patients II. Presence of immunoglobulins M which bind to lymphocytes. Diabetologia 32: 618-623, 1989
157. Kanatsuna T, Lernmark A, Rubenstein AH and Steiner DF.: Block in insulin release from column-perifused pancreatic β -cells induced by islet cell surface antibodies and complement. Diabetes 30: 231-234, 1981
158. Castaño L and Eisenbarth GS: Type I diabetes : a chronic autoimmune disease of human, mouse, and rat. Annual Rev Immunol 8: 647-679, 1990
159. Tarn AC, Thomas JM, Dean BM, Ingram D, Schwarz G, Bottazzo GF and Gale EAM: Predicting insulin-dependent diabetes. Lancet 1: 845-850, 1988
160. Kolb H, Dannehl K, Grüneklee D, Zielasek J, Bertrams J, Hübinger A and Gries AF: Prospective analysis of islet cell antibodies in children with type I (insulin-dependent) diabetes. Diabetologia 31: 189-194, 1988
161. Srikanta S, Ganda OP, Soeldner JS and Eisenbarth GS: First degree relatives of patients with type I diabetes mellitus: islet cell antibodies and abnormal insulin secretion. New Engl J Med 313: 461-464, 1985
162. Ziegler AG, Ziegler R, Vardi P, Jackson RA, Soeldner JS and Eisenbarth GS: Life table analysis of progression to diabetes of anti-insulin autoantibody: positive relatives of individuals with type I diabetes. Diabetes 38: 1320-1325, 1989
163. Nayak RC, Omar MAK, Rabizadeh A, Srikanta S and Eisenbarth GS: 'Cytoplasmic' islet cell antibodies: evidence that the target antigen is a sialoglycoconjugate. Diabetes 34: 617-619, 1985
164. Colman PG, Nayak RC, Cambell IL and Eisenbarth GS: Binding of cytoplasmic islet cell antibodies is blocked by human pancreatic glycolipid extracts. Diabetes 37: 645-652, 1988
165. Gillard BK, Thomas JW, Nell IJ and Marcus DM: Antibodies against ganglioside GT3 in the sera of patients with type I diabetes mellitus. J Immunol 142: 3826-3832, 1989
166. Dotta F, Ziegler R, O'Neill J, Nayak RC, Eisenbarth GS and Appel M: Islet autoimmunity: Identification and initial characterization of a metabolically regulable pancreatic ganglioside. Diabetologia 32: 483A, 1989
167. Palmer J, Asplin CM, Clemons P, Lyon K, Tatpati O, Raghu P and Paquette TL: Insulin antibodies in insulin-dependent diabetics before insulin treatment. Science 222: 1337-1339, 1983
168. Wilkin T, Hoskins P, Armitage M, Rodier M, Casey C, Djaz J, Pyke DL and Leslie RDG: Value of insulin autoantibodies as serum markers for insulin-dependent diabetes mellitus. Lancet 2: 480-482, 1985
169. Serreze DV, Leiter E, Kuff EL, Jardieu P and Ishizaka K: Molecular mimicry between insulin and retroviral antigen. Development of cross reactive autoantibodies in sera of NOD and C57BL/KsJ db/db mice. Diabetes 37: 351-358, 1988
170. Zhang ZJ, Davidson L, Eisenbarth GS and Weiner HL: Suppression of diabetes in nonobese diabetic mice by oral administration of porcine insulin. Proc Natl Acad Sci USA 88: 10252-10256, 1991
171. Baekkeskov S, Nielsen JH, Marner B, Bilde T, Ludvigsson J and Lernmark A: Autoantibodies in newly diagnosed diabetic children immunoprecipitate human pancreatic islet cell proteins. Nature 298: 167-169, 1982
172. Baekkeskov S, Landin M, Kristensen JK, Srikanta S, Bruining GJ, Mandrup-Poulsen T, de Beaufort C, Soeldner JS, Eisenbarth GS, 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
173. Baekkeskov S, Aanstoot HJ, Christgau S, Reetz A, Solimena M, Cascalho M, 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 347: 151-156, 1990
174. Christie MR, Vohra G, Champagne P, Daneman D and Delovitch TL: Distinct antibody specificities to a 64 kD islet cell antigen in type 1 diabetes as revealed by trypsin treatment. J Exp Med 172: 789-794, 1990
175. Lu-J, Li-Q, Xie-H, Chen ZJ, Borovitskaya AE, Maclaren NK and Notkins AL: Identification of a second transmembrane protein tyrosin phosphatase, IA-2 beta, as an autoantigen in insulin-dependent diabetes mellitus: precursor of the 37 kD trypatric fragment. Proc Natl Acad Sci USA 93: 2307-2311, 1996
176. Kaufman D, Clare-Salzler M, Tian J, Forsthuber T, Ting G, Robinson P, Atkinson MA, Sercarz EE, Tobin AJ and Lehmann PV: Spontaneous loss of T-cell tolerance to glutamic acid decarboxylase in murine insulin-dependent diabetes. Nature 366: 69-72, 1993
177. Tisch R, Yang X, Singer S, Liblau R, Fugger L and McDevitt H: Immune response to glutamic acid decarboxylase correlates with insulitis in non-obese diabetic mice. Nature 366:

72-75, 1993

178. Ko IY, Ihm SH and Yoon JW: 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
179. Ihm SH, Lee KU and Yoon JW: Studies on autoimmunity for the initiation of beta cell destruction VII. Evidence for antigenic changes on the beta cells leading to the autoimmune destruction of beta cells in BB rats. Diabetes 40: 269-274, 1991
180. Castaño L, Russo E, Zhou L, Lipes MA and Eisenbarth GS: Identification and clonig of a granule autoantigen (carboxypeptidase H) associated with type I diabetes. J Clin Endocrinol Metab 73: 1197-1201, 1991
181. Martin JM, Trink B, Daneman D, Dosch HM and Robinson BH: Milk proteins in the etiology of insulin-dependent diabetes mellitus (IDDM). Ann Med 23: 447-452, 1991
182. Beppu H, Winter WE, Atkinson MA, Maclaren NK, Fujita K and Takahashi H: Bovine albumin antibodies in NOD mice. Diabetes Res 6: 67-69, 1987
183. Glerum M, Robinson BH and Martin JM: Could bovine serum albumin be the initiating antigen ultimately responsible for the development of insulin dependent diabetes mellitus? Diabetes Res 10: 103-107, 1989
184. Johnson JH, Crider BP, McCorkle K, Alford MA and Unger RH: Inhibition of glucose transport into rat islets by immunoglobulins from patients with new-onset insulin- dependent diabetes mellitus. New Engl J Med 322: 653-659, 1990
185. Bersani G, Zanco P, Padovan D and Betterle C: Lymphocyte subpopulations in insulin-dependednt diabetics with and without serum islet-cell autoantibodies. Diabetologia 20: 47-50, 1981
186. Casali P, Nakamura M, Ginsberg-Fellner F and Notkins AL: Frequency of B cells committed to the production of antibodies to insulin in newly diagnosed patients with insulin-dependent diabetes mellitus and generation of high affinity human monoclonal IgG to insulin. J Immunol 144: 3741-3747, 1990
187. Horita M, Suzuki H, Onodera T, Ginsberg-Fellner F, Fauci AS and Notkins AL: Abnormalities of immunoregulatory T cell subsets in patients with insulin-dependent diabetes mellitus. J Immunol 129: 1426-1429, 1982
188. Becker H, Weber C, Storch S and Federlin K: Relationship between CD5+ B lymphocytes and the activity of systemic autoimmunity. Clin Immunol Immunopathol 56: 219-225, 1990
189. Casali P and Notkins AL: CD5+ B lymphocytes, polyreactive antibodies and the human B cell repertoire. Immunol Today 10: 364-368, 1989
190. Muñoz A, Gallart T, Viñas O and Gomis R: Increased CD5+ positive B lymphocytes in type I diabetes. Clin Exp Immunol 83: 304-308, 1991
191. Schatz DA, Lang F, Cantor AB, Riley WJ, Maclaren NK, Sleasman JV and Barrett DJ: CD5+ B lymphocytes in high-risk islet cell antibody-positive and newly diagnosed IDDM subjects. Diabetes 40: 1314-1318, 1991
192. Faustman D, Schoenfield D and Ziegler R: T-lymphocyte changes linked to autoantibodies: association of insulin autoantibodies with CD4+ CD45R+ lymphocyte subpopulation in prediabetic subjects. Diabetes 40: 590-597, 1991
193. Al-Kassab AS and Raziuddin S: Immune activation and T cell subset abnormalities in circulation of patients recently diagonosed type I diabetes mellitus. Clin Exp Immunol 81: 267-271, 1990
194. Alviggi L, Hopkins P, Pyke D, Johnston C, Tee D, Leslie R and Vergani D: Pathogenesis of insulin-dependent diabetes: a role for activated T-lymphocytes. Lancet 1: 4-6, 1984
195. Kontiainen S, Toomath R, Lowder J and Feldmann M: Selective activation of T cells in newly diagnosed insulin-dependent diabetic patients: evidence for heterogeneity of T cell receptor sage. Clin Exp Immunol 83: 347-351, 1991
196. DeBerardinis P, Londei M, Kahan M, Balsano F, Kontiainen S, Gale EAM, Bottazzo GF and Feldmann M: The majority of the activated T cells in the blood of insulin-dependent diabetes mellitus (IDDM) patients are CD4+. Clin Exp Immunol 73: 255-259, 1988
197. Hayward AR and Herberger M: Culture and phenotype of activated T-cells from patients with 1 diabetes mellitus. Diabetes 33: 319-323, 1984
198. Jackson RA, Morris MA, Haynes BF and Eisenbarth GS: Increased circulating Ia antigen-bearing T cells in type I diabetes mellitus. New Engl J Med 306: 785-788, 1982
199. MacCuish AC, Jordan J, Campbell CJ, Duncan LJ and Irvine WJ: Cell-mediated immunity to human pancreas in diabetes mellitus. Diabetes 23: 693-697, 1974
200. MacCuish AC, Jordan J, Campbell CJ, Duncan LJ and Irvine WJ: Cell-mediated immunity in diabetes mellitus: lymphocyte transformation by insulin and insulin fragments in insulin-teated and newly diagnosed diabetes. Diabetes 24: 36-43, 1975
201. Nerup J, Anderson OO, Bendixen G, Egeberg J and Poulsen JE: Anti-pancreatic cellular hypersensitivity in diabetes mellitus. Diabetes 20: 424-427, 1971
202. Huang SW and Maclaren NK: Insulin-dependent diabetes: a disease of autoaggression. Science 192: 64-66, 1976
203. Hänninen A, Jalkanen S, Salmi M, Toikkanen S, Nikolakaros G and Simell O: Macrophages, T cell receptor usage and endothelial cell activation in the pancreas at the onset of insulin-dependent diabetes mellitus. J Clin Invest 90: 1901-1910, 1992
204. Itoh N, Hanafusa T, Miyazaki A, Miyagawa JI, Yamagata K, Yamamoto K, Waguri M, Imagawa A, Tamura S, Inada M,

Kawata S, Tarui S, Kono N and Matsuzawa Y: Mononuclear cell infiltration and its relation to the expression of major histocompatibility complex antigens and adhesion molecules in pancreas biopsy specimens from newly diagnosed insulin-dependent diabetes mellitus patients. J Clin Invest 92: 2313-2322, 1993

205. Yoon JW, Ihm SH, Lee KU, Amano K and Pak CY: The initial step in the development of organ specific disease in BB rats. Diabetologia 31: 779-780, 1988
206. Kolb H, Kantwerk G, Treichel U, Kurner T, Kiesel U, Hoppe T and Kolb-Bachofen V: Prospective analysis of islet lesions in BB rats. Diabetologia 29(suppl 1): A559, 1986
207. Lee K, Kim M, Amano K, Pak C, Jarworski M, Mehta J and Yoon JW: Preferential infiltration of macrophages during early stages of insulitis in diabetes-prone BB rats. Diabetes 37: 1053-1058, 1988
208. Voorbij H, Jeucken P, Kabel P, DeHaan M and Drexhage H: Dendritic cells and scavenger macrophages in pancreatic islets of prediabetic BB rats. Diabetes 38: 1623-1629, 1989
209. Walker R, Bone A, Cooke A and Baird J: Distinct macrophage subpopulations in pancreas of prediabetic BB/E rats: possible role for macrophages in pathogenesis of IDDM. Diabetes 37: 1301-1304, 1988
210. Lee K, Pak C, Amano K, and Yoon J: Prevention of lymphocytic thyroiditis and insulitis in diabetes-prone BB rats by the depletion of macrophages. Diabetologia 31: 400-402, 1988
211. Oshilewski U, Kiesel U and Kolb H: Administration of silica prevents diabetes in BB rats. Diabetes 34: 197-199, 1985
212. Ihm SH and Yoon JW: Studies on autoimmunity for the initiation of beta cell destruction VI. Macrophages are essential for the development of beta cell specific cytotoxic effectors and insulitis in nonobese diabetic (NOD) mice. Diabetes 39: 1273-1278, 1990
213. Amano K and Yoon JW: Studies on autoimmunity for the initiation of beta cell destruction V. Decrease of macrophage-dependent T-lymphocytes and NK cytotoxicity in silica-treated DP-BB rats. Daibetes 39: 590-596, 1990
214. Unuaue E: Antigen-presenting function of the macrophage. Annu Rev Immunol 2: 395-428, 1984
215. Unuaue E and Allen P: The basis for the immunoregulatory role of macrophages and other accessory cells. Science 236: 551-557, 1987
216. Nagata M and Yoon JW: Studies on autoimmunity for initiation of beta cell destructuion 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
217. Mally MI, Cirulli V, Otonkoski T, Soto G and Hayek A: Ontogeny and tissue distribution of human GAD expression. Diabetes 45: 496-501, 1996
218. Tillakarantne N, Erlander M, Collard M, Grief KF and Tobin A: Glutamate decarboxylase in nonneural cells of rat testis and oviduct: differential expression of GAD65 and GAD67. J Neurochem 58: 618-627, 1992
219. Petersen JS, Russel S, Marshall MO, Kofod H, Buschard K, Cambon N, Karlsen AE, Boel E, Hagopian WA, Heijnaes KR, Moody A, Dyrberg T, Lernmark A, Madsen OD, and Michelsen BK: Differential expression of glutamic acid decarboxylase in rat and human islets. Diabetes 42: 484-495, 1993
220. Smith D, Kassam T, Singh B and Elliott J: Escherichia coli has two homologous glutamate decarboxylase genes that map to distinct loci. J Bacteriol 174: 5820-5826, 1992
221. Newell K, Ellenhorn J, Bruce D and Bluestone J: In vivo T-cell activation by staphylococcal enterotoxin B prevents outgrowth of a malignant tumor. Proc Natl Acad Sci USA 88: 1074-1078, 1991
222. Taub D, Lin Y and Rogers T: Characterization and genetic restriction of suppressor-effector cells induced by Staphylococcal enterotoxin B. J Immunol 144: 456-462, 1990
223. Ko IY, Jun HS, Kim GS and Yoon JW: Studies on autoimmunity for initiation of beta-cell destruction X. Delayed expression of a membrane-bound islet cell-specific 38 kD autoantigen that precedes insulitis and diabetes in the BB rat. Diabetologia 147: 728-739, 1994
224. Adams TE, Alpert S and Hanahan D: Non-tolerance and autoantibodies to a transgenic self antigen expressed in pancreatic beta cells. Nature 325: 223-228, 1987
225. Buschard K, Jorgensen M, Aaen K, Bock T and Josefsen D: Prevention of diabetes mellitus in BB rats by neonatal stimulation of β cells. Lancet 1: 134-135, 1990
226. Roep B, Arden S, deVries R and Hutton J: T cell clones from a type I diabetes patient respond to insulin secretory granule proteins. Nature 345: 632-634, 1990
227. Foulis A, Liddle C, Farquharson M, Richmond J and Weir R: The histopathology of the pancreas in type 1 (insulin-dependent) diabetes mellitus: a 25-year review of deaths in patients under 20 years of age in the United Kingdom. Diabetologia 29: 267-274, 1986
228. Gepts W: Islet changes in human diabetes. In the islets of Langerhans(eds. S Cooperstein and D Watkins) New York, Academic Press, 1981, pp321-356
229. Nakhleh R, Gruessner R, Swanson P, Tzardis P, Brayman K, Dunn D and Sutherland DE: Pancreas transplant morphology. A morphologic, immunohistochemical and electron microscopic comparison of allogeneic grafts with rejection, syngeneic grafts and chronic pancreatitis. Am J Surg Pathol 15: 246-256, 1991

230. Santamaria P, Nakhleh R, Sutherland D and Barbosa J: Isolation and characterization of T lymphocytes infiltrating a human pancreas allograft affected by isletitis and recurrent diabetes. Diabetes 41: 53-61, 1992
231. Sutherland D, Xu X, Micheal A, Srikanta A and Troub F: Twin-twin pancreas transplantation: reversal and reenactment of the pathogenesis of type 1 diabetes. Trans Assoc Am Physicians 97: 80-87, 1984
232. Sutherland DE, Goetz FC and Sibley RK: Recurrence of disease in pancreas transplants. Diabetes 38(suppl 1): 85-87, 1989
233. Laupacis A, Stiller CR, Cardell C, Keown P, Dupre J, Wallace AC and Thibert P: Cyclosporin prevents diabetes in BB Wistar rats. Lancet 1: 10-12, 1983
234. Like AA, Kislauskis E, Williams RM and Rossini AA: Neonatal thymectomy prevents spontaneous diabetes mellitus in the BB/W rat. Science 216: 644-646, 1982
235. Like AA, Anthony M, Cuberski DL and Rossini AA: Spontaneous diabetes in the BB/W rat: effects of glucocorticoids, cyclosporin-A and antiserum to rat lymphocytes. Diabetes 32: 326-330, 1983
236. Rossini AA, Slavin S, Woda BA, Geisberg M, Like AA and Mordes JP: Total lymphoid irradiation prevents diabetes mellitus in the Bio-Breeding/Worcester (BB/W) rat. Diabetes 33: 543-547, 1984
237. Rossini AA, Faustman D, Woda BA, Like AA, Szymanski I and Mordes JP: Lymphocyte transfusion prevent diabetes in the Bio-Breeding/Worcester rat. J Clin Invest 74: 39-46, 1984
238. Koevary s, Rossini AA, Stroller W and Chick W: Passive transfer of diabetes in the BB/W rat. Science 220: 727-728, 1983
239. Haskins K, Portas M, Bergman B, Lafferty K and Bradley B: Pancreatic islet-specific T cell clones from nonobese diabetic mice. Proc Natl Acad Sci USA 86: 8000-8004, 1989
240. Haskins K and McDuffie M: Acceleration of diabetes in young NOD mice with a CD4+ islet-specific T cell clone. Science 249: 1433-1436, 1990
241. Hayakawa M, Yokono K, Nagata M, Hatamori N, Ogawa W, Miki A, Mizoguti H and Beba S: Morphological analysis of selective destruction of pancreatic beta cells by cytocoxic T lymphocytes in NOD mice. Diabetes 40: 1210-1217, 1991
242. Nakano N, Kikutani H, Nishimoto H and Kishimoto T: T cell receptor V gene usage of islet β cell-reactive T cells is not restricted in non-obese diabetic mice. J Exp Med 173: 1091-1097, 1991
243. Pankewycz O, Strom T and Rubin-Kelley V: Islet-infiltrating T cell clones from non-obese diabetic mice that promote or prevent accelerated onset diabetes. Eur J Immunol 21: 873-879, 1991
244. Reich E, Scaringe D, Yagi J, Sherwin R and Janeway Jr C: Prevention of diabetes in NOD mice by injection of autoreactive T-lymphocytes. Diabetes 38: 1647-1651, 1989
245. Taki T, Nagata M, Ogawa W, Hatamori N, Hayakawa M, Hari J, Shii K, Baba S and Yokono K: Prevention of cyclophosphamide-induced and spontaneous diabetes in NON/Shi/Kbe mice by anti-class I K^d monoclonal antibody. Diabetes 40: 1203-1209, 1991
246. Bendelac A, Carnaud C, Boitard C, Bach J and Carnaud C: Syngeneic transfer of autoimmune diabetes from diabetic NOD mice to healthy neonate: requirement for both $L3T4^+$ and $Lyt2^+$ T cells. J Exp Med 166: 823-832, 1987
247. Bendelac A, Boitard C, Bedossa P, Barzin H and Bach J: Adoptive T cell transfer of autoimmune nonobese diabetic mouse diabetes does not require recruitment of host B lymphocytes. J Immunol 141: 2625-2628, 1988
248. Miller B, Appel M, O'Neil J and Wicker L: Both the Lyt-2+ and L3T4+ T cell subsets are required for the transfer of diabetes in non-obese diabetic mice. J Immunol 140: 52-58, 1988
249. Rabinovitch A: Immunoregulatory and cytokone Imbalances in the pathogenetis of IDDM. Diabetes 43: 613-621, 1994
250. Yagi H, Matsumono 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
251. Christianson S, Shultz L and Leither E: Adoptive transfer of diabetes into immunodeficient NOD-scid/scid mice. Relative contributions of CD4+ and CD8+ T-cells from diabetic versus prediabetic NOD, NOD-thy-la donors. Diabetes 42: 44-55.
252. Nagata M, Santamaria P, Kawamura T, Utsugi T and Yoon J: 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
253. Stuhler G and Walden P: Collaboration of helper and cytotoxic T lymphocytes. Eur J Immunol 23: 2279-2286, 1993
254. Lo D, Reilly C, Scott B, Liblau R, McDevitt H and Burkly L: Antigen-presenting cells in adoptively transferred and spontaneous autoimmune diabetes. Eur J Immunol 23: 1693-1698, 1993
255. Fisher AG, Goff LK, Lightstone L, Marvel J, Mitchison NA, Poirier G, Stauss H and Zamoyska R: Problems in the physiology of class I and class II MHC molecules and of CD45. Cold Spring Harbor Symp Quant Biol 54: 667-674, 1989
256. 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: 3803-3808. 1989

257. Prowse S, Bellgrau D and Lafferty K: Islet allografts are destroyed by disease occurrence in spontaneously diabetic NOD mice. Diabetes 35: 110-114, 1986
258. Peterson JD and Haskins K: Transfer of diabetes in the NOD-scid mouse by CD4 T-cell clones. Diabetes 45: 328-336, 1996
259. Mandrup-Poulsen T, Bendtzen K, Dinarello CA and Nerup J: Human tumor-necrosis factor potentiates human interleukin 1-mediated rat pancreatic beta cell-cytotoxicity. J Immunol 139: 4077-4082, 1987
260. Santamaria P, Gehrz R, Bryan M and Barbosa J: Involvement of class II MHC molecules in the LPS-induction of IL-1/TNF secretions by human monocytes. Quantitative differences at the polymorphic level. J Immunol 143: 913-922, 1989
261. Kimura N, Toyonaga B, Yoshikai Y, Du R and Mak T: Sequences and repertoire of the human T cell receptor alpha and beta chain variable region genes in thymocytes. Eur J Immunol 17: 375-383, 1987
262. Raulet D: The structure, function and molecular genetics of the γ/δ T cell receptor. Annu Rev Immunol 7: 175-207, 1989
263. Takihara Y, Reimann J, Michalopoulos E, Ciccone E, Moretta L and Mak T: Diversity and structure of human T cell receptor δ chain gene in peripheral blood γ/δ-bearing T lymphocytes. J Exp Med 169: 393-405, 1989
264. Wilson R. Lai E, Concannon P, Barth B and Hood L: Structure, organization and polymorphism of murine and human T-cell receptor alpha and beta chain gene families. Immunol Rev 101: 149-172, 1988
265. Matis L: The molecular basis of T-cell specificity. Annu Rev Immunol 8: 65-82, 1990
266. Esch T, Clark L, Zhang XM, Goldman S and Heber-Katz, E: Observations, legends and conjectures concerning restricted T-cell receptor usage and autoimmune disease. Crit Rev Immunol 11: 249-264, 1992
267. Maeda T, Sumida T, Kurasawa K, Tomioka H, Itoh I, Yoshida S and Koike T: T lymphocyte receptor repertoire of infiltrating T lymphocytes into NOD mouse pancreas. Diabetes 40: 1580-1585, 1991
268. Waters S, O'Neil J, Melican D and Appel M: Multiple TcR Vβ usage by infiltrates of young NOD mouse islets of Langerhans. Diabetes 41: 308-312, 1992
269. Drexler K, Burtles S and Hurtenbach U: Limited heterogeneity of T-cell receptor Vβ gene expression in the early stage of insulitis in NOD mice. Immunol Lett 37: 187-196, 1993
270. Toyoda H, Redford A, Magalong D, Chan E, Hosszufalusi N, Formby B, Teruya M and Charles NA: In situ islet T-cell receptor variable region gene usage in the nonobese diabetic mouse. Immunol Lett 32: 241-245, 1992
271. Santamaria P, Lewis C, Jesserun J, Sutherland D and Barbosa J: Skewed T cell receptor usage and junctional heterogeneity among isletitis $\alpha\beta$ and $\alpha\delta$ T cells in human type I diabetes. Diabetes 43: 599-607, 1994
272. Mandrup-Poulsen T, Bendtzen K, Nielsen JH, Bendixen G and Nerup J: Cytokines cause functional and structural damage to isolated islet of Langerhans. Allergy 40: 424-429, 1985
273. Bendtzen K, Mandrup-Poulsen T, Nerup J, Nielsen JH, Dinarello CA and Svenson M: Cytotoxicity of human p17 interleukin-1 for pancreatic islets of Langerhans. Science 232: 1545-1547, 1986
274. Palmer JP, Helqvist S, Spinas GA, Molvig J, Mandrup-Poulsen T, Andersen HU and Nerup J: Interaction of β-cell activity and IL-1 concentration and exposure time in isolated islets of Langerhans. Diabetes 38: 1211-1216, 1989
275. Sandler S, Eizirik DL, Svensson C, Strandell E, Welsh M and Welsh N: Biochemical and molecular actions of interleukin-1 on pancreatic β-cells. Autoimmunity 10: 241-253, 1991
276. Wogensen LD, Reimers J, Mandrup-Poulsen T and Nerup J: Recombinant human interleukin-1β (JL-1) induces a transient diabetic state in normal rats. Diabetes 40(suppl 1): 117A, 1991
277. Eizirik DL, Tracey DE, Bendtzen K and Sandler S: An interleukin-1 receptor antagonist protein pretects insulin-producing beta cells against suppressive effects of interleukin-1 beta. Diabetologia 34: 445-448, 1991
278. Mandrup-Poulsen T: On the pathogenesis of insulin-dependent diabetes mellitus. Thesis Dan Med Bull 35: 438-460, 1988
279. Helqvist S, Zumsteg UW, Spinas GA, Palmer JP, Mandrup-Poulsen T, Egeberg J and Nerup J: Repetitive exposure of pancreatic islets to interleukin-1β. An in vitro model of pre-diabetes? Autoimmunity 10: 311-318, 1991
280. Mandrup-Poulsen T, Egeberg J, Nerup J, Bendtzen K, Nielsen JH and Dinarello CA: Ultrastuctural studies of time course and cellular specificity of interleukin-1 mediated islet cytotoxicity. Acta Pathol Microbiol Immunol Scand C 95: 55-63, 1987
281. Sandler S, Bendzten K, Borg LAH, Eizirik DL, Strandell E and Welsh N: Studies on the mechanisms causing inhibition of insulin secretion in rat pancreatic islets exposed to human interleukin-1β indicate a perturbation in the mitochondrial function. Endocrinology 124: 1492-1501, 1989
282. Rabinovitch A, Pukel C, Baquerizo H and MacKay P: Immunological mechanisms of islet B cell destruction; cytotoxic cells and cytokines. In Frontiers in Diabetes Research: Lessons from Animal Diabetes II(eds. E Shafrir and AE Reynold) London, Libbey, 1988, pp52-57
283. Badenhoop K, Schwarz G, Trowsdale J, Lewis V, Usadel KH, Gale EAM and Bottazzo GF: TNF-α gene polymorphisms

in type 1 (insulin-dependent) diabetes mellitus. Diabetologia 32: 445-448, 1989

284. Nakamura N, Woda BA, Tafuri A, Greiner DL, Reynolds CW, Ortaldo J, Chick W, Handler ES, Mordes JP and Rossini AA: Intrinsic cytotoxicity of natural killer cells to pancreatic islets in vitro. Diabetes 39: 836-843, 1990
285. 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
286. Sandler S, Bendtzen K, Eizirik DL and Welsh M: Interleukin-6 affects insulin secretion and glucose metabolism of rat pancreatic islets in vitro. Endocrinology 126: 1288- 1294, 1990
287. Satoh J, Seino H, Abo T, Tanaka S, Shintani S, Ohata S, Tamura K, Sawai T, Nobunaga T, Oteki T, Kumagai K and Toyota T: Recombinant human tumor necrosis factor α suppresses autoimmune diabetes in nonobese diabetic mice. J Clin Invest 84: 1345-1348, 1989
288. Kolb H, Zielasek J, Treichel U, Freytag G, Wrann M and Kiesel U: Recombinant interleukin-2 enhances spontaneous insulin-dependent diabetes in BB rats. Eur J Immmunol 16: 209-212, 1986
289. Burstein D, Handler ES, Schindler J, Seals J, Mordes JP and Rossini AA: Effect of interleukin-2 on diabetes in the BB/Wor rats. Diabetes Res 5: 163-167, 1987
290. 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, 1988
291. Stewart TA, Hultgren B, Huang X, Pitts-Meek S, Hully J and Macachlan NJ: Induction of type I diabetes by interferon-α in transgenic mice. Science 260: 1942- 1946, 1993
292. Johnston Jr RB: Current concepts: immunology. Monocytes and macrophages. New Engl J Med 318: 747-752, 1988
293. Nathan CF: Secretory products of macrophages. J Clin Invest 79: 319-326, 1987
294. Ward PA and Mulligan MS: Molecular mechanisms in acute lung injury. Adv Pharmacol 24: 275-292, 1993
295. Fantone JL and Ward PA: Role of oxygen-derived free radicals and metabolites in leukocyte-dependent inflammatory reactions. Am J Pathol 107: 397-418, 1982
296. Ruddle NH: Tumor necrosis factor and related cytotoxins. Immunol Today 8: 129-130, 1987
297. Asayama K, Kooy NW and Burr IM: 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
298. Malaisse WJ, Malaisse-Lagae F, Sener A and Pipeleers DG: Determinants of the selective toxicity of alloxan to the pancreatic β cell. Proc Natl Acad Sci USA 79: 927-930, 1982
299. Abe R, Vacchio M, Fox B and Hodes R: Preferential expression of the T-cell receptor V beta 3 gene by Mls-c reactive T cells. Nature 335: 827-830, 1988
300. Berg L, Pullen A, Fazekas de St Groth B, Matis D, Benoist C and Davis M: Antigen/MHC-specific T cells are preferentially exported from the thymus in the presence of their MHC ligand. Cell 58: 1035-1046, 1989
301. Carlow D, Teh S and Teh H: Altered thymocyte development resulting from expressing a deleting ligand on selecting thymic epithelium. J Immunol 148: 2988-2995
302. Kappler J, Roehm N and Marrack P: T cell tolerance by clonal elimination in the thymus. Cell 49: 273-280, 1987
303. Karpus W and Swanborg R: Protection against experimental autoimmune encephalomyelitis requires both CD4+ T suppressor cells and myelin basic protein-primed B cells. J Neuroimmunol 33: 173-177, 1991
304. Kisielow P, Bluthmann H, Staerz U, Steinmetz M and von Boehmer H: Tolerance in T cell receptor transgenic mice involves deletion of nonmature CD4+ 8+ thymocytes. Nature 333: 742-746, 1988
305. MacDonald H, Schneider R, Lees R, Howe R, Acha-Orbea H, Festenstein H, Zinkernagel RM and Hengartner H: T-cell receptor Vβ use predicts reactivity and tolerance to MIsa-encoded antigens. Nature 332: 40-45, 1988
306. Pircher H, Burki K, Lang R, Hengartner H and Zinkernagel R: Tolerance induction in double specific T cell receptor transgenic mice varies with antigen. Nature 342: 559-561, 1989
307. Pullen A, Marrack P and Kappler J: The T cell receptor is heavily influenced by tolerance to polymorphic self-antigens. Nature 335: 796-801, 1988
308. Sha W, Nelson C, Newberry R, Kranz D, Russell J and Loh D: Positive and negative selection of an antigen receptor on T cells in transgenic mice. Nature 336: 73-76, 1988
309. Speiser D, Pircher H, Ohashi P, Kyburz D, Hengartner H and Zinkernagel R: Clonal deletion induced by either radioresistant thymic host cells or lymphohemapoietic donor cells at different stages of class 1-restricted T cell ontogeny. J Exp Med 175: 1277-1283, 1992
310. Blackman M, Gerhard-Burgert H, Woodland D, Palmer E, Kappler J and Marrack P: A role for clonal inactivation in T cell tolerance to M1s-1a. Nature 345: 540-542, 1990
311. Ramsdell F, Lantz T and Fowlkes B: A nondeletional mechanism of thymic self-tolerance. Science 246: 1038-1041, 1989
312. Roberts J, Sharrow S and Singer A: Clonal deletion and clonal anergy in the thymus induced by different cellular

elements with different radiation sensitivities. J Exp Med 171: 935-940, 1990

313. Speiser D, Stubi U and Zinkernagel R: Extrathymic positive selection of α/β T cell precursors in nude mice. Nature 355: 170-172, 1992
314. Mueller D, Jenkins M and Schwartz R: Clonal expansion versus functional clonal inactivation: a co-stimulatory signalling pathway determines the outcome of T cell antigen receptor occupancy. Annu Rev Immunol 7: 448-480, 1989
315. Qin S, Cobbold S, Benjamin R and Waldmann H: Induction of classical transplantation tolerance in the adult. J Exp Med 169: 779-794, 1989
316. Rammensee H, Kroschewski R and Frangoulis B: Clonal anergy induced in mature Vb6+ T lymphocytes on immunizing Mls-1β mice with M1s-1α-expressing cells. Nature 339: 541-544, 1989
317. Schwartz R: A cell culture model for T lymphocyte clonal anergy. Science 248: 1349-1356, 1990
318. Jones L, Chin L, Longo D and Kruisbeek A: Peripheral clonal elimination of functional T cells. Science 250: 1726-1729, 1990
319. Kawabe Y and Ochi A: Programmed cell death and extrathymic reduction of Vβ8+ CD4+ T cells in mice tolerant to *Staphylococcus aureus* enterotoxin B. Nature 349: 245-248, 1991
320. Rocha B and von Boehmer H: Peripheral selection of the T cell repertoire. Science 251: 1225-1228, 1991
321. Auphan N, Schonrich G, Malissen M, Barad M, Hammerling G, Arnold B, Malissen B and Schmitt-Verhulst AM: Influence of antigen-density on degree of clonal deletion in T cell receptor transgenic mice. Int Immunol 4: 541-547, 1992
322. Schonrich G, Kalinke U, Momburg F, Malissen M, Schmitt-Verhulst A, Malissen B, Hammerling GJ and Arnold B: Down-regulation of T cell receptors on self-reactive T cells as a novel mechanism for extrathymic tolerance induction. Cell 65: 293-304,1991
323. Allison J, Malcolm L, Chosich N and Miller J: Inflammation but not autoimmunity occurs in transgenic mice expressing constitutive levels of imterleukin-2 in islet beta cells. Eur J Immunol 22: 1115-1121, 1992
324. Burkly B, Lo D, Kanagawa O, Brinster R and Flavell R: T cell tolerance by clonal anergy in transgenic mice with nonlymphoid expression of MHC class II- I E. Nature 342: 564-566, 1989
325. Lo D, Burkly L, Widera G, Cowing C, Flavell R, Palmiter R and Brinster RL: Diabetes and tolerance in transgenic mice expressing class II MHC molecule in pancreatic beta cells. Cell 53: 159-168, 1988
326. Morahan G, Allison J and Miller J: Tolerance of class I histocompatibility antigens expressed extrathymically. Nature 339: 622-624, 1989
327. Roman L, Simons L, Hammer R, Sambrook J and Gething M: The expression of influenza virus haemagglutonin in the pancreatic beta cells of transgenic mice results in autoimmune diabetes. Cell 61: 383-396, 1990
328. Sarvetnick N, Shizuru J, Liggitt D, Martin L, McIntyre B, Gregory A, Parslow T and Stewart T: Loss of pancreatic islet tolerance induced by beta cell-expression of interferon-famma. Nature 346: 844-847, 1990
329. Allison J, Campbell I, Morahan G, Mandel T, Harrison L and Miller J: Diabetes in transgenic mice resulting from overexpression of class I histocompatibility molecules in pancreatic β-cells. Nature 333: 529-533, 1988
330. Heath W, Allison J, Hoffman M, Schonrich G, Hammerling G, Arnold B and Miller JFAP: Autoimmune diabetes as a consequence of locally produced interleukin-2. Nature 359: 547-549, 1992
331. Bohme J, Haskins K, Stecha P, van Ewijk W, LeMeur M, Gerlinger P, Benoist C and Mathis D: Transgenic mice with I-A on islet cells are normoglycemic but immunologically intolerant. Science 244: 1179-1183, 1989
332. Miller J, Daitch L, Rath S and Selsing E: Tissue-specific expression of allogenic class II MHC molecules induces neither tissue rejection nor clonal inactivation of alloreactive T cells. J Immunol 144: 334-341, 1990
333. Murphy K, Weaver C, Elish M, Allen P and Loh D: Peripheral tolerance to allogeneic class II histocompatibility antigens expressed in transgenic mice: evidence against a clonal-deletion mechanism. Proc Natl Acad Sci USA 86: 10034-10038, 1989
334. Oldstone M, Nerenberg M, Southern P, Price J and Lewicki H: Virus infection triggers insulin-dependent diabetes mellitus in a transgenic model: role of anti-self (virus) immune response. Cell 65: 319-331, 1991
335. Ohashi P, Oehen S, Buerki K, Pircher H, Ohashi C, Odermatt B, Malissen B, Zinkernagel RM and Hengartner H: Ablation of tolerance and induction of diabetes by virus infection in viral antigen transgenic mice. Cell 65: 305-317, 1991