Review

TYPE 1 DIABETES MELLITUS: PATHOGENESIS AND ADVANCES IN THERAPY

R Khardori*, ME Pauza**

INTRODUCTION:

On January 11, 1922 history was created when Leonard Thompson a 14 year old patient became the first recipient of insulin at Toronto General Hospital(1). Until insulin became available, diabetes was considered a terminal condition. Often patients were admitted dehydrated and comatose; death was generally the rule.

Diabetes is not new to mankind. Apt descriptions are found in the ancient literature of China, Egypt and India. Indian physicians classified diabetes depending the phenotype and mode of presentation. In the pre-insulin era, diabetes was classified as:

a) *Diabetes Maigre* (lean)

b) *Diabetes Gras* (big)

In the era of insulin therapy, better definitions emerged based on the stability of blood glucose control, presence or absence of ketosis, and age of onset:

a) Juvenile onset diabetes (brittle/unstable glucose control, ketosis prone, typically childhood onset)

b) Maturity onset diabetes (relatively stable glucose control, ketosis resistant, adult onset)

This classification paved the way for later use of terms such as IDDM (insulin dependent diabetes mellitus) and NIDDM (non-insulin dependent diabetes mellitus). In 1997 the American Diabetes Association simplified the classification to its current form (2). Thus, classification of diabetes is an old tradition that continues to evolve as our understanding improves.

Current Classification:

Type 1 Diabetes (previously called IDDM)

a) 1A (immune mediated)

b) 1B (non-immune mediated)

Type 2 Diabetes (previously called NIDDM)
Gestational Diabetes
Other Type Diabetes

Type 1 Diabetes Mellitus is observed in approximately 5% of patients with diabetes mellitus. Evidence of an autoimmune etiology is found in about 95% of these cases. The remaining 5% lacks defined markers of autoimmunity and therefore are classified as type 1B (3).

The incidence of type 1 diabetes varies regionally and appears to be escalating. In Finland the disease incidence is 40/100,000 per year, but it is much lower in the Zunyi region of China (0.1/100,000). Based on recent trends, the incidence of type 1 diabetes is projected to increase 40% between 1997 and 2010. The EURODIAB collaborative study suggests an annual increase of 3-4% with the highest increase expected in the 0 - 4 year age group (4).

Genetics: Type 1 diabetes is a complex polygenic disorder. It cannot be classified strictly by dominant, recessive, or intermediate inheritance, making identification of disease susceptibility or resistance genes difficult (5, 6). Furthermore it is unclear whether disease is mediated through many genes working independently, coordinated actions of susceptibility genes, or a combination of both.

Despite our current limited knowledge, important insights have been gained through studies using animal models of spontaneous type 1 diabetes such as the *BB rat*, *Tokushima rat* and *nonobese diabetic (NOD) mouse.* In both rodents and humans the most important genetic link is the major histocompatibility complex (MHC) located on chromosome 6p21.3, termed IDDM1 (7). Disease susceptibility is highly associated with inheritance of the human leukocyte antigen (HLA) alleles DR3 and DR4 as well as the associated alleles DQ2 and DQ8. More than 90% of individuals with type 1 diabetes express either DR3DQ2 or DR4DQ8. Heterozygous genotypes(DR3/DR4) are most common in children diagnosed with type 1 diabetes prior to the age of 5 (50%) (8). On the other hand, HLA class II haplotypes such as DR2DQ6 confer dominant protection (9).

**Professor-Director, **Assistant Professor, Division of Endocrinology, Metabolism & Molecular Medicine, Southern Illinois University School of Medicine, Springfield, IL 62794; U.S.A*

HLA molecules function to present peptides to T cells, a critical interaction for generating cellular immune responses. Presentation of self peptides is also essential for thymic selection, which controls the specificity of newly generated T cells that are released into the periphery.

HLA class II molecules that lack aspartic acid at position 57 of the beta chain are often found among individuals with type 1 diabetes. These HLA molecules have unique peptide binding preferences and reduced stability in vitro. Although the precise role of HLA class II molecules in disease susceptible is not fully understood, their structural and functional distinctiveness suggests a role in thymic education leading to positive selection of autoreactive T cells, negative selection of regulatory T cells, or both. In addition, these HLA molecules may exhibit enhanced presentation of beta cell derived autoantigens in the periphery thereby contributing to the increased islet specific cellular immune responses present in type 1 diabetes. Such possible roles for HLA in disease pathogenesis are not necessarily mutually exclusive.

Although MHC alleles contribute significantly to disease susceptibility, IDDM1 alone does not account for all familial disease clustering (10). Approximately 20 non-HLA loci may contribute to susceptibility. Among these, the insulin locus (IDDM2 on chromosome 11p5.5) is the best understood and contributes approximately 10% to the familial aggregation of disease (11). As the only known beta cell specific autoantigen, it is not surprising that once the insulin gene was recognized within IDDM2 an immediate search for coding region polymorphisms was undertaken. When none were found, the focus quickly shifted to surrounding regions. Upstream of the insulin gene a variable number of tandem nucleotide repeats (VNTR) was identified. The number of repeats correlates with thymic insulin expression. Large VNTR (>100, class III) are associated with high mRNA expression and protection, whereas class I VNTR (26-63 repeats) are associated with low thymic expression and susceptibility. As with HLA, peripheral T cell repertoires may be significantly influenced by polymorphisms in the insulin gene affecting thymocyte selection.

More recent evidence from rodent and human studies identifies disease associated polymorphisms within a 6.1 kb region 3' of the T-cell regulatory gene CTLA-4 (IDDM12; chromosome 2q22). In this case, the polymorphisms identified may determine susceptibility for several autoimmune disorders including type 1 diabetes (12). Disease susceptible alleles reduce the efficiency of splicing and production of soluble CTLA-4. On T cells, CTLA-4 recognizes and binds to B7 molecules present on professional antigen presenting cells. Since CTLA-4 functions to limit T cell responses, reduced soluble CTLA-4 is expected to adversely affect cellular immune regulation thereby contributing to autoimmunity.

Understanding the genetics of type 1diabetes is clearly important for predicting disease and may improve the accuracy of diagnosis, prognosis, and treatment. Unfortunately, with the above noted exceptions, the genes responsible for disease susceptibility at each of the 20 non-HLA loci remain unclear.

In some cases, genes may be unique to select families. For example, IDDM 17 was localized to chromosome 10 in a large Bedouin Arab family (13). Disease risk is 40% when HLA DR3 or DR4 are inherited with IDDM17, but the exact polymorphism remains unidentified.

Although HLA loci typically impart the greatest disease risk, diabetes is not always clearly dependent on inheritance of select HLA haplotypes. HLA genes do not appear to contribute to type 1 diabetes associated with autoimmune polyendorcrine syndrome type 1 (APS-1 (14). Rather mutations in the autoimmune regulator gene (AIRE), found on chromosome 21, are associated with disease (15). Slightly less than a fifth of patients with APS-1 develop type 1 diabetes. Once again, thymic development is implicated in pathogenesis since AIRE is primarily expressed in the thymus.

As with APS-1, type 1 diabetes found as part of X-linked autoimmunity-allergic dysregulation syndrome (XLAAD) is HLA independent and equally intriguing. The XLAAD syndrome presents early in life with autoimmunity, allergy and failure to thrive. The disease is usually fatal in first year of life. A single gene defect was first identified in *Scurfy* mice, which share a phenotype similar to humans. The gene encodes a transcription factor variably named JM2, Foxp3, or scurfin. Mutations are similarly found in the orthologous gene in humans with XLAAD and are expected to disrupt the dimerization necessary to maintain normal scurfin function. Although mutant T cells are responsible for the disease phenotype, studies

performed with bone marrow chimeric mice demonstrate that these T cells must be educated in the thymus of a *Scurfy* mouse in order to cause disease. Therefore, a defect within the thymus allows *Scurfy* T cells to escape central tolerance permitting autoimmunity as T cells enter the periphery (16-20).

TYPE 1 DIABETES — AN AUTOIMMUNE DISORDER:

Although the clinical picture of type 1 diabetes was recognized in ancient times, the autoimmune nature of this disorder is a fairly recent discovery. A role for bone marrow derived elements in disease pathogenesis is suggested by the occurrence of diabetes in patients receiving bone marrow transplants from HLA compatible siblings with type 1 diabetes and is consistent with autoimmunity. Furthermore, local inflammation, autoantibody production, specific T cell responses, and clustering with other autoimmune disorders all support an autoimmune pathogenesis.

Insulitis is a significant finding when pancreas tissue from individuals with recently diagnosed diabetes is examined (21, 22)**.** Autoimmunity is further supported by the fact that T cells are present within affected islets of humans with type 1 diabetes and dominate the islet infiltrates seen in rodents with spontaneous disease even before hyperglycemia is evident (23). In addition, increased MHC expression suggests active antigen presentation may be occurring within the islet tissue (22,24). Consistent with these findings, diabetes has also been reported in previously diabetic recipients receiving pancreas transplants from non diabetic twin or HLA identical siblings (25). In one case, T cells were isolated from the transplanted pancreas shortly after disease recurrence (26). Although not definitive, accumulation of T cells and upregulation of MHC near sites of beta cells destruction strongly suggest immune mechanisms are important for diabetes development.

Autoantibodies, called islet cell autoantibodies (ICA), are detected in individuals with type 1 diabetes and allow the clinical course of diabetes to be studied in human subjects. Antibodies directed against the islet cells were first discovered by incubating sera from type 1 diabetic patients with frozen pancreas sections from normal blood group O individuals (27,28). Over the years the specificities of several ICA have been clarified, although some still remain unidentified. Of the known specificities, glutamic acid decarboxylase (GAD65), insulinoma associated antigen-2 (IA-2 or ICA512), and insulin (IAA) are the most common target antigens. There are two isoforms of GAD: GAD65 and GAD67. Glutamic acid decarboxylase enzymatically converts glutamic acid to g aminobutyric acid (GABA) in GABAergic neurons and islet beta cells. While GAD65 is expressed by islet beta cells, in humans GAD67 is not (29). Therefore, antibodies to the GAD65 isoform are used to evaluate human disease (in rodents GAD67 is expressed by islet cells). IA-2 is a protein tyrosine phosphatase-like protein found in both and â cells of the pancreatic islet. Similarly Phogrin or IA-2b is an autoantigen with homology to IA-2 (30,31). Both IA-2 and IA-2b are highly conserved among many species, with homologues in macaques, mice, cows, zebra fish, Drosophila and Caenorhabditis elegans. Both co-localize to islet secretory granules and are phosphorylated when insulin is secreted. They associate with the cytoskeleton to facilitate exocytosis. Insulin autoantibodies are directed to b chain of insulin or proinsulin, the only known beta cell specific autoantigen. Once insulin replacement therapy begins, insulin antibodies may not be a useful marker since some patients develop antibodies to exogenous insulin. Other candidate autoantigens include *carboxypeptidase*, *hSP 60, and Ganglioside* (*GM2-1)*.

Antibodies specific for beta cell antigens are present with increased frequency among individuals recently diagnosed with type 1 diabetes (27,28). Their presence confirms a diagnosis of type 1A diabetes. Measurable autoantibody titers are also used to identify insulin-requiring older patients who are often initially diagnosed with type-2 diabetes. Older patients typically have antibodies against GAD65 (5-10%) or IA-2 (2-4%). These adult patients have a variant form of diabetes known as latent autoimmune diabetes in adult (**LADA**), **type 1.5 diabetes**, or slow progressive insulin dependent diabetes mellitus (**SPIDDM**).

Autoantibody production appears in advance (months to years) of the metabolic changes of type 1 diabetes and can be used to predict disease. Since normal individuals may produce islet specific autoantibodies, the predictive value is enhanced greatly when screening is limited to first degree relatives of a proband: the presence of 2 or more distinct antibody specificities is highly predictive of future type 1 diabetes (five year risk = 28-66%) (32, 33). However when combined with HLA typing, autoantibody screening is also useful for predicting disease in general populations (34, 35).

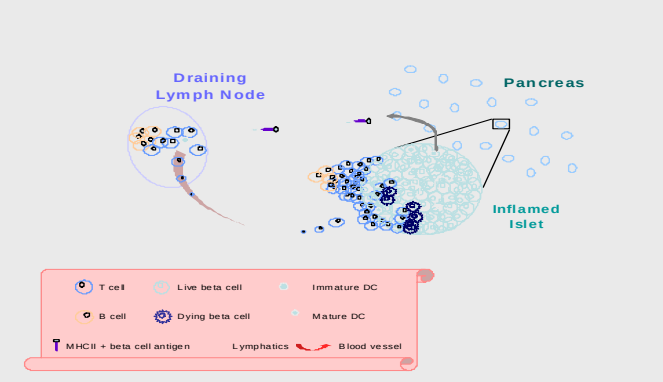
Despite the utility of autoantibodies for diagnostic purposes, their pathogenic contribution is controversial. Two studies using NOD mice deficient in peripheral B cells demonstrate a complete lack of diabetes, suggesting a possible role for autoantibodies in disease (36,37). However, transfer of autoantibodies alone did not reconstitute disease in the B-less NOD mice (36) demonstrating that the contribution of B cells to disease pathogenesis is not limited to production of autoantibodies. B cells also serve as antigen presenting cells with the ability to present a unique set of peptides (38). It is possible that their absence results in significant changes in antigen presentation which secondarily affect T cell activation. This possibility is

further supported by other studies demonstrating that diabetes cannot be transferred using serum from diabetic humans (39), that plasmapheresis provides little therapeutic benefit (40), and that incomplete disease elimination sometimes occurs in B cell deficient NOD mice (41). Although the latter report contradicts the afore mentioned studies using B-less NOD mice, a recent case report indicates that the same is true for human disease; type 1 diabetes was reported in a 14 year old male with X-linked agammaglobulinemia (42). Clearly disease can occur in the absence of B cells and autoantibodies, but do antibodies contribute to disease progression? The ability of antibodies with high affinity for insulin to accelerate diabetes onset (43) in animal disease models suggests that autoantibodies may indeed affect the time course of disease development.

While T cells are present in inflamed islets [Figure 1], the ability to study these cells in humans is limited by accessibility. T cell clones from NOD mice have proven useful in enhancing our understanding of the potential mechanisms responsible for diabetes in humans (44, 45). Since islet specific T cells with strong diabetogenic potential are present in NOD mice, it is likely that similar T cells (specific for insulin/proinsulin, GAD65, IA-2, and/or IA-2b) nfiltrate human islet tissue as well.

Elegant studies using transgenic NOD mice demonstrate that T cells are activated first in lymph nodes that drain the pancreas (46). The permissive architecture of the lymph node supports ready access of antigen on professional antigen presenting cells to circulating naïve T cells [Figure 2]. Once activated, islet specific T cells traffic to the pancreas where they proliferate and accumulate resulting in organ specific inflammation [Figure 3]. Since macrophages and dendritic cells are present in inflamed islet tissue,

Figure 1: The Immunopathology of Type 1 Diabetes. Resident antigen presenting cells phagocytose beta cells, become activated, and migrate to draining lymph nodes where they present antigen to circulating T cells. Upon activation beta cell specific T cells gain access to islet tissue through the vasculature and accumulate in the islet causing insulitis. Additional antigen presentation may occur locally leading to destruction of beta cells with subsequent hyperglycemia.



they likely function as local professional antigen presenting cells capable of presenting antigen in the context of MHC class II molecules and secreting interleukin (IL)-12, which activates antigen specific CD4 T cells and further stimulates interferon gamma secretion (47-49). Interferon gamma is a key cytokine able to inhibit Th2 cytokine production (IL-4, IL-5, IL-10) by other T cells and enhance interleukin (IL)-1β, tumor necrosis factor alpha, and free radical production by macrophages. All are toxic to islet beta cells (50-53), although pre-beta cells appear to be less sensitive to cytokine mediated destruction than mature â cells (54). In addition to direct beta cell damage, interferon gamma promotes CD8 T cell cytotoxicity. CD8 cells may cause beta cell death directly through release of perforin and granzymes or by Fas-mediated apoptosis (55, 56).

Type 1 diabetes is often associated with other well established autoimmune diseases such as chronic thyroiditis, non-destructive Addison's disease, Celiac disease, and Autoimmune Polyendocrinopathy Syndrome. Clustering of type 1 diabetes with other autoimmune diseases suggests possible defects in immune regulation may contribute to development of multiple autoimmune phenotypes.

Figure 2: Autoantigen presentation and lymphocyte activation occur in the lymph nodes draining the pancreas prior to diabetes development.

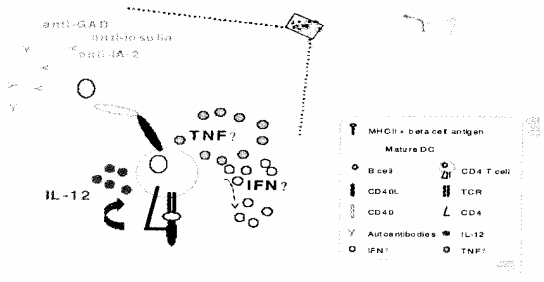
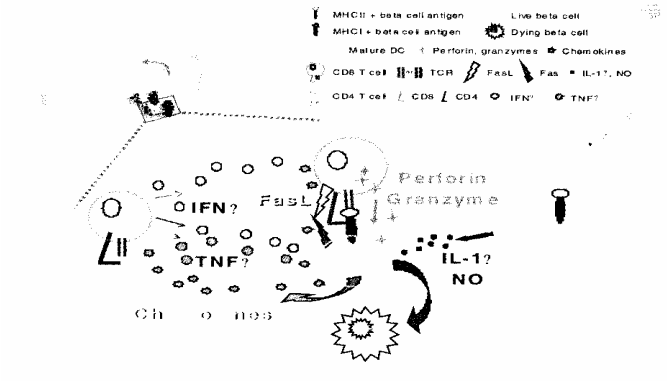


Figure 3: Local chemokine production attracts autoreactive lymphocytes that destroy beta cells. Death can be mediated by various mechanisms including Fas-FasL, perforin/granzymes, reactive oxygen species, and cytokines.



CONTROVERSIES AND QUESTIONS IN THE PATHOGENESIS OF TYPE 1 DIABETES:

Despite delineation of disease pathogenesis in animal models with the inescapable conclusion that there is autoimmune destruction of pancreatic â-cells, much remains to be learned. With improved techniques to obtain pancreatic punch biopsies our understanding of human disease will improve, however several issues remain controversial. It is unclear how disease is initiated and whether (or which) target autoantigens are abnormal. Paradigms outlining the role of T cell subsets in disease development are now being questioned and data concerning the role of Fas in beta cell death are confusing. The mechanisms responsible for leukocyte trafficking into islet tissue and the factors that limit progression from insulitis to frank diabetes are also unclear.

The lack of complete concordance among monozygotic twins (only 20-40%) indicates that both genetic and environmental factors contribute to the pathogenesis of type 1 diabetes.

Environmental factors such as *toxins (*N-nitroso derivatives*)*, *pathogens*, *food constituents*, and *drugs* have been implicated in pathogenesis. Although early Finnish studies supported a role for some of these factors, DAISY (USA) and BABYDIAB (Germany) failed to show any effect of early cow's milk exposure, breast feeding, enteroviral infections, or timing of vaccination on disease occurrence (57-59). Other epidemiological studies link viral infection to the acquisition of type 1 diabetes. Viral involvement is strongly supported in human type 1 diabetes acquired after congenital rubella. Approximately 20% of such children go on to develop type 1 diabetes. Genetics may be important here as well, determining b cell susceptibility to viral infection(s). In this way both genetic and environment factors may simultaneously contribute to disease initiation and susceptibility, but the exact contribution of each remains to be detailed.

As indicated by the various autoantibodies associated with type 1 diabetes, several islet beta cell proteins become antigenic. Is there a common antigen that initiates disease? Using T cell responses to mark early immune activation, the identification of highly diabetogenic CD8 T cell clones specific for insulin implicate insulin as a strong initiating autoantigen candidate. Upon transfer into NOD.scid mice, these clones mediate rapid disease onset (within 5-7 days) independent of CD4 cells (60). It has been argued that CD8 cells mediate early detrimental immune responses since anti-CD8 treatment is only effective at inhibiting disease if administered to NOD mice by 2-5 weeks of age (61). However, evidence of an immune response directed at insulin is not found for all humans with type 1 diabetes. Other studies suggest that GAD may be a key antigen stimulating the early autoimmune response. GAD responsive T cells are found in the periphery of humans with diabetes and those at high risk for disease (62,63). GAD specific T cells are diabetogenic when transferred into NOD.scid mice, and NOD mice immunized with purified GAD65 protein are protected from disease (64-66). As with insulin, immune responses directed at GAD are not always found in diabetic individuals and antibodies directed at insulin (not GAD) are present first among the youngest children diagnosed with diabetes (67,68). However, intriguing studies using GAD anti sense transgenic mice provide indirect evidence that the level of GAD expression in beta cells may contribute to disease susceptibility, suggesting that abnormal levels of GAD expression might be an important factor in some cases of diabetes (69). At present, it remains unclear if a single common autoantigen elicits the initial immune response directed against pancreatic beta cells. To firmly accept or refute this idea, a better understanding of the earliest events in disease as well as those events that mark the transition from insulitis to diabetes may be required.

Adoptive transfer studies using bone marrow or T cells place blame on the immune system rather than the target organ. T cells specific for islet â cells exist even in normal individuals, but tolerance is maintained by immunoregulatory mechanisms. Susceptibility to type 1 diabetes may be greatly enhanced when regulatory mechanisms fail allowing autoreactive T cells to become activated and expand clonally. A cascade of immune and inflammatory processes then culminates in b cell destruction. Many studies suggest that destructive

autoreactive T cells belong to the Th1 subset whereas protective T cells are of the Th2 subset (reviewed in 70-73). Typically, Th1 cells are produced in response to intracellular bacteria or viruses, enhance cellular immune responses, and secrete IL-2, tumor necrosis factor alpha, and/or interferon gamma. Th2 cells are generated in response to parasites and allergens, enhance humoral immune responses, and secrete IL-4, IL-5, and/or IL-10. While characterization of type 1 diabetes as a Th1 mediated disease is supported by numerous reports demonstrating the protective effects of Th2 cells, other studies question this interpretation. In vitro generated Th1 and Th2 cells, as well as Th1 and Th2 clones isolated from diabetic mice, can transfer disease in murine models (74-77). Diabetes is not fully alleviated in genetically mutant NOD mice that are deficient in interferon gamma, IL-4, or IL-10 (78,79). A purely counter regulatory role for T cell subsets would suggest Th1 diseases (such as type 1 diabetes) would not occur simultaneously with Th2 diseases (such as asthma). However, asthma is actually more prevalent in children with type 1 diabetes than non-diabetic children (80). While increased activation of Th1 cells does correlate with disease development, a causal relationship to diabetes development is questionable.

Consistent with a defect in immune regulation several studies propose that abnormalities in NKT cells might contribute to diabetes development. NKT cells are CD4+ or $CD4^{-}CD8^{-}$ (double negative) cells that express a quasi-invariant alpha/beta T cell receptor (Va14-Ja18/Vb8 in mice and Va24-Ja18/Vb11 in humans) restricted to the MHC-like molecule CD1d. The ability to produce high levels of IL-4 may be responsible for their regulatory effects, in part through the ability of IL-4 to drive Th2 cell development. NKT cells appear to be abnormal in NOD mice (81,82). Activation or transfer of additional NKT cells protects NOD mice from disease, and disease is exacerbated in NOD mice lacking CD1d (83-88). In humans, several studies demonstrate reduced NKT cell number and IL-4 secretion potential among diabetic individuals (89-91). However a more recent study using highly specific tetramer staining begs reconsideration of previous studies. Although NKT cell numbers varied between the individuals tested, they remained stable over time for each individual and were not significantly different between diabetic and pre-diabetic populations or among discordant twin pairs (92).

Given the previous large body of research supporting a role for NKT cells in protection from disease and the possibility that tetramer binding studies may exclude some NKT cells from analysis, continued exploration of the relevance of NKT cells in type 1 diabetes is warranted.

Fas mediated apoptosis is considered the ultimate mechanism of â cell death. NOD mice lacking Fas (lpr/lpr) do not develop diabetes and are resistant to disease mediated by adoptive transfer of T cells from diabetic NOD mice suggesting a key role for Fas expression on beta cells (93-95). However, islets from NOD lpr/lpr mice succumb to autoimmune destruction when transferred into diabetic wild type recipients (96-98). Even more surprising, wild type islets are not destroyed when transferred into NOD lpr/lpr recipients (97). These results suggest that Fas deficiency has other affects that may secondarily influence cell survival independent of Fas expression on beta cells directly. Such contradictory results do not necessarily mean that Fas is not involved, rather Fas may indeed play a role in beta cell death under certain circumstances (99,100). These studies highlight the need for multiple experimental approaches when using genetic mutations to study disease pathogenesis since even a single gene defect can unexpectedly affects multiple systems.

Insulitis is a consistent finding prior to destruction of beta cells in both humans and rodents, though insulitis does not always progress to hyperglycemia. This is exemplified in NOD mice, where most female mice transition from insulitis to frank diabetes but most male mice only exhibit insulitis (101). The factors that control (or limit) progression from insulitis to diabetes remain largely unknown. Understanding this transition may provide new opportunities at preventing disease among the growing population of individuals at high risk for developing type 1 diabetes.

Since insulitis is an important precursor of disease, the factors that contribute to recruitment of leukocytes to local islet tissue are of great interest. Although upregulation of vascular endothelial cell adhesion molecules such as selectins, integrins, vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1 have been appreciated for many years these provide relatively non-specific means for leukocyte entry into tissues during inflammation (102). More specific migration results from production of select small molecular weight chemoattractive cytokines known as chemokines [Figure 3]. While numerous chemokines are expressed in the pancreas of mice with insulitis (103), the chemokines responsible for early leukocyte recruitment and their coordinate actions are only just beginning to be elucidated. A recent report demonstrates that two chemokines CXCL9 (Mig) and CXCL10 (IP-10) are critical for recruitment of T cells into islet tissue (104).

Both chemokines bind the same receptor, CXCR3, which is selectively expressed on activated T cells (105). Interestingly, beta cells are a significant source of CXCL10. Our own studies demonstrate that CXCL10 expression is synergistically upregulated in beta cells by combinations of interferon gamma, tumor necrosis factor alpha, and IL-1b (Table I). This data suggests that even low levels of proinflammatory cytokines produced locally in islet tissue may profoundly upregulate chemokine production by beta cells, leading to recruitment of T cells with destructive potential. Although immunohistochemistry results suggest that CXCL9 is not produced by islet beta cells (104), these studies do not address the kinetics of expression and use only a single disease model. Thus, the source of CXCL9 is presently obscure. However, using an inducible model of diabetes Piali and colleagues demonstrate that disease is significantly delayed in mice lacking CXCR3 (104). While the cellular sources and kinetics of chemokine production are not entirely clear, CXCR3 ligands do appear to have a key role in controlling disease progression.

Table I: Proinflammatory Cytokines Synergistically Enhance CXCL10 Expression By Nit-1 Beta Cells in Vitro.*

Culture Conditions	Relative CXCL10 expression*
Media only	-
IL-1β	+/-
TNFα	+
IFNγ	+
IL-1β + TNFα	+
IL-1β + IFNγ	+
TNFα + IFNγ	+++
IL-1β + TNFβ + IFNγ	++++

- ❖ Nit-1 beta cells were cultured for 5 hours in F12K medium supplemented with 10% fetal calf serum and 1000 U/ml IL-1b, 100 U/ml TNFa, and/or 6 U/ml IFNg prior to analysis of CXCL10 expression by RNase protection assays using the mCK5 probe set (PharMingen).

- ❖ Expression was quantitated by densitometry of the phosphoimages (Cyclone phosphoimager and Optiquant software, Perkin-Elmer). All CXCL10 readings were normalized to the L32 housekeeping gene, then compared to results obtained from unstimulated Nit-1 cells.

CLINICAL TRIALS

The advent of insulin therapy completely changed the prognosis for individuals diagnosed with type 1 diabetes, however limitations still remain. Physiologic control of glucose metabolism is difficult to achieve, and therapy intensive enough to prevent disease complications is not possible for all patients.

Fortunately, the increased understanding of disease pathogenesis gained from numerous animal studies has also generated several novel therapeutic possibilities (105,106). These approaches fall into two categories based on the point of intervention: those aimed at preventing disease and others striving to inhibit disease progression.

Prevention of diabetes is relatively easy in animal models and a number of successful approaches are documented (105,106), unfortunately clinical applicability remains a challenge. Currently there is no therapy that effectively prevents diabetes, however a number of clinical trials are ongoing to test potential interventions. In the DPT-1 subjects were randomized to parenteral or oral insulin adminstration. Results from parenteral arm of study were disappointing. Insulin at the dosages used in this study failed to delay or prevent diabetes (107). Results from the group receiving oral insulin are not yet available.

Recent Diabetes Prevention Trials:

a) Diabetes Prevention Trial **(DPT-1)***

b) Finnish Type 1 Diabetes Prevention Program **(DIPP)**

c) European Nicotinamide Diabetes Intervention Trial (**ENDIT)**

d) Australian Intranasal insulin Trial **(INIT)**

e) German (Deutsc) Nicotinamide Diabetes Intervention Study **(DENIS)**

f) Trial to Reduce Diabetes In the Genetically at Risk **(TRIGER)**

Finnish trial involves nasal insulin

TRIGER involves avoidance of cow's milk

- ❖ DPT-1 has been reported as a failure, and is no longer active

In animal models, reversal of disease appears to be more difficult than prevention (106). As anticipated from the autoimmune nature of type 1 diabetes, most studies focused on modulation of immune responses. Subsequently, several immuno-regulatory agents were explored in clinical trials, though none were highly effective.

Agents That Failed To Modulate Diabetes In Clinical Trials:

Linomide
Leflunomide
Lisoflyn
IL-4 selective agonists
Vitamin D analogues
Rapamycin
Cyclooxigenase inhibitors (Indomethacin)
Mycophenolic acid

With new advances in our understanding of disease pathogenesis, additional therapeutic approaches are proposed. For example, reversal of autoimmune Th1 subset development may be accomplished following immunization with â cell autoantigens (such as GAD or insulin, the latter paving the way for the DPT-1), treatment with T cell mitogens, or application of microbial agents (such as mycobacterium avium, bacillus Calmette-Guerin(BCG), or Q fever vaccine (106, 108). A small randomized, double-blind, phase II study suggests that an immunomodulatory peptide from heat shock protein 60 (DiaPep 277) may preserve endogenous insulin production in newly diagnosed (< 6 months) patients with type 1 diabetes, perhaps through a shift from Th1 to Th2 immune responses (109). Another interesting study used complete Freund's adjuvant (CFA) together with islet transplantation or repeated exposure to allogeneic splenocytes to restore endogenous beta cell function in previously diabetic female NOD mice (110). Though the exact mechanisms responsible for correction of autoimmunity are not entirely clear, changes in immune regulation are apparent. More importantly, this study demonstrates that blockade of the disease process may be sufficient to allow recuperation and nearly complete restoration of beta cell function.

Future Prevention Trials Using Novel Approaches:

a) Insulin Peptide B9-23
b) Insulin B chainx
c) Insulin B25 aspart
d) Hsp 60

CURRENT CLINICAL STATUS OF TYPE 1 DIABETES MELLITUS:

With the introduction of insulin in 1922, the outlook for patients changed dramatically. What was a death sentence became a survivable chronic disease (111,112). Diabetic patients were then looking forward to relatively good glucose control and freedom from repeated episodes of ketoacidosis. However early insulin preparations were relatively short acting, forcing patients to take multiple injections of insulin daily. It was not until several years later that long acting versions of insulin were introduced.

At present we have a wide range of insulin preparations that can be used to achieve the desired glucose control. Although animal sources of insulin are still available, synthetic insulin made possible by recombinant DNA technology is also widely used (typically synthetic human insulin or its analogs). Advances in insulin delivery have also been made and include sophisticated devices such as pens and pumps. Implantable pumps have been tried in France and are currently undergoing trials in the United States. As newer insulin preparations and delivery systems were developed, advances in technologies for blood glucose monitoring were also made.

In the last two decades these efforts have resulted in a huge range of glucose monitoring devices that are easy to handle for self monitoring purposes. Physicians can also take advantage of continuous glucose monitoring devices (**CGMS**: Minimed or Glucowatch-Cygnus). This is particularly helpful for managing patients who have brittle disease and experience frequent hypoglycemia or hypoglycemia unawareness.

Another major advance in monitoring glucose control occurred with the ability to measure *glycosylated hemoglobin* (HbA_1c). This allows physicians and patients to determine the durability of glucose control over the previous 2-3 months. This innovation made it possible to study the efficacy of newer insulin regimens and helped determine the impact of stricter glycemic control. We now possess the ability to monitor *fructosamine* for determining glycemic control over a somewhat shorter time period (4-6 weeks).

There have been major improvements in our ability to measure *microalbumin* and *advanced glycation endproducts* (AGE's). This should enable undertaking measures to slow down the progression of complications such as nephropathy and neuropathy.

Much has been learned about the importance of intensive glucose control; the landmark DCCT study revealed beyond any doubt the significance of ensuring near normoglycemia in patients with type 1 diabetes mellitus. With intense glucose control (target HbA_{1c} of 7%), retinopathy risk was reduced by 63%, nephropathy by 54 %, and neuropathy by 60% (113). Strict glycemic control is now the standard of care. With such strict control comes an increased need for patients to understand their disease. This is best

accomplished through diabetes education, including nutrition counseling. All patients must understand the principles of nutrition planning. Educated patients have significantly better glycemic control. This is particularly important since the benefits of strict glycemic control last even beyond the period of most intensive implementation (114).

Pancreas Transplant in Type 1 Diabetes Mellitus:

Pancreas transplant is a widely viable option. It requires a dedicated and experienced team. Long term immunosuppression is required and quite expensive. The first pancreas transplant was performed on December 16, 1966. Since then, approximately 12,000 transplants have been performed worldwide. Most of these (10%) were performed at the University of Minnesota. The three year patient survival is 90%. Pancreas graft survival is 60% and graft rejection about 24% (115). Most pancreas transplants are performed as dual organ transplants with a kidney. The long term improvements have been remarkable.

Islet Cell Transplant in Type 1 Diabetes Mellitus:

There is a resurgent interest in islet transplantation due to recent successes reported by a group in Edmonton, Canada. Although the first clinical attempt occurred more than a century ago in Bristol, U.K. [*B Med J 1894; 2: 1303]*, poor graft survival limited its usefulness. The Edmonton group instituted changes in the immunosuppressive regime, which greatly improved transplant outcome. A steroid free immunosuppression cocktail (sirolimus, tacrolimus, daclizumab and anti-IL-2R antibody) was initiated immediately before transplantation. By June of 2001, fifteen type 1 diabetic patients had undergone islet transplantation. The patients were chosen based on a profile of brittle diabetes or frequent hypoglycemia. As of June 2001, all patients had sustained insulin production. The mean HbA_1c decreased from 8.3% to 5.8 $\pm$ 0.1%. Five patients in this series are back on insulin (requiring half of pre-transplant doses). Over 400 islet cell allografts were performed worldwide within the past decade. Presently, a multicenter study is underway across the United States at 9 major medical centers. Although the simplicity of administration (intra portal injection of islets) makes it attractive, patients require islets from at least two donor pancreas (an average 11,000 islet equivalents per kilogram body weight). The necessary islet cell mass required to achieve normoglycemia in the recipient and difficulties in recovering islet tissue from donor pancreas can be limiting factors.

FUTURE DIRECTIONS IN TYPE 1 DIABETES MELLITUS:

Due to a shortage of available donor pancreas, alternative sources of islets are of keen interest.

Engineered insulinoma cell may become a viable alternative for transplantation (116). Other potential alternative sources of islets are being explored and these include (117,118):

Xenogenic islet cells (humanized pig islet cells)

Expansion and transdifferentiation of pancreatic duct cells

Fetal pancreatic stem cells and b cell precursors

Embryonic stem cells

The natural senescence and apoptosis observed among developed islet tissue may be prevented through the use of telomerase and caspase. It may also be possible to manipulate cultured beta cells using recombinant viral vectors (adeno-associated virus) to make them resistant to assault. This strategy remains to be proven in humans. Recently researchers at the Joslin Diabetes Center (Boston,MA) have claimed existence of a putative islet cell growth factor that could be used to expand islet cell clusters (119).

Engineering other Cells to Produce Insulin (b cell Copy Cats)

In last few years a new era has dawned, exploring the ability to engineer non-islet cells to produce insulin through forced expression of the insulin gene (120-122). So far **duodenal K cells**, **hepatocytes** and **pituitary** cells have been successfully transfected.

Most of the time glucose was able to cause release of insulin in these systems. However, insulin delivery should be modulated in response to changing periods of food intake (feeding/fasting), and exercise. Unless such regulation is ensured, these approaches will not likely be applicable to humans. The fact that experiments have been successful in generating surrogate beta cells is a major step forward (123-125). The pancreatic and duodenal homeobox gene 1 (PDX-1) encodes protein that is central to pancreatic development and insulin gene expression. Recombinant transfer of PDX-1 to the livers of mice led to an increase in immunoreactive insulin levels by reprogramming hepatocytes causing them to acquire a b cell phenotype (126).

SUMMARY

Type 1 Diabetes Mellitus has undergone a sea of changes in our understanding of its pathobiology. Advances in detection/diagnosis and monitoring of patients with disease have resulted in our ability to institute intensified medical management that in turn has resulted in marked risk reduction (retinopathy, neuropathy and nephropathy). The chapter has briefly touched upon areas of immunogenetics, prevention trials and newer treatment modalities such as pancreas transplant, islet cell transplant and future directions in providing alternate sources for b cells and tantalizing prospects for converting cells other than islet b cells to insulin producing cells. Meanwhile, the importance of strict glycemic control, better patient education, and frequent monitoring for any emerging micro- or macro-vascular complications cannot be underemphasized. It is imperative on physicians to keep their patients under the best possible control without too much intrusion on patient lifestyle.

REFERENCES

1. Bliss M. The discovery of insulin.Chicago: University of Chicago press, 1982

2. American Diabetes Association: Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 2001;24: S5-S20.

3. Todd JA. From genome to aetiology in a Multifactorial disease type-1 diabetes. Bio Assays 21999;21;164-73

4. Anonymous. EURODIAB ACE Study Group. Variation and trends in incidence of childhood diabetes in Europe. Lancet 2000; 355: 873-6.

5. Atkinson MA, Eisenbarth GS. Type-1 diabetes: new perspectives on disease pathogenesis and treatment. Lancet 2001;358:221-9.

6. Rabinovitch A. Autoimmune diabetes. Science & Mediicne 2000:7(3):18-27.

7. Pociot F, McDermott MF. Genetics of type1 diabetes mellitus. Genes Immun 2002(5):235-49.

8. Atkinson MA, Eisenbarth GS. Type 1 diabetes: new perspectives on disease pathogenesis and treatment. Lancet 2001;358(9277):221-9

9. Todd JA, Wicker LS. Genetic protection from the inflammatory disease type 1 diabetes in humans and animal models. Immunity2001. 15:387-95.

10. Todd JA. Genetics of type 1 diabetes. Pathol Biol (Paris) 1997;45(3):219-27

11. Bennett ST, Todd JA. Human type 1 diabetes and the insulin gene: principles of mapping polygenes. Annu Rev Genet 1996;30:343-70

12. Ueda H, Howson JM, Esposito L, Heward J, Snook H, Chamberlain G, Rainbow DB, Hunter KM, Smith AN, Di Genova G, Herr MH, Dahlman I, Payne F, Smyth D, Lowe C, Twells RC, Howlett S, Healy B, Nutland S, Rance HE, Everett V, Smink LJ, Lam AC, Cordell HJ, Walker NM, Bordin C, Hulme J, Motzo C, Cucca F, Hess JF, Metzker ML, Rogers J, Gregory S, Allahabadia A, Nithiyananthan R, Tuomilehto- Wolf E, Tuomilehto J, Bingley P, Gillespie KM, Undlien DE, Ronningen KS, Guja C, Ionescu-Tirgoviste C, Savage DA, Maxwell AP, Carson DJ, Patterson CC, Franklyn JA, Clayton DG, Peterson LB, Wicker LS, Todd JA, Gough SC. Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. Nature 2003 ;423 (6939):506-11.

13. Verge CF, Vardi P, Babu S, Bao F, Erlich HA, Bugawan T, Tiosano D, Yu L, Eisenbarth GS, Fain PR. Evidence for oligogenic inheritance of type 1 diabetes in a large Bedouin Arab family. J Clin Invest 1998;102(8):1569-75

14. Meyer G, Badenhoop K. Autoimmune regulator (AIRE) gene on chromosome 21: Implications for autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) and more common manifestations of endocrine autoimmunity. J Endocrinol Invest. 2001;25(9):804-11

15. Nagamine K, Peterson P, Scott HS, Kudoh J, Minoshima S, Heino M, Krohn KJ, Lalioti MD, Mullis PE, Antonarakis SE, Kawassaki K, Asakawa S, Ito F, Shimizu N. Positional cloning of the APECED gene. Nat Genet. 1997;17(4):393-8

16. Patel DD. Escape from tolerance in the human x-linked autoimmunity-allergic dysregulation syndrome and the Scurfy mouse. J Clin Invest 2001;107(2):155-7.

17. Brunkow ME. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. Nat Genet 2001 (1):18-20

18. Chatila TA Blaseser F, Ho N, Lederman HM, Voulgaropoulos C, Helms C, Bowcock AM. JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic dysregulation syndrome. J Clin Invest 2000;106(12):R75-81

19. Wildin RS, Ramsdell F, Peake J, Faravelli F, Casanova JL, Buist N, Levy-Lahad E, Mazzella M, Goulet O, Perroni L, Bricarelli FD, Byrne G, McEuen M, Proll S, Appleby M, Brunkow ME. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. Nat Genet 2001;27(1):18-20.

20. Godfrey VL, Wilkinson JE, Russell LB. X-linked lymphoreticular disease in the scurfy (sf) mutant mouse. Am J Pathol 1991:138(6):1379-87.

21. Bennett CL, Christie J, Ramsdell F, Brunkow ME, Gerguson PH, Whitesell L, Kelly TE, Saulsbury FT, Chance PF, Ochs HD. The immune dysregulation polyendocrinopathy, enteropathy, x-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat Genet 2001; 27(1):20-1.

22. Gepts W. Pathologic anatomy of the pancreas in juvenile diabetes mellitus. Diabetes 1965; 14(10):619-33.

23. Imagawa A, Hanafusa T, Tamura S, Moriwaki M, Itoh N, Yamamoto K, Iwahashi H, Yamagata K, Waguri M, Nanmo T Uno S, Nakajima Pancreatic biopsy as a procedure for detecting in situ autoimmune phenomena in type 1 diabetes: close correlation between serological markers and histological markers and histological evidence of cellular autoimmunity. Diabetes 2001;50(6):1269-73.

24. Pauza ME, Nguyen A, Wolfe T, Ho IC, Glimcher LH, von Herrath M, Lo D. Variable effects of transgenic c-Maf on autoimmune diabetes. Diabetes 2001;50(1):39-46.

25. Grening JE, Tree TI, Kotowicz KT, van Halteren AG, Roep BO, Klein NJ, Peakman M. Processing and presentation of the islet autoantigen GAD by vascular endothelial cells promotes transmigration of autoreactive T-cells. Diabetes 2003; 52(3):717-25.

26. Sutherland DE, Goetz FC, Sibley RK. Recurrence of disease in pancreas transplants. Diabetes. 1989:38 Suppl 1-85-7

27. Santamaria P, Nakhleh RE, Sutherland DE, Barbosa JJ. Characterization of T lymphocytes infiltrating human pancreas allograft affected by isletitis and recurrent diabetes. Diabetes. 1992;41(1):53-61.

28. Bottazzo GF, Florin-Christensen A, Doniach D. Islet-cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. Lancet 1974;2((7892):1279-83.

29. MacCuish AC, Irvine WJ, Barnes EW, Duncan LJ. Antibodies to pancreatic islet cells in insulin-dependent diabetics with coexistent autoimmune disease. Lancet 1974 28; 2(7896):1529-31.

30. Mally MI, Cirulli V, Otonkoski T, Soto G, Hayek A. Ontogeny and tissue distribution of human GAD expression. Diabetes 1996;45(4):496-501

31. Wasmeier C, Hutton JC. Secretagogue-dependent phosphorylation of the insulin granule membrane protein phogrin is mediated by cAMP-dependent protein kinase. J Biol Chem2001; 276 :31919 –1928,

32. Cui L, Yu WP, DeAizpurua HJ, Schmidli RS, Pallen CJ. Cloning and characterization of islet cell antigen-related protein-tyrosine phosphatase (PTP), a novel receptor-like PTP and autoantigen in insulin-dependent diabetes. *J Biol Chem* 271 :24817 –24823,1996

33. Maclaren N, Lan M, Coutant R, Schatz D, Silverstein J, Muir A, Clare-Salzer M, She JX, Malone J, Crockett S, Schwartz S, Quattrin T, DeSilva M, Vander Vegt P, Notkins A, Krischer J, Only multiple autoantibodies to islet cells (ICA), Insulin GAD65, IA-2 and IA-2beta predict immune –mediated (Type 1) diabetes in relatives. J Autoimmun 1999; 12(4):279-87

34. Bingley PPJ, Bonifacio E, Williams AJ, Genovese S, Bottazzo GF, Gale EA. Prediction of IDDM in the general population: strategies based on combinations of autoantibody markers. Diabetes 1997;46(11):1701-10.

35. Mire-Sluis AR, Gaines Das R, Lernmark A. The World Health Organization International collaborative Study for islet cell antibodies. Diabetologia 2000; 43(10):1282-92

36. Lernmark A. Contolling the controls: GAD65 autoreactive T cells in type 1 diabetes. J clin Invest 2002; 109(7):869-70

37. Serreze DV, Chapman HD, Varnum DS, Hanson PC, Reifsnyder SD, Richard SA, Fleming EH, Lelither EH, Shultlz LDB lymphocytes are essential for the initiation of T cell-mediated autoimmune diabetes: analysis of a new "speed congenic" stock of NOD. Ig mu mull mice. J. Exp. Med 1996; 184:2049,

38. Akashi T, Nagafuchi S, Anzai K, Kondo S, Kitamura D, Wakana S, Ono J, Kikuchi M, Niho Y, Watanabe T. Direct evidence for the contribution of B cells to the progression of insulitis and the development of diabetes in non-obese diabetic mice. Int. Immunol. 1997; 9:1159.

39. Drake JR, Lewis TA, Condon KB, Mitchell RN, Webster P. Involvement of MIIC-like late endosomes in B cell receptor-mediated antigen processing in murine B cells. J Immunol. 1999; 15; 162(2):1150-5.

40. Wrede G, Kiesel U, Freytag G, Kolb H. Chronic insulitis after partial islet damage by passive insulin antibody transfer. Horm. Metab. Res 1984; 16 (Supple.1), 97-101.

41. Marner B, Lernmark A, Ludvigsson J, MacKay P, Matsuba I, Nerup J, Rabinovitch A. Islet cell antibodies in insulin-dependent (Type 1) diabetic children treated with plasmapheresis. Diabetes Res 1985; 2, 231-236

42. Yang M, Charlton B, Gautam AM. Development of insulitis and diabetes in B cell-deficient NOD mice. J Autoimmun 1997;10: 257-260.

43. Martin S, Wolf-Eichbaum D, Duinkerken G, Scherbaum WA, Kolb H, Noordzij JG, Roep BO. Development of type 1 diabetes despite severe hereditary B-cell deficiency. N Engl J Med 2001; 345:1036-1040.

44. Hulbert C, Riseili B, Rojas M, Thomas JW. B cell specificity contributes to the outsome of diabetes innonobese diabetic ice. J Immunol 2001, 167:5535-5538.

45. Haskins K, Wegmann D, Diabetogenic T-cell clones. Diabetes 45:1299-1305, 1996

46. Wong FS, Karttunen J. Dumont C, Wen L, Visintin I, Pilip IM,Shastri N, Pamer EG, Janeway CA Jr. Identification of an MHC class I-restricted autoantigen in type 1 diabetes by screening an organ-specific cDNA library. Nat Med 1999; 5:1026-1031.

47. Hoglund P, Mintern J, Waltzinger C, Heath W, Benoist C, Mathis D. Initiation of autoimmune diabetes by developmentally regulated presentation of islet cell antigens

in the pancreatic lymph nodes. J Exp Med 1999;189(2):331-9

48. Alleva DG, Pavlovich RP, Grant C, Kaser SB, Beller DI. Aberrant macrophage cytokine production is a conserved feature among autoimmune prone mouse strains: elevated IL-12 and an imbalance in TNF andIL-10 define a unique cytokine profile in macrophages from young non-obese diabetic (NOD) ice. Diabetes 2000; 49:1106.

49. Trembleau S, Penna G, Bosi E, Mortara A, Gatel MK, Adorini. L. Interleukin 12 administration induces T helper type 1 cells and accelerates autoimmune diabetes in NOD ice. J Exp Med 1995; 181(2):817-21.

50. Zipris D. Evidence that Th1 lymphocytes predominate in islet inflammation and thyroiditis in the BioBreeding (BB) rat. J Autoimmun 1996; 9(3):315-9.

51. Campbell IL, Iscaro A, Harrison LC. IFN-gamma and tumor necrosis factor-alpha. Cytotoxicity to murine islets of Langerhans. J Immunol 198 1, 141(7):2325-9.

52. Mandrup-Poulsen T, Spinas GA, Prowse SJ, Hansen BS, Jorgensen DW, Bendtzen K, Nielsen JH, Nerup J. Islet cytotoxicity of interleukin 1. Influence of cultures conditions andislet donor characteristice. Diabets. 1987; 36(5):641-7

53. Matsuoka T, Kajimoto Y, Watada H, Kaneto H, Kishimoto M, Umayahara Y, Fujitani Y, Kamada T, Kawamori R, Yamasaki Y. Glycation-dependent, reactive oxygen species-mediated suppression of the insulin gene promoter activity in HIT cell. J Clin Invest. 1997;99(1): 155-50

54. Kaneto H, Xu G, Song KH, Suzuma K, Bonner-Weir S. Sharma A, Weir GC. Activation of the hexosamine pathway leads to deterioration of pancreatic beta-cell function through the induction of oxidative stress. J Biol Chem. 2001; 276:31099-104.

55. Lortz S, Tiedge M, Nachtwey t, Karlsen AE, Nerup J and Lenzen S. Protection of insulin-producing RINm5F cells against cytokine-mediated toxicity through over expression of antioxidant enzymes. Diabetes Vol 49, Issue 7, 1123-30.

56. Kagi D, Odermatt B, Ohashi PS, Zinkernagel RM, and Hengartner H. Development of insulitis without diabetes in transgenic mice lacking perforin-dependent cytoxicity. Journal of Experimental Medicine 183: 2143-52.

57. Chervonsky AV, Wang Y, Wong FS, Bisintin I, Flavell RA, Janeway CA Jr, Matis LA. The role of Fas in autoimmune diabetes. Cedll. 1997;89(1):17-24.

58. Norris JM, Beaty B, Lkingsmith G et al. Lack of association between early exposure to cow's milk protein and â-cell autoimmunity. Diabetes Autoimmunity Study in the Young (DAISY). JAMA 1996;76:609-14.

59. Graves PM, Barriga KH, Norris JM et al. Lack of association between early childhood immunization and b cell autoimmunity. Diabetes Care 1999; 22:1694-7.

60. Hummel M, Fuchtenbusch M, Schenker M et al. No major association between breast feeding, vaccinations, and childhood viral diseases with early autoimmunity in the German BABYDIAB Study. Diabetes Care 2000;23: 969-74.

61. Wong FS, Visintin I, WEn L, Flavell RA, Janeway CA. CD8T cell clones from young nonobese diabetic (NOD) islets can transfer rapid onset of diabetes in NOD mice in the absence of CD4cells. J. Exp. Med. 1996; 183, 67-76.

62. Wang B, Gonzalez A, Benoist C, and Mathis D. The role of CD8+ T cells in the initiation of insulin –dependent diabetes mellitus. Eur. J. Immunol. 1996; 26, 1762-9.

63. Honeyman MC, Cram DS, Harrison LC. Glutamic acid decarboxylase 67-reactive T cells: a maker of insulin-dependent diabetes. J. Exp. Med. (1993), 177; 535-40.

64. Panina-Bordignon P, Lang R, van Endert PM, Benazzi E, Felis AM, Pastore RM, Spinas GA and Sinigaglia. Cytotoxic T cells specific for glutamic acid decarboxylase in autoimmune diabetes. J. Exp. Med. (1995), 181; 1923-7.

65. Kaufman D, Clare-Salzler, 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.

66. Tisch R, Yang XD, Singer SM, Liblau R, Fugger L, and McDevitt H. Immune response to glutamic acid decarboxylase correlates with insulitis in non-obese diabetic mice. Nature (1993), 366; 72-5.

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

68. Vardi P, Ziegler AG, Mathews JH, Dib S, Keller RJ, Ricker AT, Wolfsdorf JJ, Herskowitz RD, Rabizadeh A, and Eisenbarth GS. Concentration of insulin autoantibodies at onset of type 1 idabetes. Inverse loglinear correlation with age. Diabetes Care (1988), 11; 736-739.

69. Eisenbarth GS, Jackson RA and Pugliese A. Insulin autoimmunity: the rate limiting factor in pre-type-I diabetes. J. Autoimmun. (1992), 5; 241-6.

70. Ji-Won Yoon, Chang-Soon Yoon, Hye-Won Lim, Qi Quan Huang, Yup Kang, Kwang Ho Pyun, Kensuke Hirasawa, Robert S. Sherwin and Hee-Sook Jun. Control of Autoimmune Diabetes in NOD Mice by GAD Expression or Suppression I â cells. Science 1999; 284: 1183-7.

71. Rabinovitch A. Immunoregulatory and cytokine imbalances in the pathogenesis of IDDM: therapeutic intervention by immunostimulation? Diabetes 1994; 43:613.

72. Tisch R, McDevitt H. Insulin-dependent diabetes mellitus. Cell 1996;85:291.

73. Dellovitch TL, Singh B, The nonobese diabetic mouse as a model of autoimmune diabetes: immune dysregulation gets the NOD. Immunity. 1997; 7:291.

74. Sluarez-Pinzon WL, Rabinovitch A. Approaches to type 1 diabetes prevention by intervention in cytokine immunoregulatory circuits. Int J Exp Diabetes Res. 2001; 2(1):3-17.

75. Akhtar L, Gold JP, Pan, L-Y, Ferrara JLM, Yang X-D, Kim JI, Tan KN. CD4+b islet cell-reactive T cell clones that suppress autoimmune diabetes in nonobese diabetic mice. J Exp Med. 1995.; 182:87.

76. Pakala SV, Kurrer MO, Katz JD. T helper 2(Th2) T cells induce acute pancreatitis and diabetes in immune-compromised nonobese diabetic (NOD) mice. J. Exp. Med. 1997. 186:299

77. Zekzer D, Wong FS, Wen L, altieri M, Gurlo T, von Frafenstein H, Sherwin RS. 1997. Inhibition of diabetes by an insulin reactive CD4 T-cell clone in the nonobese diabetic mouse. Diabetes 46: 1124.

78. Poulin M, Haskins K. Induction diabetes in nonobese diabetic mice by Th2 T cell clones from a TCR transgenic mouse. J. Immunol. 2000; 164:3072.

79. Hultgren B, Huang X, Dybdal N, Stewart TA. Genetic absence of ã-interferon delays but does not prevent diabetes in NOD mice. Diabetes 1996; 45:812.

80. Serreze DV, Chapman HD, Post CM, Johnson EA, Suarez-Pinzon WL, Rabinovitch A, Th1 to Th2 cytokine shifts in nonobese diabetic mice: sometimes an outcome, rather than the cause, of diabetes resistance elicited by immunostimulation. J Immunol. 2001; 166(2):1352-9.

81. Kero J, Gissler M, Hemminki E, Isolauri E. Could TH1 and TH2 diseases coexist: Evaluation of asthma incidence in children with celiac disease, type 1 diabetes, or rheumatoid arthritis: a register study. J Allergy Clin Immunol. 2001;108(5):781-3.

82. Pakala, S. V., M. O. Kurrer, J. D. Katz. T helper 2 (Th2) T cells induce acute pancreatitis and diabetes in immune-compromised nonobese diabetic (NOD) mice. J. Exp. Med. 1997; 186:299.

83. Zekzer, D., F. S. Wong, L. Wen, M. Altieri, T. Gurlo, H. von Grafenstein, R. S. Sherwin. Inhibition of diabetes by an insulin reactive CD4 T-cell clone in the nonobese diabetic mouse. Diabetes 1997;46:1124.

84. Poulin, M., K. Haskins. Induction of diabetes in nonobese diabetic mice by Th2 T cell clones from a TCR transgenic mouse. J. Immunol. 2000;164:3072.

85. Hultgren, B., X. Huang, N. Dybdal, T. A. Stewart. Genetic absence of g-interferon delays but does not prevent diabetes in NOD mice. Diabetes 1996.; 45:812.

86. Serreze DV, Chapman HD, Post CM, Johnson EA, Suarez-Pinzon WL, Rabinovitch A. Th1 to Th2 cytokine shifts in nonobese diabetic mice: sometimes an outcome, rather than the cause, of diabetes resistance elicited by immunostimulation. J Immunol. 2001; 166(2):1352-9.

87. Chervonsky, A. V. et al. The role of Fas in autoimmune diabetes. Cell (1997); 89, 17-24.

88. Loweth, A. C., Williams, G. T., James, R. F. L., Scarpello, J. H. B. & Morgan, N. G. Human islets of Langerhans express Fas ligand and undergo apoptosis in response to interleukin-1b and Fas ligation. Diabetes (1998). 47, 727-32

89. Itoh, N. et al. Requirement of Fas for the development of autoimmune diabetes in nonobese diabetic mice. J. Exp. Med. (1997). 186, 613-8

90. Thomas, H. E., Darwiche, R., Corbett, J. A. & Kay, T. W. H. Evidence that b cell death in the nonobese diabetic mouse is Fas independent. J. Immunol. (1999); 163, 1562-9

91. Allison, J. & Strasser, A. Mechanisms of beta cell death in diabetes: a minor role for CD95. Proc. Natl Acad. Sci. USA (1998). 95, 13818-22

92. Kim, Y. H. et al. Apoptosis of pancreatic beta-cells detected in accelerated diabetes of NOD mice: no role of Fas-Fas ligand interaction in autoimmune diabetes. Eur. J. Immunol. (1999); 29, 455-65.

93. Watanabe, D., Suda, T., Hashimoto, H. & Nagata, S. Constitutive activation of the Fas ligand gene in mouse lymphoproliferative disorders. EMBO J. (1995). 14, 12-8

94. Chervonsky AV et al. The role of Fas in autoimmune diabetes. Cell (1997). 89, 17-24

95. Loweth AC, Williams GT, James RFL, Scarpello JHB and Morgan NG. Human islets of Langerhans express Fas ligand and undergo apoptosis in response to interleukin-1b and Fas ligation. Diabetes (1998).47, 727-32

96. Itoh N et al. Requirement of Fax for the developemnt of autoimmune diabetes in nonobese diabetic mice. J. Exp. Med. (1997). 186, 613-8

97. Thomas HE, Darwich R, Corbett JA, Kay TWH. Evidence that â cell death in the nonobese diabetic mouse is Fas independent. J. Immunol. 1999.163, 1562-9

98. Allison J & Strasser A. Mechanisms of beta cell death in diabetes: a minor role for CD95. Proc. Natl Acad. Sci. USA 1998. 95, 13818-22

99. Kim Y H et al. Apoptosis of pancreatic beta-cells detected in accelerated diabetes of NOD mice: no role of Fas-Fas ligand interaction in autoimmune diabetes. Eur. J. Immunol. 1999. 29, 455-65

100. Watanabe D, Suda T, Hashimoto H, and Nagata S. Constitutive activation of the Fas ligand gene in mouse lymphoproliferative disorders. EMBO J. 1995. 14, 12-8.

101. Kim S et al. Inhibition of autoimmune diabetes by Fas ligand: the paradox is solved. J. Immunol (2000). 164, 2931-6

102. Cheta D. Animal models of type 1 (insulin-dependent) diabetes mellitus. J Pediatr Endocrinol Metab. 1998;11 (1):11-9.

103. Yang XD, Michie SA, Mebius RE, Tisch R, Weissman I, McDevitt HO. The role of cell adhesion molecules in the development of IDDM: Implications for pathogenesis and therapy. Diabetes. 1996 ;45 (6):705-10.

104. Bradley LM, Asensio VC, Schioetz LK, Harbertson J, Krahl T, Patstone G, Woolf N, Campbell IL, Sarvetnick N. Islet-specific Th1, but not Th2, cells secrete multiple chemokines and promote rapid induction of autoimmune diabetes. J Immunol. 1999;1612(5):2511-20.

105. Frigerio S, Junt T, Lu B, Gerard C, Zumsteg U, Hollander GA, Piali L. Beta cells are responsible for CXCR3-mediated T-cell infiltration in insulitis. Nat Med. 2002;8(12):1414-20.

106. Loetscher M, Gerber B, Loetscher P, Jones SA, Piali L, Clark-Lewis I, Baggiolini M, Moser B. Chemokine receptor specific for IP10 and mig: structure, function, and expression in activated T-lymphocytes. J Exp Med. 1996:184 (3):963-9.

105.(B) Atkinson MA & Leiter EH. The NOD mouse model of type 1 diabetes: as good as it gets? Nature Medicine, 1999; 5 601-4.

107. Thivolet C. New therapeutic approaches to type 1 diabetes: from prevention to cellular or gene therapies. Clin Endocrinol (Oxf). 2001;55(5):565-74

108. Diabetes Prevention Trial-Type 1 Diabetes Study Group. Effects of Insulin in Relatives of Patients with Type 1 Diabetes Mellitus. New Engl J Med 2002. 346:1685-91.

109. Martins TC, Aguas AP. Mechanisms of Mycobacterium avium-induced resistance against insulin-dependent diabetes mellitus (IDDM) in non-obese diabetic (NOD) mice: role of Fas and Th1 cells. Clin Exp Immunol. 1999;115 (2):248-54.

110. Raz I, Elias D, Avron A, Tamir M, Metzger M, Cohen IR. Beta-cell function in new-onset type 1 diabetes and immunomodulation with a heat-shock protein peptide (DiaPep277): a randomized double-blind, phase II trial. Lancet. 2001;358(9295):1749-53.

111. Ryu S, Kodama S, Ryu K, Schoenfeld DA, Faustman DL. Reversal of established autoimmune diabetes by restoration of endogenous beta cell function. J Clin Inves. 2001;108(1):63-72.

112. Notkins AL, Lernmark A. Autoimmune type-1 diabetes: resolved and unresolved issues. J. Clin Invest 2001; 108:1247-52

113. Wucherperg KW, Eisenbarth GS. Type-1 Diabetes. Nature Immunology 2001; 2:767-8.

114. The Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications ininsulin-dependent diabetes mellitus. N Engl J Med 1993;329: 977-86.

115. The Writing Team for the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group: effect of intensive therapy on the microvascular complications of type 1 diabetes mellitus. JAMA 2002;287:2563-9.

116. Sutherland DER, Gruessner RWG, Dunn DL et al. Lessons learned from more than 1,000 pancreas transplants at a single institution. Ann Surg 2001;233:463-501.

117. Newgard CB. Cellular engineering and gene therapy. Strategies for insulin replacement indiabetes. 1994;43;341-50.

118. Thivolet C. New Therapeutic approaches to type 1 diabetes: from prevention to cellular or gene therapies. Clin Endocrinol 2001; 55:565-74.

119. Efrat S. Development of engineered pancreatic B-cell lines for cell therapy of diabetes. Adv Drug Del. Rev 1998:33-45

120. Flier J, Kulkarni RN, KahanCE. Evidence of a circulating islet cell growth factor in insulin-resistant states. PNAS 2001;98: 7475-80.

121. Cheung AT, Dayanandan B, Lewis JT et al. Glucose dependent insulin release from genetically engineered K cells. Science 2000; 290:1959-62.

122. Lee HC, Kim S-J, Shin H-C et al. Remission in models of type-1 diabetes by gene therapy using single chain insulin analogue. Nature 2000;408: 483-8.

123. von Herrath M, Holz A. Pathological changes in the islet milieu precede infiltration of islets and destruction of a-cells by autoreactive lymphocytes in a transgenic model of virus-induced IDDM. J Autoimmunity 1997:10:231-8.

124. Peck AB, Chaudhari M, Cornelius JG et al. Pancreatic stem cells: Building blocks for a better surrogate islet to treat type 1Diabetes. Ann Med 2001:33:186-92.

125. Alleva DG. Crowe PD, Jin L et al. A disease associated cellular immune response in type 1 diabetes to an immunodominant epitope of insulin. J Clin Invest 2001;107: 173-80.

126. Tamborlane WV, Bonfig W, Boland E. Recent advances in treatment of youth with Type-1 diabetes: better care through technology. Diabetic. Med 2001;18: 864-70.

127. Ferber S, Wlkin A, Cohen H et al. pancreatic and duodenal homeobox gene 1 induces expression of insulin genes in liver and ameliorates streptozotocin-induced hypoglycemia, Nat. Medicine 2000:6:568-72.