MEDICAL GRAND ROUNDS

"PREVENTION OF AUTOIMMUNE DIABETES"

Marilyn Alford, R.N., M.S., takes blood samples from diabetic Jeanette Wilkinson and her sister Ann Marie. Both girls will be listed in the Diabetes Registry, a list of siblings of diabetics, which identifies potential diabetics before they get the disease. (A brother, Brad Wilkinson, was not available for the photo.)

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SEPTEMBER 15, 1988

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INTRODUCTION

The most recent major therapeutic advance in diabetes, the isolation of insulin by Banting and Best, took place in 1921, 6 years before Charles A. Lindbergh made his epochal transatlantic flight. Whereas aerospace technology has advanced at a near-miraculous pace, treatment of diabetes has remained virtually unchanged for the past 3 generations despite substantial scientific progress in our understanding of the disease, and, ironically, despite the fact that both its curability and preventability have already been established.

PREVENTION OF TYPE I DIABETES:

I. Criteria of Disease Preventability (Table I).

TABLE I. CRITERIA FOR POTENTIAL PREVENTABILITY OF DISEASE

A. The type of disease process, if not the actual cause, must be known.

B. High risk groups must be identifiable.

C. The preovert phase (the time between the onset of the process and the onset of overt disease) must be sufficiently long to provide time for diagnosis and treatment.

D. There must be clinical tests to diagnose and track the preovert destructive process.

E. There must be an effective intervention with a low risk-reward ratio.

II. Does Type I Diabetes Qualify?

1. The type of disease process, if not the specific cause of the disease, is known: Type I diabetes is now universally accepted as an autoimmune disorder, although the specific precipitating factor or factors have yet to be identified. Of the volumes of evidence for this the most irrefutable comes from twin-to-twin pancreatic transplantation (Table II) in which transplantation of pancreas from a nondiabetic to a diabetic monozygotic twin cured diabetes in four recipients (1). However, those of the recipients who did not receive

immunosuppressive therapy all developed biopsy-proven insulitis; islet cell antibodies and hyperglycemia recurred within 6 weeks without any evidence of graft rejection. In the fourth recipient cyclosporine A treatment prevented reenactment of the diabetes.

TABLE II. REVERSAL AND RE-ENACTMENT OF TYPE 1 DIABETES IN PANCREAS TRANSPLANTED FROM A NONDIABETIC TO A DIABETIC MONOZYGOTIC TWIN AFTER MORE THAN 15 YEARS OF DISCORDANCE

Twin Pair	Immuno-suppression	Initial Cure of Diabetes	Recurrence of Diabetes Within Four Weeks	Bx† Evidence of Rejection	Insulitis	Appearance of Islet Cell Antibodies*
1	0	+	+	0	+	+
2	0	+	+	0	+	+
3	0	+	+	0	+	+
4	+	+	0	0	no bx	–

*Appeared at approximately eight weeks. Summarized from Sutherland DER, Sibly R, Chinn P, et al. Twin to twin pancreas transplantation (TX): reversal and reenactment of the pathogenesis of type 1 diabetes. Clin Res 1984; 32:561A.
†Bx = biopsy

Conclusion: Type I diabetes is an autoimmune disease and can be prevented with immunosuppression.

2. High risk groups are identifiable: Over half of the inherited predisposition to insulin-dependent diabetes maps to the region of the short arm of chromosome 6 that contains the highly polymorphic class II genes that determine immune responsiveness (Figure 1). It was previously believed that there was linkage between a susceptibility gene and the DR3/DR4 loci because 95% of Caucasian autoimmune diabetics have one or both of these antigens (2). Today it is believed that alleles of HLA-DQβ determine diabetes susceptibility and resistance; structure of the β chain of the DQ molecule, in particular residue 57 of the β chain, specifies the autoimmune response against beta cells (Table III) (3). Of interest is the fact that there are homologies between the section of the peptide that includes position 57 encoded by DQβ3.2 (DR4) allele and EBV which encodes a 6-fold repeat GPPAA, which is also in the envelope protein of rubella (4). The significance of this observation is unknown.

TABLE III.
PREDICTED SUSCEPTIBILITY OF AUTOIMMUNE DIABETES OF DR3 AND/OR DR4 SUBJECTS IN GENERAL POPULATION BASED ON DNA SEQUENCE OF DQ β CHAIN GENE

Susceptibility	Asp-57
Full	Neg/Neg
10%	Neg/Pos
0	Pos/Pos

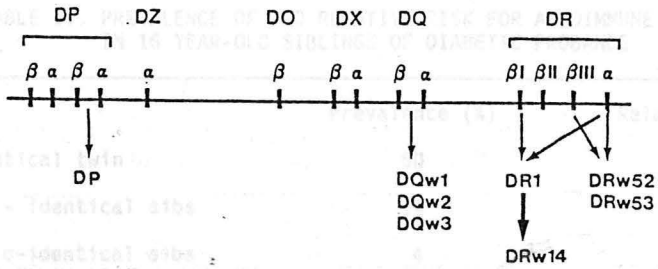


Figure 1: Map of the class II loci of the human HLA-D region on the short arm of chromosome 6. The $DR_{\beta I}$ and DR_{α} gene products associate to form a cell-surface heterodimeric glycoprotein. This molecule reacts with the alloantisera that define the major serological allotypes HLA-DR1 to DRw14. The relatively non-polymorphic $DR_{\beta III}$ and DR_{α} gene products encode the DRw52 and DRw53 serological determinants, which are each associated with several DR allotypes. For the DR2, DR5, DR7 and DRw9 haplotypes we assume that the $DR_{\beta I}$ gene encodes the more polymorphic chain. The DR1 and DRw8 haplotypes appear to have only one expressed DR_{β} gene that corresponds to the $DR_{\beta I}$ locus. On the DR3 and DR4 haplotypes the $DR_{\beta II}$ gene is not expressed. The DQ_{α} and DQ_{β} genes encode the DQ serological specificities (DQw1, DQw2 and DQw3). A fourth determinant called DQ Blank, has recently been defined by an alloantiserum, TK2. The DX_{α} and DX_{β} and the second DP_{α} and DP_{β} genes are not expressed, and the DO_{β} and DZ_{α} are expressed only in very small amounts (3).

In NOD mice at least three recessive loci are required for autoimmune diabetes, the HLA-linked locus and a Thy-1/Alp-1-linked locus on chromosome 9 (5). If IDDM susceptibility in man is also polygenic, a locus proximal to Thy-1 and Alp-1 on the long arm of chromosome 11 should be looked for. Hoover and Capra (personal communication) have identified a potential susceptibility locus within the T-cell receptor β-chain gene. Heterozygosity of TCR Bgl-2 is increased in autoimmune diabetics. Recent studies suggest that a point mutation within the D-segment coding region in linkage disequilibrium with this polymorphism. Heterozygosity may reflect the requirement for 2 different T-cell types $CD4^+$ and $CD8^+$. Assuming that only 2 loci are involved in humans, a homozygous asp-57-negative patient with diabetogenic T-cell receptor alleles should confer maximal susceptibility to the disease approaching that of an identical twin of a Type I diabetic (Table IV)(6).

Conclusion: High risk groups are identifiable both in selected subsets (such as diabetic families [Table IV] or congenital rubella victims) and in random populations.

TABLE IV. PREVALENCE OF AND RELATIVE RISK FOR AUTOIMMUNE DIABETES IN 16 YEAR-OLD SIBLINGS OF DIABETIC PROBANDS

	Prevalence (%)	Relative Risk
Identical twin	50	
HLA - identical sibs	14	118
Haplo-identical sibs	4	31
Non-identical sibs	1	NS
All sibs	5	36

3. There is sufficient time between initiation of the destructive process and serious damage or depletion of target cells: Although rare cases of fulminating destruction occur (7) it is now believed that the usual course is from months to 3 years in duration.

Conclusion: The prodromal period is almost always sufficient to permit the diagnosis of preovert autoimmune β-cell destruction and to undertake almost any conceivable intervention.

4. There are tests to track the disease process:

A) Immunologic tests: a) Islet cell surface antibodies: ICSA requires viable dispersed islet cells and is therefore not ideal for routine clinical use. It has been claimed that it gives a higher percent of positives than ICA-cyt in new onset diabetics (about 80%)(8). These antibodies are cytotoxic in the presence of complement (9). b) Islet cell cytoplasmic antibodies: ICA-cyt are easily measured on sections of pancreas from Type O humans or from monkey or rat. These are positive in 60% of new onset diabetics (Figure 2). They probably reflect immune response to β-cell proteins extruded during their destruction. c) Insulin autoantibodies: IAA determination involves relatively simple RIA methodology.

B) Functional tests: a) Glucose stimulated insulin secretion. Loss of the first phase response to insulin secretion is regarded as the most sensitive indicator of beta cell depletion. When the sum of the 1 and 3 minute specimens is below 40 μU/ml, significant functional loss is present. Because of wide variability in absolute insulin levels in the normal population, we prefer to base decisions concerning need to intervene upon evidence of progressive loss of function. If over a two year period first phase insulin response to intravenous glucose has declined to 50% or less of the initial response in the presence of positive immunologic tests, the person is classified as probably in an intervention-appropriate category.

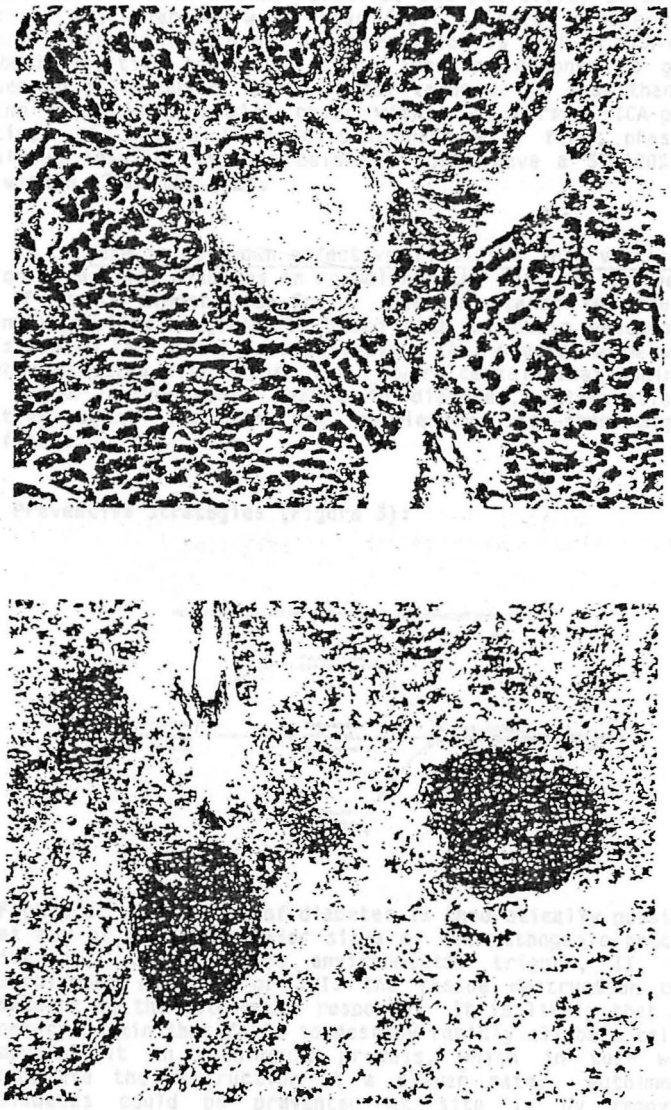


Figure 2: A negative (top) and positive (bottom) stain for ICA-cyt using the glucose oxidase coupled antibody method (10).

In NOD mice conversion to diabetes never occurs until after both ICA-cyt and IAA have become positive, but positivity of both does not guarantee that diabetes will occur (11). Careful prospective studies are not available in man but the prediction is that if ICA-cyt and IAA are both positive and first phase insulin response to glucose has dropped to below 50% of the original value or is less than 40 µU/ml, fasting hyperglycemia will occur within 2 years. ICA-positive 1° relatives of a type I diabetic proband with first phase glucose-stimulated insulin response below 25 µU/ml have a 59-100% chance of IDDM within 12 months (12).

5. <u>There is no known effective risk-free intervention:</u> Intervention to prevent diabetes in normal healthy children can be justified only if it is almost risk-free. The reluctance to intervene with presently available agents is based both on their unacceptable risk levels and on the lack of proven indices that predict with precision if and when diabetes will occur. If it were certain that nonintervention in a given person would result in diabetes, intervention with an effective agent that is not completely risk-free might become justifiable.

III. Preventive Strategies (Figure 3):

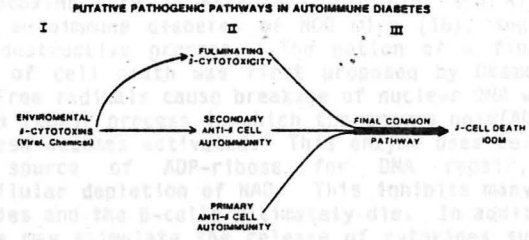


Figure 3: Prevention of diabetes is theoretically possible at any of the three major sites of the pathogenic process i.e., I. the putative environmental trigger, II, the autoimmune response or III. the tissue destruction consequent to the autoimmune response. It is likely that any betacytotoxin that fails to destroy rapidly all beta cells, may elicit an autoimmune process, which in turn will complete the destruction at a slower rate. Autoimmune diabetes could be prevented at site I. by removing identifiable environmental betacytotoxins and vaccinating high risk groups against potential viral agents if these were to be implicated in externally triggered cases. Environmental factors such as viruses and betacytotoxins rarely cause IDDM by direct, rapid and massive beta cell

Figure 3 (continued): damage. They more commonly cause subclinical β-cell damage which in turn elicits an autoimmune response to β-cell antigens that continues the destructive process. This is true of such diverse betacytotoxins as Vacor, a rat poison sometimes used in suicide attempts or inadvertently ingested by children. Streptozotocin, alloxan and viruses in animals and can induce either rapid fulminating β-cell damage or slow autoimmune-mediated damage in the genetically predisposed. Such autoimmunity can be classified as "secondary". The autoimmune diabetes caused by low dose streptozotocin (13) can be blocked with mAb against either $L3T4^+$ or $Lyt2^+$ T-cells, the cells that combine to cause insulitis and β-cell loss in spontaneous rodent diabetes (14). Most autoimmune diabetes arises without any apparent external trigger, however. It is possible that in many such cases an endogenous β-cell protein is antigenic in susceptible individuals; if so, the 50% discordance in monozygotic twins must reflect extensive genetic rearrangement of immunoglobulin and T-cell receptor genes rather than environmental discordance. The process could be prevented at site II. by blocking at one or more points the autoimmune process or at site and III. by protecting against tissue injury via the "final common pathway", damage by oxygen-derived free radicals (Figure 4). Agents like nicotinamide protect both against such different betacytotoxins such as streptozotocin (15) and alloxan and against autoimmune diabetes of NOD mice (16), suggesting a common destructive process. The notion of a final common pathway of cell death was first proposed by Okamota et al (17). Free radicals cause breakage of nuclear DNA which initiates a repair process in which the enzyme poly(ADP-ribose) synthetase becomes activated. This enzyme uses cellular NAD as a source of ADP-ribose for DNA repair, causing intracellular depletion of NAD. This inhibits many cellular activities and the β-cells ultimately die. In addition, free radicals may stimulate the release of cytokines such as TNF and interleukin I which have been reported to be betacytotoxic (18). Unfortunately long-term use of nicotinamide is discouraged by the possibility that it can act as a carcinogen by inhibiting DNA repair enzyme - at least when given in combination with streptozotocin (19).

TABLE V. PREVENTING AUTOIMMUNE DIABETES. The agents used or proposed for use in autoimmune diabetic models grouped according to their site of action (cf Figure 3).

AGENT OR PROCEDURE	DIABETES MODEL	RESULT	MECHANISM	
I. VACCINATION				
Vaccine	Virus-induced	Prevents	Prevents infection	Notkins NEJM 306:486, 1982
II BLOCKING AUTOIMMUNITY				
a. Blocking Presentation of Antigen				
Anti-Ir gene product antibody	Low dose SZ mice	Prevention	Anti T-cell or macrophage effect that prevents antigen recognition	Kiesil Diabetes 32:869, 1983
Silica	BB rats	Marked reduction in diabetes	Macrophage depletion or inactivation	Ochilewski et al Diabetes 34:197,'85
b. Blocking recognition				
Strepto-coccal Prep OK-432	NOD mice	Complete prevention of diabetes and marked reduction of insulitis	↑ suppressor T-cells (?)	Toyota et al. Diabetes 32:496,1986
LCM virus	NOD mice BB/W rats	Complete prevention of diabetes and insulitis	Alteration of T-cell subsets	Oldstone Science 239:500, 1988
Mab to L3T4⁻	NOD mice	Prevention & long-term reversal of insulitis and hyperglycemia	Elimination of L3T4⁺ helper T-cells	Shizuru et al Science 240:659-40, 1988
c. Blocking Effector Cells				
Neonatal Thymectomy	BB/W rats	Reduces frequency of diabetes	Prevents cell-mediated β-cell destruction	Like et al. Science 216:644, 1982
Azothioprine	alloxanized mice	Prevention		Sandler Diabetologia 23:374, 1982
Total lymphoid irradiation	BB/W rat	Marked reduction in frequency of diabetes	Immunosuppression	Rossini et al. Diabetes 33:543,1984
Cyclosporine	NOD mice	Complete prevention of insulitis	Immunosuppression	Formly et al. J Pharm Exp The 241: 1106,1987
Pertussis vaccine	SZ rats	Complete prevention	?	Huang Clin Exp Immun 48:375, 1982
Lymphocyte transfusions	BB/W rat	Marked reduction in diabetes and insulitis	? Restores suppressor T cells	Rossini et al J Clin Invest 74:39, 1984
Allogenic bone marrow transplan-tation	NOD mice	Prevention of diabetes and insulitis	?	Ikehara et al. PNAS 82:7743, 1985
III BLOCKING β-CELL NECROSIS				
Amygdalin	Alloxanized rats	Prevents diabetes	Radical scavenging	Heikhila Life Sci 27:659, 1980
Superoxide dismutase	Low dose SZ rats	Prevents glucose intolerance and ↓ of pancreatic insulin	Radical scavenging	Robbins et al, Diabetologia 118:59, 1980
Superoxide dismutase	Alloxanized rats	Prevents glucose intolerance and ↓ of pancreatic insulin	Radical scavenging	Grankviat et al, Nature 294: 158, 1981
(-)-epicatechin	Alloxanized rats	Prevents diabetes	Radical scavenging	Chahrovarthy et al.Life Sci 29:2043, 1988
ICRF-187	Alloxanized rats	Prevents diabetes	Radical scavenging	El-Hage et al Res Commun 33:509,1981
DMSO	Alloxanized rats	Prevents diabetes	Radical scavenging	El-Hage et al Res Commun 33:509,1981
Iron Chelator (DETAPAC)	Alloxanized rats	Prevents diabetes	Radical scavenging	Heikkila Experientia 38:379, 1982
Diethyl urea	SZ mice	Partial protection	Radical scavenging	Sandler Diabetologia 23:374, 1982
Nicotinic acid	NOD mice	complete protection	Radical scavenging	Yamada et al Diabetes 31:749, 1982

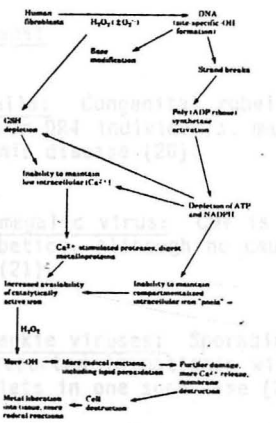


Figure 4. Interrelationship of oxidant-damaging mechanisms (Halliwell, B. A radical approach to human disease. In "Oxygen radicals and tissue injury." Proc. of a Brook Lodge Symposium. Augusta, Michigan. pp. 139-43, 1988.

A. Blocking Putative Environmental Triggers (Table VI).

TABLE VI. POSSIBLE ENVIRONMENTAL TRIGGERS

1. Viruses

   - Mumps
   - Rubella
   - CMV
   - Coxsackie B_4 and B_5
   - Retroviruses with Type C Particle
   - Retrovirus with Type A particles and p73 antigen production
   - Reoviruses
   - ECM

2. Betacytotoxins

   - Nitroso compounds
   - Vacor
   - Others ?

1. The Viruses:

a. Rubella: Congenital rubella will induce diabetes in up to 40% of DR3 and/or DR4 individuals, making it one of the highest risk categories for this disease (20).

b. Cytomegalic virus: CMV is incorporated in the genome of 22% of type I diabetics, although no cause and effect relationship has been established (21).

c. Coxsackie viruses: Sporadic instances of Coxsackie B_4 infection have been reported in patients with ketoacidosis and it has been isolated from islets in one such case (22).

d. Retroviruses: Retrovirus-like particles, Type C, have been identified in pancreatic beta cells of NOD mice and are presumed to be vertically transmitted (23) (Figure 3). Type C particles are found in db/db mice and in streptozotocin-induced diabetes. In the former group they are linked to the insulin promoter and mild hyperglycemia may therefore increase expression of a viral proteins (24). Retroviral P73, a group-specific antigen of the intracisternal Type A particle may share a common epitope with insulin; insulin autoantibodies may in fact be directed against the P73 antigen (25).

Conclusion: Prospects for intervention at this level are poor because of the lack of the understanding of the relationship of viruses to the disease, their mode of transmission, the virtual absence of any real epidemics and the broad spectrum of suspect viruses. Prevention of congenital rubella is an obvious measure that is mandatory for reasons more compelling than diabetes prevention.

2. Nonviral environmental factors: The increased incidence of autoimmune diabetes in Icelandic boys born in the month of October is thought to be related to maternal ingestion at the time of conception of N-nitroso compounds contained in cured mutton (26,27). Vacor, a rat poison used in suicide attempts may produce autoimmune diabetes if all β-cells are not destroyed by the poison.

B. Blocking the Autoimmune Process

The autoimmune process can be separated into three components each of which theoretically could be interrupted.

1. Blockade of antigen presentation:

Antigen presentation can be blocked in at least 2 ways: a) By antibodies to class II antigens at the time that the immune response is initiated. This strategy has been used to prevent experimental allergic encephalitis (28). b) By administration of a nonimmunogenic peptide homologous with the β-cell antigen that can inhibit priming for T-cell responses in mice by competitively outbinding the β-cell antigen, thereby replacing it in the cleft of the MHC molecule (29).

2. Blockade of Antigen recognition. (Figure 5):

1) Mab blockade: Recognition of antigen by T-cells can be blocked a) by antibodies against the L3T4 determinant ($CD4^+$ in humans) on the surface of T-lymphocytes in mice, the helper subset responsible for MHC class II restricted antigen recognition, (30,31). This effectively prevents the appearance of autoimmune diabetes in NOD mice. After 100-150 days of treatment therapy can be discontinued and diabetes does not supervene. $L3T4^+$ $Lyt2^-$ T-lymphocytes are the principle cells that follow macrophages as infiltrators of islets in NOD mice with insulitis (32). Adoptive transfer of $L3T4^+$ $Lyt2^-$ T-lymphocytes will cause insulitis, although in young irradiated NOD mice adoptive transfer of splenic T-cells must include both $L3T4^+$ and $Lyt2^+$ ($CD8^+$ in humans) subsets of T-cells to produce both insulitis and diabetes (33); $L3T4^+$ T-cells recognize antigen in the context of class II MHC molecules while $Lyt2^+$ T-cells recognize antigen in conjunction with class I MHC molecules. $L3T4^+$ T-cells are also thought to serve as lymphokine-secreting helper cells that activate cytotoxic T-cells bearing the $Lyt2^+$ antigens. $L3T4^+$ cells are essential for insulitis and for activating $Lyt2^+$ T cell-dependent β-cell destruction. Insulitis is not prevented by anti-$L3T4^+$ monoclonal therapy but destruction is (34). Thus if beta cells do not express class II antigens *in situ* the requirement for $Lyt2^+$ T-cells for beta cell destruction may reflect the need for a class I restricted cytotoxic T-

PUTATIVE AUTOIMMUNE PATHWAYS OF β-CELL DESTRUCTION (†)

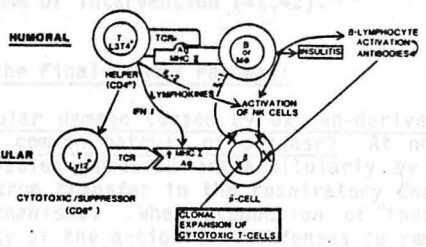


FIGURE 5

cell as the final effector cell. Both Lyt2$^+$ and L3T4$^+$ T-cell subsets are observed around the ducts and vessels of the pancreas. Whereas class I antigens are found on all cells, class II antigens are expressed only on ductal epithelial cells, endothelial cells, fibroblasts, dendritic cells and macrophages. Work in mice (35) and rats (36) points to NK cells as a likely participant in the destructive process. This would explain why NOD mice destroy cultured islets from BALB/C mice, indicating a lack of MHC antigen restriction, i.e. the T-lymphocytes were not interacting with MHC-processed antigens (35).

2) Blockade by a virus: Lymphocytic choriomeningitis virus infects primarily the T-helper subset Thy 1.2$^+$, L3T4$^+$ Lyt2$^-$, abolishing virus specific H-2-restricted cytotoxic T-lymphocyte function throughout the animal's life. LCMV infection initiated in NOD mice at birth or in adulthood abrogated the expected incidence of autoimmune diabetes and blood glucose and insulin levels were normal (37). Moreover transfer of lymphocytes from LCMV-infected NOD mice into uninfected NOD recipients prevented or minnimized autoimmune lesions within islets. Thus viral products could be used to attack or treat specific cells on the basis of tropism of the virus or its proteins. Another virus, the lactic dehydrogenase virus, aborts immune complex disease in lupus-prone New Zealand mice and autoimmune allergic encephalitis in Lewis rats (38,39). LDV is believed to bind to the Ia receptor and replicate in macrophages, presumably interfering with antigen processing (41).

3) Effector function blockade: β-cell destruction mediated by L3T4$^+$ Lyt2$^-$ T-lymphocytes could be brought about by one or more of the following mechanisms: 1) The L3T4$^+$ T-lymphocytes themselves could exert a cytotoxic effect (doubtful), 2) They could stimulate cytotoxic T-cells, 3) They could stimulate B-lymphocytes to produce cytotoxic antibodies, 4) They could activate macrophages or NK cells that would act as nonspecific effectors for β-cell damage or 5) IL-1, TNF and other lymphokines could be β-cytotoxic (18).

a) Blockade with cyclosporine: Cyclosporine clearly is effective in the prevention of autoimmune diabetes in NOD mice (39) and BB rats (40). In man cyclosporine has been restricted to treatment of new onset Type I diabetics in whom 85 - 95% of beta cells have been destroyed at the time of intervention (41,42).

C. Blocking the Final Common Pathway:

Does cellular damage caused by oxygen-derived free radicals constitute a final common pathway of disease? At normal oxygenation reactive oxygen species produced intracellularly by the reaction of oxygen during electron transfer in the respiratory chain are scavanged by antioxidant mechanisms. When production of these free radicals exceeds the capacity of the antioxidant defenses to remove oxidants and of the repair processes to restore oxidized components, disorganization

of intracellular enzymes and membranes will result. The bulk of oxygen reduction occurs in the mitachondrial cytochrome oxidase pathway without the release of partially reduced intermediates. However, several other oxidations result in univalent reduction of oxygen to produce superoxide or divalent reduction to generate hydrogen peroxide. Superoxide can generate hydrogen peroxide by dismutation and hydroxyl radicals and perhaps singlet oxygen by interaction with hydrogen peroxide in the presence of iron. While hydrogen peroxide is not a free radical and singlet oxygen is a spin-altered form of oxygen, these metabolites are included because of their increased reactivity for molecules. The primary defense against damage by these reactive radicals is a team of enzymes superoxide dismutase, catylase and peroxidase. Increased steady state levels of superoxide, whether due to increased production or decreased enzymatic scavanging will result in cessation of growth, mutagenesis and cell death. For a complete review see reference 43.

In addition to antioxidants, quenchers such as glutathione and Vitamins A,C and E protect against damage (Figure 6). Oxidants attack lipids, proteins, nucleotides, NADPH and carbohydrates and impair metabolism, transport, growth, secretion, motility and permeability in various cells. Damage results in edema and inflammation. Neutrophils and other phagocytes such as macrophages can manufacture large quantities of powerful oxidizing agents when they encounter opsonized bacteria or complement fragments C5a or other appropriate stimuli (44). Oxidant production is associated with an abrupt increase in phagocyte oxygen consumption, a metabolic event known as the respiratory burst. Its function is to aid in the destruction of invading microorganisms. Although intended for internal use by the phagocyte, leakage of oxidants into surrounding tissues causes unintended damage therein. The targets of oxidants are shown in Figure 5 and include cellular membranes, DNA, glyceraldehyde-3-phosphate dehydrogenase in the glycolytic pathway, calcium^{2+} reservoirs and mitochondria. The inhibition of glyceraldehyde-3-phosphate dehydrogenase results in reduced synthesis of ATP. NAD depletion also contributes to the reduced glycolytic synthesis of ATP. It has been shown that oxidants damage cellular DNA, activating poly (ADP ribose) polymerase which depletes NAD by converting it to nicotinamide in the course of ADP ribosylation. Cellular DNA is damaged within 20 seconds after exposure of cells to H_2O_2 and repair takes hours. H_2O_2 is the most important oxidant causing damage to DNA in physiologically relevant circumstances. It does so by generating OH^- radicals (45). Neutrophils are activated by platelet activating factor which results in excessive neutrophil adhesion to endothelial cells, superoxide generation and elastase release. PAF inhibitors may therefore have therapeutic role in certain types of injury.

While oxygen oxidants have been implicated in ischemic injury, reperfusion injury and various other forms of trauma, the relationship to immune-mediated damage is less certain. IgG immune complex induced injury depends on the presence of neutrophils whereas IgA related injury does not. Superoxide dismutase has been useful in preventing certain forms of immune complex induced lung damage. In macrophage-dependent lung injury superoxide, H_2O_2 and OH^- radicals are all involved.

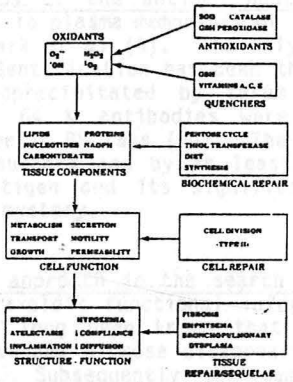


Figure 6: Schematic representation of the sequence of changes in pulmonary oxygen toxicity. The boxes on the left indicate levels of response to oxidants with oxidation of cell components followed by alteration of cell function and organ structure. The boxes on the right indicate defense mechanisms correspondening to each level of oxidant stress.

Free radicals may also act indirectly through cytokines such as TNF (46). TNF release is enhanced by H_2O_2 and inhibited by desferroxiamine. Since TNF and IL-1 have been suspected as initiators of betacytotoxicity in diabetes the foregoing relationships, however tenuous, warrant further study.

IV. Strategies Requiring Identification of the Primary Antigen: If the specific primary antigen is available other strategies can be used.

1) Tolerance to the antigen might be induced prior to beta cell destruction as has been done for myelin basic protein and thyroglobulin coupled to autologous lymphocytes in blocking experimental allergic encephalitis (47) or experimental parotitis (48).

2) Antigen coupled to a lymphotoxic substance (eg. ricin) and presented to T-cells could theoretically destroy the clone of cytotoxic T-cells.

3) Antigen presented to T-cells might permit identification of the clone and the production of specific monoclonal antibodies against it and no other T-cells.

Current status of the antigen hunt in autoimmune diabetology: Surface antibodies to plasma membranes of islet cells (ICSA) were first reported by Lernmark et al (8). The only significant subsequent step towards antigen identification has been the identification of a 64,000 M_r protein immunoprecipitated by serum from new onset autoimmune diabetics (49). 64 K antibodies were subsequently identified in spontaneously diabetic BB rats (50). The work has been confirmed both by the original authors and by at least one other laboratory. The nature of the antigen and its significance in the pathogenesis of diabetes remain a mystery.

A functional approach in the search for the antigen: Our group has attempted to exploit functional information in searching for the antigen. It has long been known that the acute phase of insulin response to intravenous glucose disappears before overt type II NIDDM supervenes (51). Subsequently the same observation was made in autoimmune prediabetes (52). It should be emphasized that in both cases this is a glucose-restricted loss of beta cell function because the insulin response to other secretagogues remains unimpaired. The same observation was subsequently made in the perfused pancreatic remnant of rats previously subjected to 90% subtotal pancreatectomy. The ubiquity of this defect led to the tacit view that it was a nonspecific manifestation of overworked or sick β-cells.

We have considered an alternative possibility, namely that this selective loss of a single beta cell function may be pointing to the locus of the earliest defect, a defect somewhere in the glucose recognition - transport - metabolism - signaling apparatus (Figure 6). Common sense suggests that loss of glucose-stimulated insulin secretion is an obligatory precursor to any hyperglycemic state, i.e., it would be impossible to have steady state hyperglycemia if the beta cell response to circulating glucose were not seriously impaired. In autoimmune beta cell disease the glucose-insulin secretory apparatus may be attacked by antibodies at any point required for its normal function. In nonimmune diabetes mellitus it is postulated that other types of lesions at unknown sites would impair the function of this apparatus. After surgical removal of 90% of the pancreas one would have to assume that most of the glucose-responsive β-cells had been resected.

Our group has studied the glucose-stimulated insulin response in the preovert and overt phases of autoimmune beta cell destruction in BB/W rats (53). The glucose-induced insulin response in isolated perfused pancreata removed from non-diabetic diabetes-prone BB/W rats at 60, 80 and 140 days of age declined progressively below age-matched diabetes-resistant controls while the response to arginine in the same pancreata did not decline in either group (Figure 7).

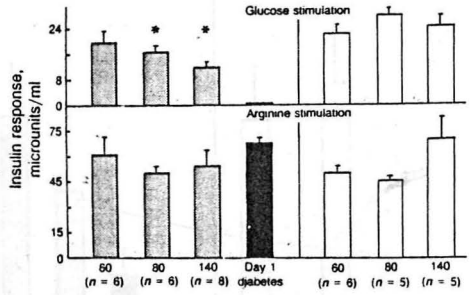


Fig. 7: Baseline levels of insulin and the insulin response to glucose (10 mM) and to arginine (10 mM) in perfused pancreata of nondiabetic diabetes-prone BB/W rats (stippled bars) at 60, 80, and 140 days of age: age-matched diabetes-resistant BB/W controls (open bars); and diabetic BB/W rats (solid bars) on the first day of diabetes (●, P<0.05 vs. preceding age; ●●, P<0.01 vs. preceding age; *, P<0.05 vs. age-matched diabetes resistant group; o, not statistically significant). There was no response to glucose or to arginine on day 14 of diabetes (data not shown); baseline insulin secretion on that day averaged 2.1 ± 0.1 microunits/ml.

During the first 24 hours of overt diabetes (fasting hyperglycemia >199 mg/dl) glucose-stimulated insulin secretion was 0 while arginine-stimulated insulin secretion in the same pancreata was no different than that of age-matched nondiabetic controls (Figure 7 & 8). On the

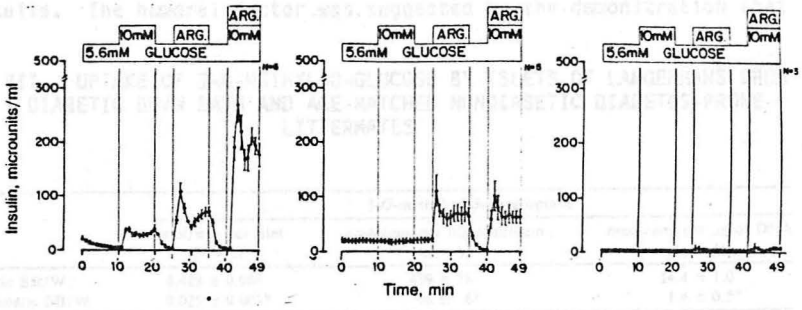


Fig. 8. Insulin levels in venous effluent of perfused pancreata of 60-day-old nondiabetic diabetes-prone BB/W rats (left) and diabetic BB/rats on the 1st (center) and 14th days of the diabetes during perfusion with 10 mM of glucose, 10 mM arginine, and a combination of both (right).

first day of overt hyperglycemia, volume density of beta cells immunostainable for insulin had fallen to 20% of normal (Figure 9). In

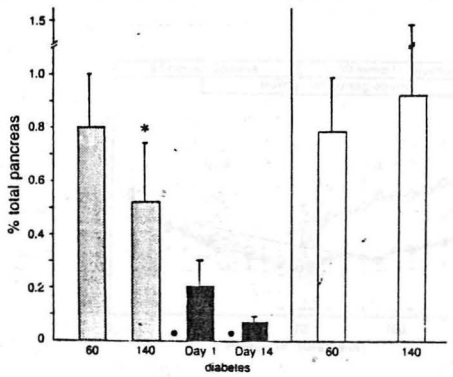


Figure 9: Volume density of endocrine pancreas as percent of total pancreas in diabetes-prone (stippled bars) and diabetes -resistant (open bars) rats at 60 and 140 days of age and diabetic rats (solid bars) on the 1st and 14th days of diabetes. For all experiments, n = 4.●, P<0.05 vs. preceding age; o, not statistically significant.

these islets the glucose transport rate was profoundly reduced compared to age-matched nondiabetic controls (Table VII). Arginine transport was normal. These results could be interpreted in several ways 1) there are no functional differences between beta cells; the loss of glucose response simply represents a progressive loss of functioning β-cells leaving an increasing work burden on an ever-shrinking and increasingly unhealthy population of survivors. 2) A humoral or cellular factor interferes with glucose transport and/or recognition at the surface of beta cells. The humoral factor was suggested by the demonstration that

TABLE VII. UPTAKE OF 3-O-METHYL-D-GLUCOSE BY ISLETS OF LANGERHANS FROM DAY 1 DIABETIC BB/W RATS AND AGE-MATCHED NONDIABETIC DIABETES-PRONE LITTERMATES

	3-*O*-methyl-D-glucose uptake		
	nmol/min per islet (n = 6)	nmol/min per mg of protein (n = 6)	nmol/min per μg of DNA (n = 4)
Prediabetic BB/W	0.419 ± 0.084	429 ± 76	14.4 ± 1.0
Day 1 diabetic BB/W	0.025 ± 0.003*	36 ± 6*	1.4 ± 0.5*

Values are means ± SEM.
*P < 0.005.

immunoglobulins from children with IDDM inhibit glucose-induced insulin release from isolated dispersed and perifused rat islets (Figure 10) (54). Cellular inhibition was suggested as well (55). Johnson, JH et

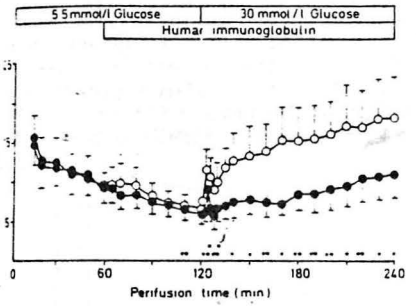


Figure 10: Inhibition of glucose-stimulated insulin release from column-perifused rat islet cells by human immunoglobulin prepared from the sera of Type I diabetic children positive for ICSA. Following a 60-min perifusion period in the presence of D-glucose (5mmol/1) alone, the dispersed islet cells were exposed either to immunoglobulin from healthy control subjects (o—o) or from ICSA-positive Type I diabetic patients (●——●). Mean ± SEM values for six different individuals in each group are shown. *p<0.05, **p<0.025.

al are studying with our group glucose transport kinetics of islets with and without immunoglobulins from new-onset autoimmune diabetic children. Uptake of 3-O-methyl-β-D-glucose by islets is consistent with facilitated diffusion, the very rapid uptake reaching equilibrium in about 60 seconds. (Figure 11) (56). To explore the possibility of

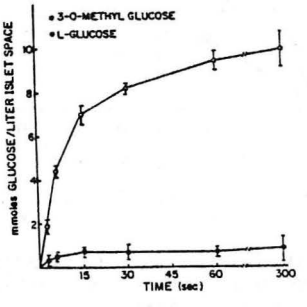


Fig. 11: Uptake of 3-O-methyl-$^{\beta}$-D-glucose by isolated islets of normal rats.

interference by a humoral factor, protein A-sepharose purified IgG from 8 new onset autoimmune diabetic humans and 11 normal subjects were incubated with isolated islets of normal rats prior to measurement of 3-O-methyl-β-D-glucose transport. Significant inhibition was obtained (Figure 12) with islets but not with red blood cells or hepatocytes (Johnson JH et al unpublished observations, 1988). The data must be extended further but these initial results are extremely promising. Interestingly, Graeme Bell's group has just reported that the hepatocyte glucose transporter, which is believed to be closely related to one of the β-cell glucose transporters, has a molecular weight when fully glycosylated remarkably similar to the 64 k antigen of Baekkeskov et al (57). It is localized by immunostaining only to β-cells and hepatocytes and is as good a marker for β-cells as insulin (Orci, L. personal communication).

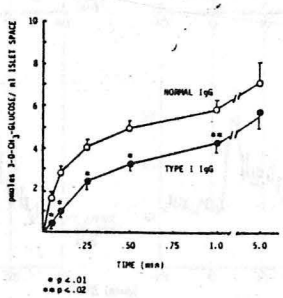


FIGURE 12

3) A third interpretation is that there are functionally heterologous beta cells some of which respond to glucose and some of which do not. If this were true, beta cells still present on the first day of overt diabetes must be in a glucose-unresponsive subset of β-cells. Since they too are rapidly destroyed one must invoke an immunologic cascade, that is, destruction of glucose-responsive cells elicits secondary autoimmune response to shared antigens common to all beta cell subsets. Figure 13 puts the glucose-responsive β-cell hypothesis

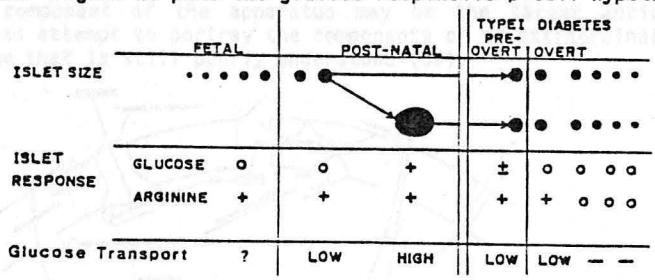


Figure 13: A scheme for the natural history of islet size and function before and after the onset of autoimmune diabetes based on our recent findings. The small round circles represent glucose-responsive islets and the large ovals, glucose-responsive islets. Large and small islets coexist only in the post-natal period. By "post-natal" we mean the period between birth and the onset of autoimmune diabetes.

in a broad ontologic perspective. Figure 14 shows suggestive differences in glucose versus arginine response of large and small islets but these differences were not significant (58).

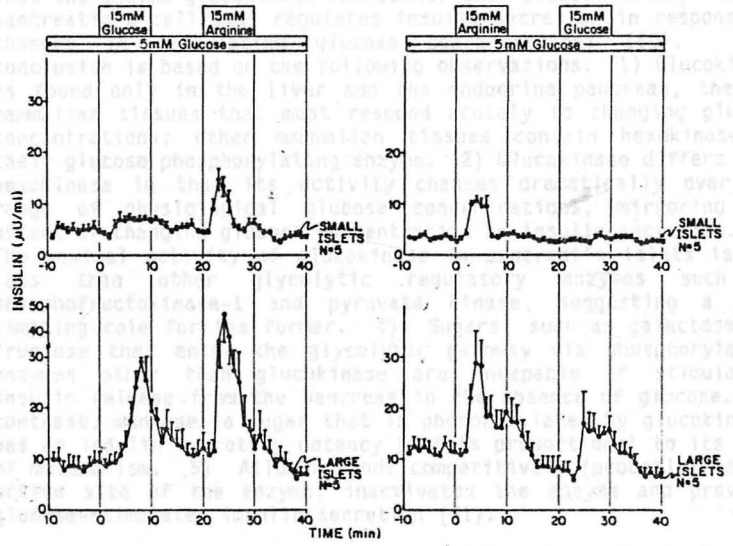


Figure 14: Comparison of arginine and glucose stimulated insulin release in isolated perifused large (>300 μm in diameter) and small (<100 μm in diameter) of normal rats (58).

V. The Glucose-Insulin Secretory Apparatus

If the glucose-stimulated insulin secretion is under immunologic attack, any component of the apparatus may be the target antigen. Figure 15 is an attempt to portray the components of an extraordinarily complex system that is still poorly understood (59).

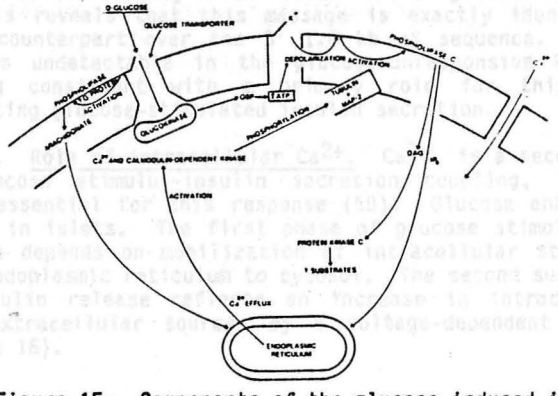


Figure 15: Components of the glucose-induced insulin secretory system.

1. Glucose transporters: Lodish has cloned several islet transporters (personal communication of unpublished information).

2. Islet Glucokinase: Matschinsky and coworkers believe that the enzyme glucokinase represents the "glucose sensor" of the pancreatic β-cell that regulates insulin secretion in response to changes in circulating glucose concentrations (60). This conclusion is based on the following observations: 1) Glucokinase is found only in the liver and the endocrine pancreas, the two mammalian tissues that must respond acutely to changing glucose concentrations; other mammalian tissues contain hexokinase as their glucose phosphorylating enzyme. 2) Glucokinase differs from hexokinase in that its activity changes dramatically over the range of physiological glucose concentrations, mirroring the effect of changing glucose concentration on insulin secretion. 3) The maximal activity of glucokinase in pancreatic islets is far less than other glycolytic regulatory enzymes such as phosphofructokinase-1 and pyruvate kinase, suggesting a rate limiting role for the former. 4) Sugars such as galactose and fructose that enter the glycolytic pathway via phosphorylating enzymes other than glucokinase are incapable of stimulating insulin release from the pancreas in the absence of glucose. In contrast, mannose, a sugar that is phosphorylated by glucokinase, has an insulin secretory potency that is proportional to its rate of metabolism. 5) Alloxan binds competitively (probably) to the active site of the enzyme, inactivates the enzyme and prevents glucose-stimulated insulin secretion (61).

The cloning of cDNA encoding liver glucokinase (62) has allowed characterization of the glucokinase mRNA in liver, and has led to an evaluation of the regulation of its steady-state levels in response to different physiological and developmental conditions in this tissue. Specifically, Northern blot hybridization studies demonstrate a single cross-hybridizing mRNA species in liver induced by administration of insulin or by ingestion of a meal. Recently, Newgard's group et al have shown that the islets of Langerhans contain a larger glucokinase mRNA than found in liver. It is also induced by a fasting/refeeding regimen. Construction of a cDNA library from pancreatic islet RNA has led to the cloning of the islet form of glucokinase. Sequence analysis reveals that this message is exactly identical to its liver counterpart over the 3' 1.8 kb of sequence. Glucokinase mRNA is undetectable in the glucose-unresponsive RIN cells, a finding consistent with a primary role for this enzyme in regulating glucose-stimulated insulin secretion.

3. Role of intracellular Ca^{2+}. Ca^{2+} is a second messenger in glucose stimulus-insulin secretion coupling, its presence being essential for this response (59). Glucose enhances $^{45}Ca^{2+}$ uptake in islets. The first phase of glucose stimulated insulin release depends on mobilization of intracellular stores of Ca^{2+} from endoplasmic reticulum to cytosol. The second sustained phase of insulin release reflects an increase in intracellular Ca^{2+} from extracellular sources by a voltage-dependent Ca^{2+} influx (Figure 16).

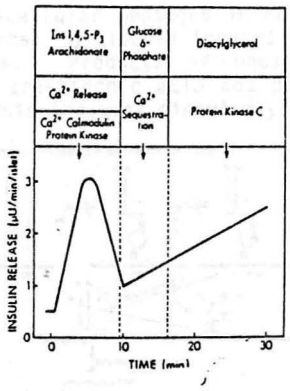


Figure 16: Dynamic representation of intracellular events involved in glucose-induced insulin secretion.

4) Protein kinases:

a) Ca^{2+} and calmodulin-dependent protein kinase: It is present in both membranes and cytosol of islets and its substrates include microtubule associated protein-2, synapsin I, glycogen synthase and α-tubulin (59). Its activity in islets corellate with the first phase of glucose-induced insulin secretion (Figure 15). Alloxan potently inhibits this protein kinase in islets (but not in other tissues) and simultaneously abolishes glucose induced insulin release. (Alloxan also binds to and inactivates glucokinase of islets).

b) Protein kinase C (Ca^{2+} and phospholipid dependent protein kinase). Less is known about this protein kinase in islets. Phorbol esters stimulate insulin secretion from intact islets and activate protein kinase C in islet homogenates. Its substrates in islets remain to be identified.

c) Transduction and messenger systems in glucose-induced insulin secretion. There being no evidence of a membrane receptor for D-glucose, current opinion favors unrestricted transport of D-glucose via one of the several glucose transporters believed to be present in islet cells. A novel still unidentified GTP-binding protein analogous to the G proteins (G_i,G_s) that mediate adenylate cyclase regulation may be involved in coupling to phospholipase C activation. Hydrolysis of plasma membrane polyphosphoinositides serves as a transducing mechanism for

generation of intracellular messages in response to extracellular signals in the islets. D-glucose induces phospholipid hydrolysis in isolated islets. Products of phospholipase A_2 catalyzed hydrolysis include arachidonic acid and product of phospholipase C-catalyzed hydrolysis includes diacyl glycerol (Figure 17). The

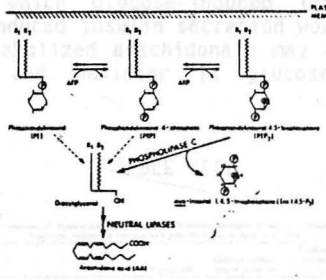


Figure 17: Schematic representation of phosphoinositide metabolism (59).

effects of products of cyclooxygenase products on insulin secretion are still uncertain. However lipooxygenase inhibitors suppress glucose-induced insulin secretion from isolated islets. It is likely that an arachidonate-linked 12-lipooxygenase product participates in the secretory process. 12-HETE production and insulin secretion (Figure 18) appear to be linked in a number of

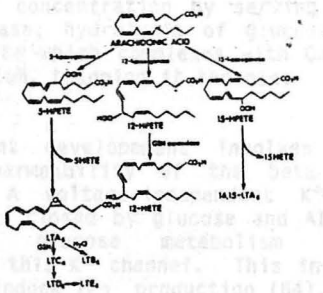


Figure 18: Metabolism of arachidonic acid by mammalian lypooxygenase (59).

ways (63). Inositol 1,4,5-triphosphate (IP_3) is the second messenger for the release of Ca^{2+} clustered in the endoplasmic reticulum. IP_3 accumulation occurs within a few seconds following a stimulus to the plasma membrane. The mobilizing effect of IP_3 is specific both for the target organelles and for the inositol phosphate metabolite. Glucose induces accumulation of IP_3 in

islets with sufficient rapidity and in sufficient concentration to induce Ca^{2+} release from endoplasmic reticulum. But if glucose activation is linked to IP_3 release it would be the first example in which phospholipase C hydrolysis of phosphoinositide is not coupled to receptor activation. This is the only conceptual problem, without which glucose-induced insulin secretion and carbamyl choline-induced insulin secretion would be identical (see Table VIII). Unmetabolized arachidonate may also serve as a Ca^{2+} mobilizing agent and mediator in glucose-stimulated insulin secretion.

TABLE VIII

Comparison of Agonist-induced Responses by Receptor Mediated Events in a Typical Cell with Responses Induced by Glucose or Carbamylcholine in Pancreatic Islets

	Typical cell	Glucose-induced insulin secretion	Carbamylcholine-induced insulin secretion
Agonist-specific receptor	+	−	+
GTP binding protein	+	+	+
PIP$_2$ hydrolysis	+	+	+
Ins-1,4,5-P$_3$ accumulation	+	+	+
Pathways for Ins-1,3,4,5-P$_4$ and Ins-1,3,4-P$_3$ 5-phosphomonoesterase	+	+	+
Ins-1,4,5-P$_3$ induced Ca^{2+} release from the endoplasmic reticulum	+	+	+
Increase in extracellular Ca^{2+}	+	+	+
Physiological response	+	+	+

Glucose 6-phosphate may provide a link between glucose metabolism by glucokinase, the rate limiting enzyme of islet glycolysis, and IP_3 induced Ca^{2+} efflux. Glucose-6-phosphate may be the "off" signal (see Figure 16) which decreases intracellular Ca^{2+} concentration by serving as a substrate for glucose 6-phosphotase; hydrolysis of glucose 6-phosphate yields an organic phosphate which complexes with Ca^{2+} within the islet endoplasmic reticulum, trapping it therein.

A more recent developement involves a glucose-mediated decrease in K^+ permeability of the beta cell and membrane depolarization. A voltage-independent K^+ channel has been identified which is closed by glucose and ATP. This raises the possibility that glucose metabolism regulates membrane depolarization via this K^+ channel. This in turn could activate phospholipase and induce IP_3 production (64).

VI. Miscellaneous Strategies to Prevent Diabetes:

1. Antigen concealment: Insulin excess in BB/W rats for an extended period beginning well before the onset of autoimmune diabetes and extending beyond the end of the window of vulnerability will prevent diabetes in BB/W rats. This has been reported both by insulin injection and by transplanting insulinomas to hybrid BB/NEDH rats. (M. Appel, unpublished

observations.) Although the mechanism is unknown and could involve an effect of insulin on immunocytes, it may well be that the remarkable contraction of beta cell volume during hypoglycemia removes most of the antigenic stimulation during the period of maximum of immune responsiveness. As shown in Figure 19 (C) by in situ hybridization histochemistry, first employed to study islet behavior by Drs. Chen, Alam, Inman and Komiya in our laboratory, the islets are few, small and do not express insulin gene. The idea of preventing diabetes in humans by this strategy is probably impractical because of the many years that would be required to test its efficacy. However an extremely long-acting insulin preparation injected only once or twice a week to produce a similar atrophy of the islets, might be of interest.

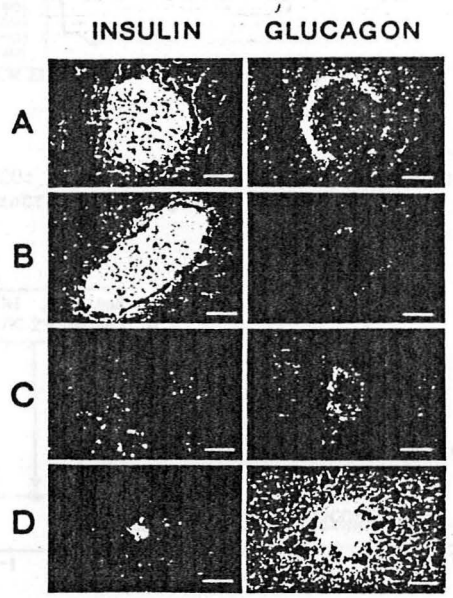


FIGURE 19

2. Overcoming compensatory failure of beta cells; Stimulation of beta cell regeneration: The ability to meet the challenge of hyperglycemia we consider to be the ultimate test of the integrity of the glucose-insulin secretory apparatus described above. If one maintains a hyperglycemic clamp in dogs with 50% pancreatectomy one rapidly induces permanent alloxan-like destruction of β-cells and severe IDDM (65) (Figure 20). In the rat with 50% pancreatectomy, compensation occurs (Figure 21) (Komiya et al).

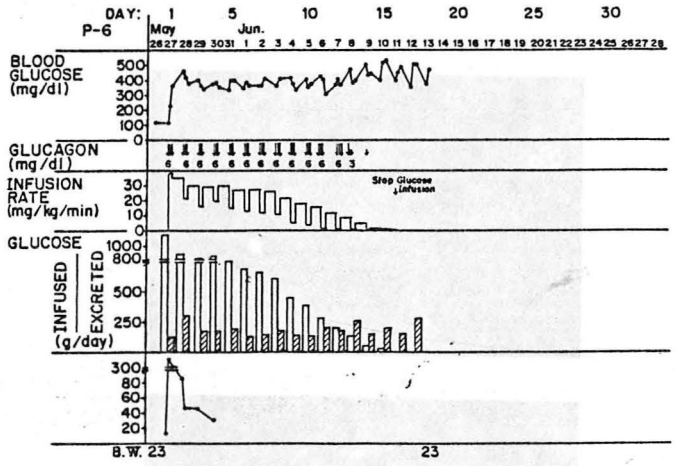


Figure 20: Hyperglycemic clamp rapidly induces IDDM in 50% depancreatized dogs.

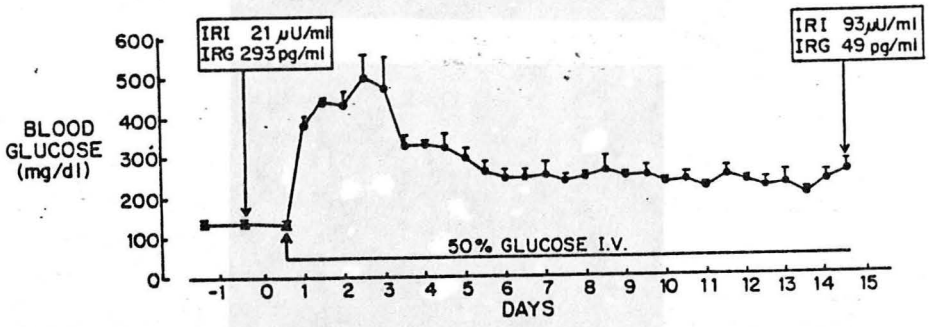


Figure 21: Glucose profile and hormone levels of 50% depancreatized Wistar rats subjected to chronic hyperglycemic clamping for 14d.

Drs. L. Chen, T. Alam and L. Inman have characterized the compensatory response of such pancreata at the molecular and cellular levels (Figure 22). In contrast to the vigorous response at both molecular and cellular levels in normal rats (Table IX), in streptozotocin diabetic animals the surviving β-cells were unresponsive to glucose with inappropriately low insulin gene expression (Figure 19,D). Compensatory

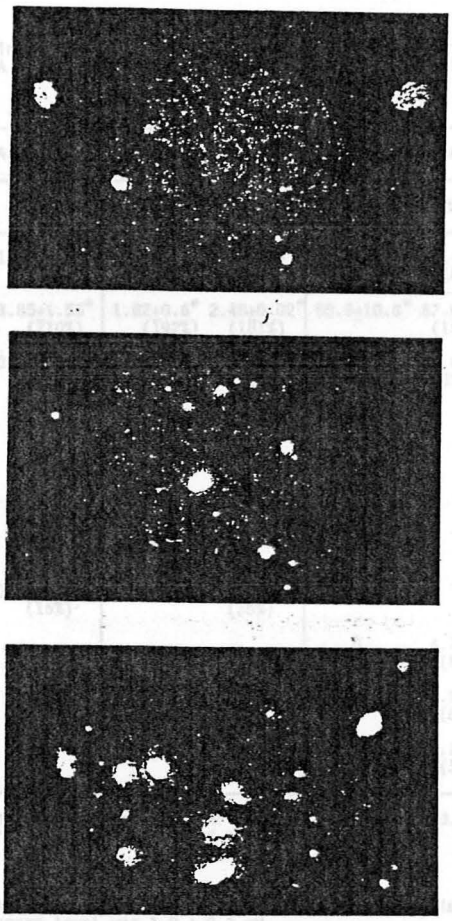


Figure 22: Dark field photomicrograph at low magnification of pancreas sections hybridized in situ with the proinsulin oligonucleotide. Top panel - duodenal half of an intact pancreas of a normal rat. Middle - Pancreatic remnant of a rat 1 mo. after 50% pancreatectomy. Bottom - Pancreatic remnant 1 mo. after 50% pancreatectomy during which time 50% glucose was infused continuously for the final 14 d. Note the "mini-islets" near pancreatic ducts. The bar in figure is 500 µm.

TABLE IX

Total endocrine area per exocrine area (%), number of islets per mm^2 of exocrine area and density of proinsulin and proglucagon mRNA signal per area of beta and alpha cells in various experimental groups.

Group	Endocrine Area (%) (N=12)		Islets/mm^2		(N = 6) mRNA density Proinsulin		(N = 6) Proglucagon	
	Head	Tail	Head	Tail	Head	Tail	Head	Tail
A. Normal controls	0.92±0.4 (100%)	1.83±0.21 (100%)	0.95±0.7 (100%)	1.37±0.34 (100%)	23.2±9.1	57.4±14.7 (100%)		44.1±12.8 (100%)
B. 50% glucose infused X 5d	2.1±0.98* (228%)	3.85±1.55* (210%)	1.82±0.6* (192%)	2.48±0.02* (181%)	58.9±10.0*	87.0±4.3 (153%)		8.2±2.3* (19%)
C. Insulinoma†		0.13±0.01 (7%)		0.73±0.07 (53%)		37.0±31 (65)		63±1.6 (145%)
D. Controls†† for P PX	1.70±0.34 (100%)		0.91±0.30 100%)		59.0±26.2 (100%)		8.2±7.0 (100%)	
E. P PX	3.49±0.45* (205%)		1.43±0.19* (157%)		80.8±13.1* (136%)		43.5±24.7* (531%)	
F. P PX plus 50% I.V. glucose	3.62±1.03* (213%)		2.11±0.29* (232%)		80.2±15.6* (135%)		3.0±5.0* (36%)	
G. Streptozotocin diabetes		0.27±0.15* (15%)		0.48±0.19* (35%)				
Insulin-treated*					0	4.7±3.7* (8.3%)		53.5±17.0 (121%)
Insulin-deprived**					19.8±5.0	49.5±20.0 (86%)	0	79.5±6.1* (180%)
Insulin-deprived + repleted***					8.2±6.7*	18.1±14.6* (32%)		60.1±15.6 (136%)

N = number of pancreatic sections. (Four sections per pancreas were examined. Every islet was counted.) P PX = partial pancreatectomy. (%) = % of control value.

*$p<0.01$

†NEDH rats, a gift of Dr. Michael Appel (Worcester, MA) with subcutaneously implanted radiation-induced insulinoma (RINs). Glucose level was 1.9 ± 0.2 mM.

††Duodenal half of intact pancreata of normal rats.

*Insulin-treated rats (N=3): Daily insulin treatment was continued for 10d and rats were sacrificed. Glycosuria was minimal or absent. At the time of sacrifice plasma glucose level averaged 5.6 ± 1.3 mM and glucagon 294 ± 51 pg/ml.

** Insulin-deprived rats: Insulin treatment was given for 8 days as above but was then stopped for 2-3d and rats were sacrificed after the appearance of ketonuria and glycosuria. At the time of sacrifice their mean blood glucose was 23.7 ± 0.4 mM.

***Insulin-deprived acutely repleted with insulin: Rats received insulin treatment for 8 d. and it was discontinued for 2 d. as above. However, 5 U of regular porcine insulin was administered intraperitoneally 1 h before sacrifice. Plasma glucose declined from 25.9 ± 1.2 mM to 6.1 ± 2.8 mM at the time of sacrifice and plasma glucagon to 248 ± 16 pg/ml.

failure in the surviving beta cells seems to be present in the autoimmune diabetes of BB/W rats at the time of the onset of their diabetes. Normal islets of Langerhans compensate for hyperglycemia in two ways, 1) at the molecular level by increasing expression of the proinsulin gene and decreasing expression of the proglucagon gene and 2) at the cellular level by increasing the number of β-cells by increasing both the size and number of islets.

Potential regulators of compensation by β-cells: A potential regulator of proinsulin gene expression is provided by the recent description of a beta cell specific protein that binds to the regulatory sequences of the insulin gene enhancer. It has been designated insulin enhancer binding factor 1 (IEF1) (66). Perhaps anomalies in this interaction account for part of the inability of diabetic islets, both autoimmune and nonautoimmune, to meet the challenge of hyperglycemia. Compensatory expansion in beta cell volume is brought about both by an increase in the size and number of islets. In situ hybridization permits not only an assessment of biosynthetic activity on an islet-by-islet basis but a better means of detecting islets responding to challenge because of the fact that hypersecreting islets secrete their hormonal product as fast as they sythesize it, which leaves them degranulated and relatively invisible by immunofluorescent staining technics. The factor that controls expansion of the islets is unknown. However, a novel gene expressed in a cDNA library made from regenerating islets has been described by Okamato's group in Osaka (67). It has been found in nicotinamide-treated rats following 90% resection of the pancreas and in aurothioglucose-treated mice. The gene has been termed the "reg" or "regeneration" gene. It is believed that it encodes a 165 amino acid protein of 18,656 kd. A human homologue encoding a 166 amino acid protein has been obtained. The gene is not expressed in insulinomas or regenerating liver, suggesting that its expression is highly specific to the normal replication of pancreata beta cells. Preliminary in situ hybridization by Drs. Ling Chen and Tausif Alam suggest that the reg gene mRNA is located in the exocrine tissue surrounding the islet rather than the islet itself but this must be reexamined. While it must be stressed that the available data does not yet establish a reg gene product as a factor in compensatory regeneration by beta cells, the work of Okamoto's group is consistent with this possibility. If indeed there is a reg factor that controls normal compensatory regeneration of beta cells it might be useful in many forms of diabetes, including immunosuppressed new onset autoimmune diabetes.

More recently Milburn and Newgard at this institution have shown that the "reg" mRNA is also found in normal rat islets. In addition, they demonstrated cross-hybridization of the "reg" cDNA probe with two other mRNA species in islets. Finally, they showed that the expression of these mRNAs is regulated by diet, with powerful induction occurring when animals are fasted for 48 hours and refed for four hours (Milburn,

Hughes, and Newgard, unpublished) (Figure 23). The physiological role and molecular relationship of this putative family of islet genes could involve a role in the compensatory response lost in diabetes. A relationship to the Ohlsson IEF is also under scrutiny.

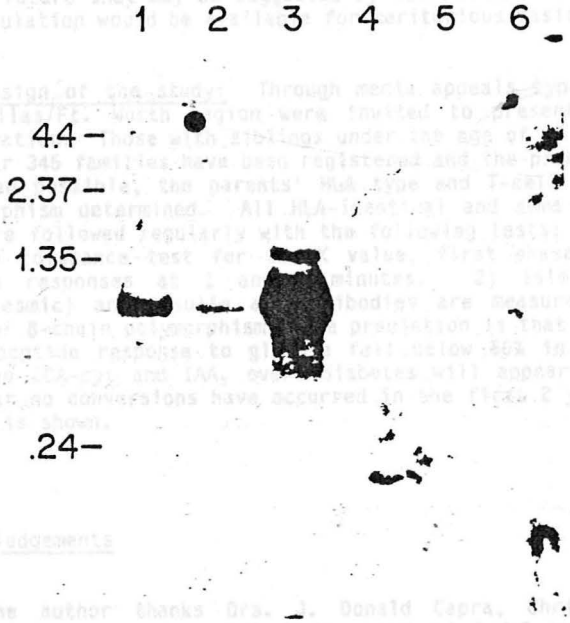


Figure 23: Northern blot analysis with the "reg" cDNA. The lanes contain the following types and amounts of poly A^+ mRNA: Lane 1, 5 µg islet RNA isolated from ad-lib fed rats; Lane 2, 2 µg islet RNA isolated from ad-lib fed rats; Lane 3, 1 µg islet RNA isolated from rats fasted for 48 hours and refed on standard chow for 4 hours; Lane 4, 5 µg liver RNA from fasted rats; Lane 5, 5 µg liver RNA from ad-lib fed rats; Lane 6, 5 µg liver RNA from fasted/refed rats.

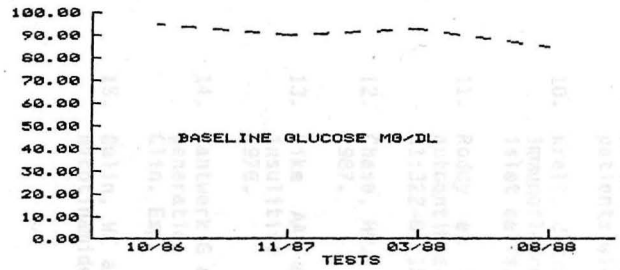
VII. Greenwall Foundation Diabetes Prevention Project: Two years ago, the Greenwall Foundation of New York funded a program in Dallas designed to help in the prevention of diabetes. The program was implemented only after considerable debate concerning wisdom of commiting funds for a goal that was then and is not now clearly attainable. The decision to proceed was based on the following considerations: 1) Identification within the community of those children at maximum risk for autoimmune diabetes would permit prompt

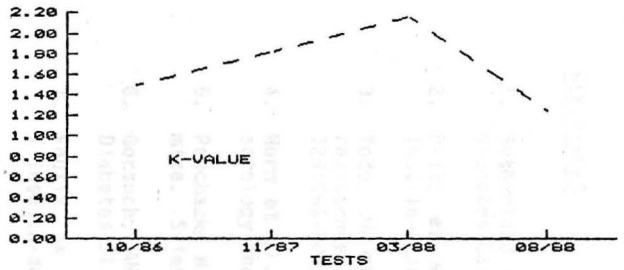
intervention if and when a sufficiently safe and effective agent should become available. 2) Until then prospective studies of a high risk group would generate in reliable criteria for predicting if and when overt diabetes would supervene. (A control group for testing an eventual intervention with an innocuous agent might be precluded on ethical and political grounds.) 3) Serum and DNA from probands, HLA-identical and HLA-nonidentical siblings would be banked for studies of the future that may be suggested by new scientific discoveries. 4) The population would be available for meritorious basic studies.

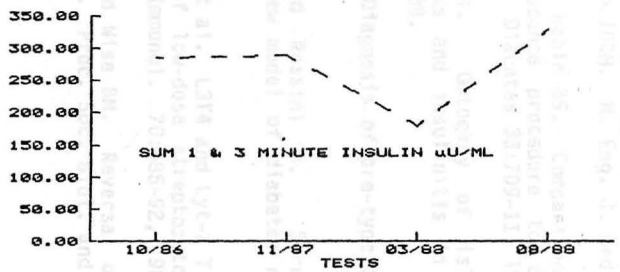
Design of the study: Through media appeals type I diabetics in the Dallas/Ft. Worth region were invited to present themselves for registration. Those with siblings under the age of 25 were registered. Thus far 345 families have been registered and the probands', siblings' and when possible, the parents' HLA type and T-cell receptor β-chain polymorphism determined. All HLA-identical and some HLA-nonidentical sibs are followed regularly with the following tests: 1) Intravenous glucose tolerance test for the K value, first phase insulin and C-peptide responses at 1 and 3 minutes. 2) Islet cell antibody (cytoplasmic) and insulin autoantibodies are measured. 3) T-cell receptor β-chain polymorphism. The prediction is that when the insulin and C-peptide response to glucose fall below 50% in the presence of positive ICA-cyt and IAA, overt diabetes will appear within 2 years. Thus far no conversions have occurred in the first 2 years. A typical record is shown.

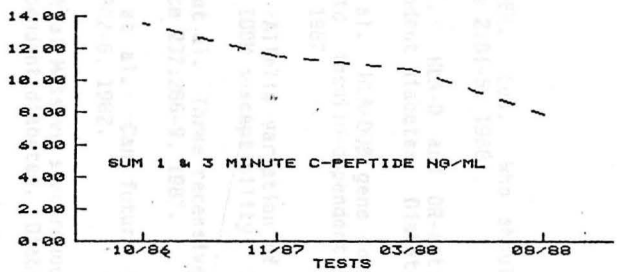
Acknowledgements

The author thanks Drs. J. Donald Capra, Chris Newgard, J.H. Johnson, Peter Lipsky, and Marie Hoover for helpful comments and Teresa Autrey and Vicky Robertson for excellent secretarial assistance.









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