The Contributions of Epidemiology to the Understanding of the Etiology of Insulin-Dependent Diabetes Mellitus

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Why does insulin-dependent diabetes mellitus occur? What are the factors that account for the remarkable variability in the age and timing of onset of clinical disease? Are there adverse environmental factors that may stimulate early disease expression? Conversely, are there environmental influences that, if enhanced, may prevent or delay expression? Can insulin-dependent diabetes mellitus be prevented or cured?

The advances in knowledge of the multi-factorial nature of the etiology of insulin-dependent diabetes mellitus (IDDM) over the past two decades is one of the highlights of modern medicine. We now know that IDDM is not genetic in the usual Mendelian sense. Rather, several genetic alterations, most but probably not all of which are located on chromosome six within the major histocompatibility complex, result in an increased likelihood of β-cell damage (1–10).

The mechanism of β-cell damage and destruction is autoimmune. Substantial evidence suggests that antigens released from the β cell are seen as foreign proteins by the macrophage or antigen presenting cell (APC) that presents the altered antigen to a highly specialized HLA-linked receptor in a helper T cell. This process then initiates an active cellular and humoral response involving antibody production and lymphokine release. The final biochemical mediator of this toxic process is probably nitric oxide (11–16).

**EPIDEMIOLOGIC VARIABLES THAT PROVIDE INSIGHT INTO IDDM ETIOLOGY**

The original epidemiological inquiries set out to define the magnitude of the problem of diabetes in our society by determining incidence and prevalence rates. As these studies began to appear in various parts of the world, comparisons led to the surprising observation that this disease did not occur uniformly over time or space.
Attempts to define these curious observations further led to a variety of interlocking studies looking closely at geographic variation, secular trends, evidence of migrant drift, and epidemics. These studies have been extraordinarily important in defining new directions for research for clinical and basic scientists, geneticists and molecular biologists (17).

GEOGRAPHIC DISTRIBUTION OF IDDM

A conference on the epidemiology of IDDM held in 1983 identified clearly, and for the first time, the remarkable variation in expression of this disorder in various parts of the world (18). Many follow-up studies have confirmed this, with the finding of a high incidence of IDDM in the Scandinavian countries, intermediate levels in much of the West, and very low levels in the Far Eastern countries, including Japan, China, and Korea (19–25). This distribution is extraordinarily similar to that seen in the worldwide distribution of atherosclerosis and of morbidity and mortality from coronary artery disease. The latter is closely linked to the ingestion of saturated fat and cholesterol and to the dietary intake of dairy products. Conversely, atherosclerotic risk and IDDM incidence are inversely correlated with the content of naturally occurring antioxidants in the diet, traditionally high in the diet of the Orient and quite low in that of individuals living in Western countries (26–35).

Genetic studies carried out by our laboratories, in collaboration with many other investigators, have documented a remarkable parallel between the population distribution of the homozygous non-aspartic-acid status at codon 57 of the HLA-DQ β antigen and the national incidence of IDDM. That is, in many of the countries studied to date, the incidence of diabetes, either high or low, is directly correlated with this diabetes susceptibility gene distribution. The addition of the HLA-DQ α variation with arginine found at position 52 further defines diabetes susceptibility. However, it appears that somewhat less than one individual in 20 carrying gene alterations that increase susceptibility to diabetes will in fact develop overt diabetes, so environmental factors are still very important for actual disease induction (36–38).

SECULAR TRENDS IN IDDM INCIDENCE

If the incidence of IDDM were exclusively dependent upon the frequency of the diabetes susceptibility genes within the population, one would expect that the annual incidence would be remarkably similar year after year, reflecting the absence of or only very slow change in the distribution of diabetes-related genes in the population over time. That, in fact, is not the case. There is now substantial evidence, based on carefully constructed registry data extending in many countries over the last 20–40 years, that there have been substantial increases in IDDM incidence in many locations, particularly in the Scandinavian countries. The incidence of diabetes among Finnish children has almost doubled during the past 20 years. Similar observations
have been made in Norway, Denmark, and Sweden. In our own experience in Allegheny County, Pennsylvania, we had observed an increase of approximately 1% per year in the incidence of IDDM until a more recent acute acceleration (39-44).

Probably the most dramatic evidence of a secular increase is given by the experience on the Italian island of Sardinia. The incidence of IDDM in Sardinia currently approaches 30/100,000 per year, just below that in Finland and approximately five times greater than the incidence of diabetes in Italian children living on the mainland of Italy. This high incidence is explained by the finding of a very high frequency of homozygous non-aspartic-acid genetic status in the population of Sardinia (36,37,45).

However, a recent review of the earlier incidence of diabetes in Sardinia, not yet published or verified, suggests that the attack rate for this disease among Sardinian school children 20–30 years ago was in the neighborhood of 4–5/100,000 per year, the incidence rate now seen in the mainland of Italy. Obviously, there has been no significant increase in the genetic susceptibility to diabetes in Sardinia over the past quarter of a century. The increase in attack rate, if it is accurate, must reflect significant environmental changes that have occurred during that period, almost certainly associated with industrialization, changes in lifestyle patterns, and changes in dietary habits.

DIABETES EPIDEMICS

While secular changes in IDDM incidence over a period of several years stress the validity of a totally genetic-immunologic explanation for IDDM, acute increases in incidence, referred to by some as diabetes epidemics, truly defy genetic explanation and must be viewed as a consequence of acute environmental changes. There are now several well-documented examples of abrupt increases in IDDM incidence followed by a return to the baseline incidence in several countries, including Poland, Latvia, and England (46–50).

Our own recent experience in Pittsburgh is particularly important in this regard. Both in our Children's Hospital and in Allegheny County registry we have continued surveillance, both retrospectively and prospectively, covering approximately 40 years. While we have documented a slight and just statistically significant incidence increase of about 1% per year from 1965 through 1985, we have recently observed a major increase in incidence during the interval 1985–1989. This increase in incidence is seen predominantly in males, in younger children, and in African-Americans. It is statistically correlated with chicken pox epidemics, with a lag phase of between 2 and 3 years. Although we do not suggest that chicken pox is a primary etiologic factor in IDDM, we strongly believe that our recent observations of a highly significant increase in incidence among children in western Pennsylvania indicates changing environmental stressors. Our documentation of increasing frequency of IDDM in younger children is consistent with the clinical experience of many pediatric diabetologists in various parts of the world and again suggests environmental stressors appearing at earlier ages (51).
MIGRANT STUDIES

Although classical prospective migrant studies have not been carried out in IDDM, there are a number of important and valuable observations that strongly suggest that susceptibility to IDDM may be altered by changes in geography and life style. These conclusions result from the study of individuals of a particular geographic or nationality group living at a distance from their natural home, within a population that has an IDDM incidence significantly different from that of the study population. Such observations have documented that Japanese living in Hawaii have an IDDM incidence approximately five times greater than Japanese children living in their homeland. Similarly, there is an approximate doubling of the incidence of French and Italian children in Montreal when compared with the incidence in France and Italy (52,53). The incidence of IDDM in Indian children following migration from South Africa to England showed a dramatic increase from very low levels to that compatible with children in England (54).

These studies and others strongly support the thesis that environmental factors may either increase or decrease the expression in diabetes in susceptible individuals. The migrant study observations have been appropriately criticized by the absence of experiences documenting reduced risk in certain populations. However, the natural migrant drift in the past several decades is from east to west, from developing countries to Western countries, from poverty to affluence, and from tropical to temperate zones. All of these moves in general involve migration of individuals from countries at lesser risk for the development of diabetes to countries where the incidence rate is higher.

EVIDENCE FOR ENVIRONMENTAL FACTORS IN THE ETIOLOGY OF INSULIN-DEPENDENT DIABETES MELLITUS

Environmental factors may play one of several possible roles in processes involving $\beta$-cell destruction. At one extreme, there are undoubtedly cases of diabetes occurring as a direct result of ingestion of $\beta$-cytotoxic agents, with no intervening genetic susceptibility or autoimmune mechanisms. At the other extreme, $\beta$-cell destruction results exclusively from genetically mediated autoimmune processes that are internally triggered without interaction with specific environmental stimulants. It seems likely, however, that most cases of IDDM fall in a middle ground where environmental factors, rather than being directly causative, act as stimulants or provocateurs of the immune system. Further it is my belief that, in the great majority of cases, IDDM does not result from a single environmental insult leading to a relentless autoimmune destructive process but rather that there are multiple hits from the environment, resulting in waxing and waning of the inflammatory process. What is the evidence? (55–57,10).

Four general environmental categories have been proposed as potential causative or provocative agents in the expression of IDDM. These include infectious agents,
environmental toxins, nutrient factors, and physical and emotional stress. An additional interesting observation is that children who experience maternal-child blood group incompatibility are apparently at a fourfold increased risk for IDDM (57). Although it is likely that genetically susceptible individuals finally develop diabetes after multiple environmental insults, possibly involving adverse experiences from each of the four general environmental categories, these categories are discussed separately below.

ANIMAL STUDIES

In medicine, we have traditionally looked to animal models for insights and understanding of disease processes that cannot be elucidated fully in the human subject. Animal research has played a major role in the study of diabetes for well over 100 years. Strangely, those stringent advocates of an exclusively internally mediated autoimmune disease process accept the evidence of disease mechanisms from genetically susceptible models as important to the human condition. The animal experience can be summarized as follows.

1. Toxic agents, for example alloxan and streptozotocin, can regularly induce β-cell destruction and diabetes in both genetically susceptible and normal animals. Overt diabetes can be rapidly induced by a large dose of streptozotocin by direct toxic action of the drug, or probably by autoimmune mechanisms, using small doses of the toxin repeatedly. (This is reminiscent of the human situation with the drug Vacor® in which the acute ingestion of a large dose results in rapid β-cell destruction, while chronic ingestion of small doses appears to induce autoimmune β-cell destruction) (58).

2. Various infectious agents can induce β-cell destruction in diabetes-susceptible and non-susceptible animal models. The identified agents include encephalomyocarditis (EMC) virus, Mengo virus, and coxsackie B virus variants. The β-cell destructive process may be either directly cytolytic or may cause the induction of β-cell damage leading to eventual autoimmune destruction (59).

3. Environmental manipulation will alter the expression of diabetes in genetically susceptible animal strains. The BB rat and the NOD mouse strains are immunologically defective animals that are at markedly increased risk for the development of β-cell destruction or overt diabetes, mimicking human IDDM in many respects. Environmental manipulation, particularly dietary alteration, can result in marked variation in the expression of disease in both of these animal models (60–62). This is particularly true in relation to cow’s milk protein and/or beef in the animal chow. Adding bovine serum albumin or whole milk or beef protein to the chow increases the incidence of diabetes, while replacing the animal chow with synthetic proteins and amino acids reduces the expression of diabetes to near zero. This evidence strongly suggests that cow’s milk protein or beef has a specific provocative effect on the immune system of these animals, resulting in progressive β-cell destruction.

4. Various immune suppressive or modulating strategies introduced well before
β-cell damage will reduce the expression of diabetes in genetically susceptible animal models. These observations provide cause for optimism that immunologic intervention before the onset of diabetes may prevent disease in the human situation (63–66).

INFECTION DISEASES

Several viral infections have been associated with the development of IDDM, on the basis either of epidemiologic surveys or of individual case reports. The association between mumps infection and IDDM has been known for 100 years, and there have been periodic case reports of diabetes occurring in individual cases following mumps infection a few weeks previously. Coxsackie infections, particularly serotypes B3 and B4, have been associated with IDDM both in disease surveillance statistics and in a few isolated but highly important cases. Cytomegalovirus (CMV) has also been associated with carbohydrate intolerance and diabetes, and insulitis has been identified in the pancreas of infants dying with disseminated CMV infection. The best documented association between a specific viral infection and IDDM is found in the congenital rubella syndrome. Rubella infection, acquired by the fetus as a result of transplacental passage of the virus, results in a generalized rubella infection that may have devastating effects on the infant. In the great majority of cases, the infection burns out soon after delivery. However, it leaves behind an autoimmune destructive process that over a period of 5 to 20 years will result in β-cell destruction and overt diabetes. HLA studies indicate that diabetes eventually develops in those children with rubella syndrome who have a genetic predisposition to disease. The eventual development of diabetes may approach 100% in those individuals who are HLA DR3 and/or DR4. No DQ α or β studies have been reported on this group of patients but they should be done. The congenital rubella syndrome-diabetes association is strong evidence of a virus-induced autoimmune phenomenon leading to β-cell destruction. The recent studies of Yoon et al. documenting an increased frequency of CMV viral fragments in the genome of children with IDDM suggest that such patients either acquired CMV infections very early in life, which then initiated a β-cell destructive process, or that IDDM may be associated with persistence of the infection (67–74).

ENVIRONMENTAL TOXINS

It is well documented that several chemical agents have β-cell destructive properties. Streptozotocin, the agent used to induce diabetes in laboratory animals, has been used to destroy the pancreas in humans with severe hypoglycemia resulting from inoperable islet cell malignancy. The rodenticide Vacol® is highly toxic to the β cell, resulting in an acute induction of diabetic ketoacidosis following oral ingestion of a large dose (75,76). Inadvertent chronic small-dose ingestion, as apparently occurred in Korea when the rat poison was mixed with feed, is strongly reminiscent of low-dose streptozotocin-induced autoimmune destruction in laboratory animal models. Nitrosamines, produced when foods are cured by smoking techniques, have
been implicated in cases of IDDM in Iceland (77,78). The possibility that the widely used technique of grilling or barbecuing beef may be a factor needs investigation. Furthermore, almost nothing is known about the health hazards of a large number of industrial environmental contaminants as they enter our food and water supply. The recent finding that the apparently benign inert silicone breast implant may be associated with autoimmune disease should raise increasing concern about the health hazards of so-called biochemical advances in our society.

**NUTRIENTS**

There is increasing evidence that nutrients may play either a protective or a provocative role in the development of IDDM. Of special interest is the accumulating evidence that the early introduction of cow's milk protein may be an important factor in the later expression of diabetes in genetically susceptible infants (79–81).

The recent studies of Karjalainen et al. (82) have focused attention on the possible relationship between early ingestion of bovine serum albumin and the later development of diabetes in genetically susceptible individuals. These investigators found that antibody to a specific fragment of bovine serum albumin, referred to as Abbos, is present in 100% of newly diagnosed diabetic Finnish children, while it is hardly ever present in non-diabetic children or normal adults. The Abbos epitope is immunologically cross reactive with a β-cell autoantigen, P69, which may, by virtue of molecular biological mimicry, explain why ingestion of this compound can induce β-cell inflammatory responses (82). Recent attempts in another laboratory to confirm the Abbos finding have been unsuccessful (83).

It is well documented that the antioxidant potential of the islet tissue of the pancreas is inherently deficient when compared with most of the other tissues and organs of the body. This means that the islet tissue is at increased risk of free radical damage. The balance between oxidant and antioxidant concentrations within the body fluids and tissues is a delicate one. Free radical excess or antioxidant deficiency will result in disease. Nutritional intake is an important factor in this balance, and antioxidant ingestion is achieving increasing prominence in the prevention of human disease. It is possible that the lower content of naturally occurring antioxidants that characterizes the Western diets may be inadequate to neutralize free radical accumulation, thus increasing the likelihood of β-cell damage and clinical diabetes. Conversely, the considerably lower incidence of diabetes in Oriental countries may be at least partially related to a high dietary intake of antioxidant compounds.

**STRESS**

The relationship between acute emotional stress and the induction of hyperthyroidism has been widely accepted among endocrinologists for many years. The anecdotal association between the two is convincing. Only recently has research begun to
explore the complexities of the interrelationship between the endocrine and the immunologic systems. It is now quite clear that alterations in the hypothalamic-pituitary-adrenal axis, through the release of both ACTH and adrenal steroids, can result in major alterations in the effectiveness of the immunologic surveillance system. While the anecdotal association between emotional trauma and the onset of IDDM is not as strong in diabetes as it is in hyperthyroidism, there are, nonetheless, several studies suggesting such a cause-and-effect relationship (84–87).

THE FUTURE

The possibility of curing or preventing insulin-dependent diabetes mellitus rests solely on the continued accumulation of knowledge into causation. This includes a more complete understanding of the several genetic alterations that may be associated with either increasing risk for diabetes or protection against β-cell inflammation. Much more must be learned about the normal immunologic system and the alterations that occur in individuals who later develop IDDM. There is increasing focus on the development of new immunosuppressive or immunomodulatory interventions that may alter the immune reaction and thus prevent β-cell destruction. In addition, efforts must continually be directed toward further defining environmental risk factors and toward the development of epidemiologic methods for reducing these risks, with a major collaborative effort involving basic and clinical scientists. One can look to the future with optimism (88–90).

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Dr. Cowett: Would you discuss the antioxidants?

Dr. Drash: I am very interested in antioxidants, as are several other people in the field. We need to look more toward the use of antioxidants as preventives. We also need to examine the distribution of antioxidants in the diet, since in the Western world the frequency distribution of diabetes is negatively correlated with the natural occurrence of dietary antioxidants. So I would raise the question with you as to whether the level of naturally occurring dietary antioxidants may be a factor in the initiation of β-cell damage in a genetically predisposed individual. We should consider the pediatric experience; the first two years of life is the only time when nearly everyone is given vitamins, and diabetes is very uncommon in this period. Could this be a factor in the low incidence of diabetes in early life? Should we consider vitamin supplementation on a routine basis later in life?

Dr. Bergman: All sorts of factors may challenge the β-cell. For example, insulin sensitivity diminishes if you take an Indian population and move them to Leicester, so past history cannot be ruled out. Insulin resistance develops during puberty and adolescence. Infectious diseases also cause insulin resistance.

Dr. Drash: I agree that there are multiple mechanisms for β-cell damage, and I personally feel that this is a “multiple-hit” disease. I think it is very rare for an individual to be exposed to something that sets off the whole process which then progresses inevitably to disease. I think there is a waxing and waning of the process leading eventually to damage, but if the precipitating factors don’t occur often enough the disease does not develop. I think milk is very important. We have just come to some firm conclusions about the danger of early introduction of cow’s milk protein. I believe that in some individuals this is a significant factor. However, I don’t think we have any idea what proportion of the childhood population gets β-cell damage following early exposure to cow’s milk protein.

Dr. Assan: If you adjust for the DR3 antigen from the Finnish data and the data from all the European countries, can you assess the part that is due to the DR3 gradients from the north to the south of Europe and can you then delineate better the possible part played by nitrosamines or cow’s milk, for instance? In a particular community, do DR3 individuals who drink cow’s milk become diabetic more frequently than the others?

Dr. Drash: The study that will answer this is, we hope, about to get under way in the coming months, led by a group in Canada. This will examine newborns in families in which there are diabetics, and in which the parents have been definitively HLA typed. The newborns will also be typed, so one will be able to say which are at very high risk. They will then be randomized to an exclusively breast-fed group or, if the mother does not want to breast feed, to a standard formula preparation group or to a Nutramigen group. The study is still on the drawing board but it really needs to move forward to define the relative roles of milk factors and high genetic sensitivity.

Dr. Dakou: Overall, Greece has among the lowest incidences of diabetes in the world, but the incidence is quite high in Athens and the Piraeus, almost double that in towns of less than 10,000 inhabitants. This supports an environmental cause because we don’t think there is a different genetic population in Athens from that in the cities of less than 10,000 inhabitants. In certain other areas of Greece, we also see a localized high incidence of the disease. We do not find a seasonal variation. What I would like to ask is whether you have broken down your data on “bad genes” according to age of onset. In the old fashioned HLA-ABC studies we have done in our population we found that children with onset of diabetes below the age of 4 years had almost double the risk if they had bad genes.
**Dr. Drash:** Yes, we are currently working on that precise question. Early analysis suggests that individuals with early onset do have more "bad genes." An important paper has appeared in *Diabetes Care* from Switzerland. This is a study by Schoenle EJ et al. from Zürich based on military records. In Switzerland, all males have to do military service. Schoenle has been able to go back about 15 or 20 years in this population and determine the development of diabetes, and he has obtained data that may be similar to the Greek data. The first analysis shows that individuals growing up in urban communities in larger cities had a much higher incidence of diabetes than those growing up in rural communities (about twice as high). This supports the view that stress and the hurly burly of lifestyle in the city are factors in the pathogenesis, which might be consistent with your data. Because Schoenle had the military dataset, which stays in the system until the person dies, he was able to examine the continued expression of diabetes over time. He has found that by 40 or 45 years of age there is essentially an equal distribution of the incidence of type 1 diabetes in urban and rural areas. What the data seem to be saying is that if you are genetically susceptible the disease will catch up with you eventually, but if you live in a stressful environment the expression of the disease may occur earlier. This is very important information that I think will soon be verified by other groups (1).

**Dr. Zoupas:** How can you measure stress?

**Dr. Drash:** This is obviously a difficult area for quantitative data. However, we have accepted for many years that stress can provoke the expression of hyperthyroidism, for example, and we know a great deal more now in terms of the hypothalamic-pituitary-adrenal relationship and its interaction with the immunologic system. So certainly there is good evidence of an effect of the endocrine system on the immunologic system. How to quantitate this is a different matter. I think Fuller reported several years ago on the development of diabetes after parental death or divorce, both stressful life events, and there are several papers on this subject in the literature. But I agree with you, it is very difficult to quantitate.

**Dr. Bartsocas:** Athens has a particularly high incidence of diabetes compared with the rest of Greece. For example, in Macedonia the incidence is only 4.5 per 100,000, while in Athens it is almost 9.5 per 100,000. My second point is that DR2 does not protect Greek people from developing IDDM since the same incidence of HLA-DR2 exists in the IDDM population.

**Dr. Bottazzo:** I wish everything were as clear cut as the Swiss data, but it isn't. For example, a recent paper from Yorkshire, UK, which appeared in *Diabetologia*, showed exactly the opposite (2). Apparently in that area, there are more cases of IDDM in rural than in urban areas. We have also looked at this aspect in Sardinia (Songini, personal communication) and we found there is no difference between rural and urban areas. Although stress may be important, it should not be assumed that city dwelling is necessarily a risk factor for IDDM.

**Dr. Scott:** It is very difficult to determine what people eat, and I don't think we should be too ready to correlate certain dietary habits with the risk of type 1 diabetes.

**Dr. Drash:** I agree that it is a very difficult task. I think the reason it has not been done before is that it is so difficult to characterize eating habits in a way that is precise enough to obtain scientific data.

**Dr. Alivisatos:** Would you like to comment on the risk of developing diabetes in small-for-dates babies (babies with intrauterine growth retardation)?

**Dr. Drash:** I am not sure that this has been adequately looked at. We know that the very
small-for-dates baby is at risk of transient diabetes in the newborn period. This is really a reflection of intrauterine starvation. I don’t know whether true diabetes is seen with greater frequency in that population.

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