The Etiology of Type 1 Diabetes:

Nature and Nurture

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The development of type 1 (insulin-dependent) diabetes mellitus requires genetic susceptibility (1). This disease is thought to be the result of an organ-specific autoimmune process in which the immune system reacts abnormally against the body’s own insulin secreting β cells in the pancreatic islets. Several elements have been studied in the search for causes of type 1 diabetes, such as diabetes-related genes, immune factors, and environmental factors [mainly diet, viruses, and diabetogenic chemicals; for reviews see (1–5)]. The exact identity of the factors involved in the etiology of type 1 diabetes in humans is difficult to ascertain because the prodromal period is measured in years (6), and it is not yet possible to predict who in the general population will become insulin dependent. Only 10% of all patients with type 1 diabetes have a first-degree relative with the disease. If there is no family history of diabetes, the risk of developing type 1 diabetes is about 1% by the age of 50 years (7).

As a result, most studies have been carried out in first-degree relatives of patients with type 1 diabetes. In this group, the risk of developing the disease is increased and depends on which relative had the disease (7). It ranges from ~1–2% for mother and ~6% for father to 10% by 50 years if a sibling had the disease. The highest risk is reported in identical twins, 34–50%. The lack of concordance in identical twins has been taken as evidence that environmental factors play a role in the etiology of the disease.

Not all genetically susceptible people develop diabetes and, despite much greater genetic homogeneity, neither do all diabetes-prone BioBreeding (BBdp) rats or non-obese diabetic (NOD) mice. However, in all three species it is genes in the major histocompatibility complex (MHC) region that impart the main risk of developing diabetes. Moreover, there is marked geographic variation in diabetes incidence in countries around the world, and this is also evident in colonies of BBdp rats and NOD mice. The highest incidence of human type 1 diabetes occurs in Finland, the Scandinavian countries, and certain “hot spots” such as Sardinia and Prince Edward
Island, Canada, while the lowest incidence is seen in China, Japan, Korea, and Macedonia (8). There are also indications that migrant populations, such as the Japanese who moved to Hawaii and the French Canadians in Montreal, have an increased diabetes incidence compared with their country of origin (9). There have been reports that the incidence of diabetes is increasing in several areas, and there may even be "epidemics" (10). All these data are generally taken as further indication that environmental factors are involved.

The purpose of this review is to discuss briefly the evidence of a role for both genetics and the environment, with a particular emphasis on dietary factors, in the etiology of type 1 diabetes in humans and in the two spontaneous animal models of this disease, the BBdp rat and the NOD mouse.

NATURE

Susceptibility Genes for Type 1 Diabetes

Type 1 diabetes is not inherited in a Mendelian fashion, rather, it is the susceptibility to develop type 1 diabetes that is inherited (11). It is a polygenic disease and, as with many autoimmune diseases, susceptibility or resistance is closely associated with polymorphism in the class II genes of the major histocompatibility complex (MHC) (12).

In Caucasians, type 1 diabetes was originally found to be associated with MHC class I HLA-B8 and HLA-B15. The focus then shifted to the MHC class II HLA-DR locus, which showed a strong association of DR3 or DR4 with the disease; of those who become diabetic, approximately 90% have DR3 or DR4 genes. The presence of DR2 was shown to be protective. In 1987, an important discovery was reported by Todd et al. showing that HLA-DQ alleles with aspartic acid at position 57 of the β chain were associated with decreased susceptibility, protection, or neutral effects. Susceptibility was associated with the presence of an uncharged amino acid at this position; hence HLA-DQβ (non-Asp57) was identified as a significant risk factor. Studies of various racial groups support the concept that one of the strongest gene associations with diabetes is the DQA1*0301-DQB1*0201 combination and having both DR2 and DQB1*0602 confers strong protection (13,14).

The picture is, in fact, even more complex (15). Having an arginine at position 52 of the HLA-DQα molecule (DQα, Arg52) as well as DQβ, non-Asp57 is a combination that results in high genetic risk for type 1 diabetes. Still further data will be required to explain why 10–20% of type 1 diabetic patients do not have the common DQA1-DQB1 high-risk combinations.

Nonetheless, certain gene combinations, particularly of the cis- and trans-encoded DQ molecules, are associated with susceptibility or protection from diabetes and DQ-determined resistance is dominant over susceptibility. These associations could permit the identification of approximately 2% of the general population who are HLA-DR3 and DR4 (DQw2 and DQw8) heterozygous, with risk as high as 8%, a figure
similar to siblings of diabetic patients (3). Strong linkage disequilibrium, which Thorsby & Rønningen (15) define as specific allelic variants often present together on the same chromosomal complex, means that, even if these MHC associations are not the actual susceptibility genes, the risk genes are likely to be located in or close to this area (16).

It is only recently that yeast artificial chromosome (YAC) and cosmid clones for the whole of the MHC have been isolated. In this region, there are at least 70–100 other expressed sequences, some of which affect the immune response, including certain complement proteins, cytokines such as tumor necrosis factor (TNF) α and β, and the peptide transporters TAP1 and TAP2 (17). Recent studies of gene polymorphism in this region indicate that two proteins believed to be involved in antigen processing and presentation by class I molecules, large multifunctional protease (LMP) and the transporter associated with antigen processing (TAP), are unlikely to be associated with development of type 1 diabetes, but some controversy exists (16). Genes outside the MHC region may also be important, but less is known about this connection. Non-MHC-region genes such as insulin and insulin-like growth factor 2 may modify susceptibility in DR4 individuals (18).

In the NOD mouse and BBdp rat, the genetics are no less complex than in humans, and several genes predispose these animals to diabetes. The NOD mouse was discovered serendipitously in 1974 in Japan during an inbreeding program established to try to isolate a strain with high cataract development (19). Approximately 80–100% of females and about 20–30% of males become diabetic by 16–20 weeks. Macrophages and mononuclear cells surround and infiltrate the islets (insulitis) beginning at around weaning several weeks before diabetes onset. The MHC haplotype of the NOD mouse, designated H-2g<sup>7</sup>, has a unique H-2 (MHC class II) I-A complex, I-A NOD, and I-E expression is lacking. NOD mice have an I-Aβ chain with a serine at position 57 and are therefore "non-Asp57" similar to human type 1 patients who are HLA-DQβ, non-Asp57. However, it should be noted that the diabetogenic I-Aβ of NOD is also present in the Swiss (ICR) mice from which NOD was derived, yet these mice are diabetes resistant.

Including the previously mentioned genes, there are at least eight other genes, designated idd-2 to idd-9, involved in NOD diabetes (2,20). Most of these influence immune reactivity [for example, genes are linked to the IL-1 receptor (idd-5), the Thy1/Alp-1 genes (idd-2), impaired IgG Fc receptor (idd-3), and infection response genes (Lsh/Ity/Bcg)] or they are thought somehow to control the progression of insulitis and diabetes. Another gene that is linked to the Bcl-2 locus outside the MHC region has a role in apoptosis and is involved in insulinitis and sialitis (2).

The BBdp rat was discovered in an outbred colony of Wistar rats at the BioBreeding Laboratories near Ottawa, Canada, in 1974 (21). In most colonies, 70% or more of BBdp rats develop insulin-dependent diabetes at between 60 and 120 days of age and mononuclear cells can be seen at the periphery and inside the islets 2–3 weeks prior to overt diabetes. The genetics of the BBdp rat have been extensively studied (22–24), and it appears that the diabetes trait is an autosomal recessive with incomplete penetrance, related to both MHC and non-MHC genes. There are at least three genes
required: a class II MHC (DR equivalent), RT1\textsuperscript{a}, a lymphopenia gene, Lyp, and an as yet uncharacterized gene. The BBdp rat has a serine at position 57 of both class II \( \beta \) chains, RT1.B\( \beta \) and RT1.D\( \beta \), in keeping with the "non-Asp" model, but the diabetes resistant BB rat (BBdr), the Lewis rat (RT\( \text{I} \text{I} \)) and the BUF rat (RT\( \text{I} \text{I} \text{b} \)) also have either serine or tryptophan (i.e., non-Asp) at this position. Therefore, the non-Asp residue at position 57 of the class II \( \beta \) chain is probably not a diabetes susceptibility marker in the rat.

Thus in humans, BBdp rats, and NOD mice, there is a requirement for certain diabetes susceptibility MHC haplotypes that are insufficient alone to produce diabetes (Table 1). As suggested by Leiter (37), it is likely that diabetes is the result of several common alleles in the presence of unfavorable conditions. This emphasizes again the fact that not all genetically susceptible individuals will develop diabetes, and there are possibly other diabetes susceptibility genes and non-genetic factors involved.

Prediction

Much progress has been made in the ability to predict who among the first-degree relatives of patients with type 1 diabetes will develop diabetes and when the disease will appear (3,38). There is general agreement that type 1 diabetes is a cell-mediated disease, but the pre-diabetic period is also associated with the appearance of several autoantibodies. In the years since islet cell antibodies were first reported by Bottazzo \textit{et al.} (39), it has become clear that type 1 diabetic patients have autoantibodies to numerous antigens such as glutamic acid decarboxylase (GAD, formerly 64 kDa), a 38 kDa protein, insulin (IAA), proinsulin, carboxypeptidase H, amylin, heat shock proteins, glucose transporter, thymic hormones, thyroid, adrenal cortex, and others (1,2,38). These antibodies can be measured several years before overt diabetes, and they are probably a secondary phenomenon resulting from the progressive destruction of \( \beta \) cells (2). They are nonetheless useful in predicting the course of \( \beta \)-cell destruction and may well provide clues to the nature of the autoantigens that trigger or sustain the autoimmune process.

Attempts to predict who among high-risk subjects will develop diabetes have focused on the presence of certain islet cell antibodies, insulin autoantibody (IAA), anti-GAD (64 kDa), and metabolic signs of \( \beta \)-cell destruction (3,38). Eisenbarth and colleagues have developed a linear regression model in which measurements of competitive insulin autoantibodies and first-phase insulin response to intravenous glucose can predict with a high degree of success when islet cell antibody positive first-degree relatives of patients with type 1 diabetes will themselves become diabetic. At the present time, the tests available to predict diabetes susceptibility are useful in those at high risk, namely first-degree relatives. However, these tests lack the predictive power (4) to use in screening for the 85–90% of patients who do not have a family history of type 1 diabetes. More recent data suggest that measurement of antibodies
### TABLE 1. Characteristics of human, BB rat and NOD mouse diabetes

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Human</th>
<th>BBdp rat</th>
<th>NOD mouse</th>
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<tbody>
<tr>
<td>Insulin-dependent</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Insulitis</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<td>Lymphopenia</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>Gender distribution</td>
<td>Equal</td>
<td>Equal</td>
<td>Usually ?</td>
</tr>
<tr>
<td>Non-MHC genes</td>
<td>Possible&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>MHC susceptibility genes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>HLA-DQ&lt;sup&gt;b&lt;/sup&gt;, non-Asp57 or equivalent</td>
<td>Yes</td>
<td>Yes, but also in BBdr</td>
<td>Yes</td>
</tr>
<tr>
<td>HLA-DQx, Arg52 or equivalent</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Viruses ↑ incidence</td>
<td>Some evidence&lt;sup&gt;a&lt;/sup&gt; for rubella virus, coxsackie, cytomegalovirus mumps (?)</td>
<td>No evidence of virus involvement in BBdp but Kilham rat virus can cause diabetes in BBdr rats.&lt;sup&gt;0&lt;/sup&gt;</td>
<td>Endogenous β cell retrovirus present but role in pathogenesis not known</td>
</tr>
<tr>
<td>Viruses ↓ incidence</td>
<td>Not known</td>
<td>LCMV injection protects; gnotobiotic BBdp still get diabetes</td>
<td>LCMV injection protects; immunization with retroviral proteins protects (?)&lt;sup&gt;a&lt;/sup&gt; Injection of complete Freund's adjuvant protects. BCG injected I.V. protects, as does OK 432.</td>
</tr>
<tr>
<td>Other microbial agents</td>
<td>Not known, but role for bacteria proposed&lt;sup&gt;f&lt;/sup&gt;</td>
<td>OK 432 (lyophilized streptococcus prep) protects; complete Freund's protects</td>
<td>Injection of complete Freund's adjuvant protects. BCG injected I.V. protects, as does OK 432.</td>
</tr>
<tr>
<td>Diet</td>
<td>Possible connection with early diet&lt;sup&gt;b&lt;/sup&gt;, meta-analysis shows significant but weak OR (&lt;~1.5); cow milk protein (BSA) link proposed&lt;sup&gt;d&lt;/sup&gt; but remains controversial&lt;sup&gt;i&lt;/sup&gt;</td>
<td>Major determinant of BBdp diabetes&lt;sup&gt;c&lt;/sup&gt;; casein and hydr. casein protect; no effect of high starch, sugars or energy restriction or fat source (except EFA deficiency); wheat, soy, occasionally milk and possibly alfalfa diets are diabetogenic&lt;sup&gt;k&lt;/sup&gt;</td>
<td>Major determinant of NOD diabetes: casein&lt;sup&gt;1&lt;/sup&gt;, hydr. casein&lt;sup&gt;1-n&lt;/sup&gt; and high vitamin E&lt;sup&gt;e&lt;/sup&gt; diets protect; wheat flour&lt;sup&gt;m&lt;/sup&gt; diabetogenic</td>
</tr>
</tbody>
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<sup>a</sup> From Rossini et al. (1); Yoon J-W et al. (2); Thai A-C et al. (3); Skyler et al. (4); Scott FW et al. (5).
<sup>b</sup> From Julier C et al. (18).
<sup>c</sup> From Yoon J-W et al. (2); Yoon J-W et al. (25).
<sup>d</sup> From Crisa L et al. (23).
<sup*e</sup> From Yoon J-W et al. (25).
<sup>f</sup> From Morris JA (26).
<sup>g</sup> From Gerstein H (27).
<sup>h</sup> From Karjalainen J et al. (28).
<sup>i</sup> From Atkinson MA et al. (29).
<sup>j</sup> From Scott et al. (30); Hoorfar J et al. (31); Scott FW et al. (32).
<sup>k</sup> From Rossini AA et al. (1).
<sup>l</sup> From Coleman DL et al. (35).
<sup>m</sup> From Hoorfar J et al. (33).
<sup>n</sup> From Elliott RB et al. (34).
<sup>o</sup> From Hayward AR et al. (36).

**Note:**
- Hydr, hydrolyzed; LCMV, lymphocytic choriomeningitis virus; BSA, bovine serum albumin; BCG, bacille Calmette-Guérin, attenuated *mycobacterium tuberculosis*; EFA, essential fatty acids
- MHC, major histocompatibility complex
against the β-cell autoantigen, GAD, has greater predictive value in the general population than islet cell antibodies or competitive insulin autoantibodies. Thus, with more relevant autoantibodies [GAD, 38 kDa (?)], better genetic data, and measurements of remaining β-cell function, it may be possible in the near future to predict who in the general population will become diabetic.

This brings up the question of what preventive measures can then be offered to these individuals. Skyler and Marks have reviewed the numerous immunotherapies that have been or are being tried (4). Several of these approaches are relatively nonspecific, of unknown efficacy, and not without risk. They reflect the incompleteness of our current understanding of how the immune system functions as well as the lack of knowledge of gene-environment interaction, particularly the timing of initiation, dose, duration, and frequency of exposure to diabetogenic agents in the environment. It is likely that immune interventions in newly diagnosed type 1 diabetic patients occur too late in the process to spare sufficient β-cell function to provide a lasting cure. For this reason, prevention strategies that avoid initiation or that dampen the early phases of the destructive immune process are most likely to provide long-term success.

NURTURE IDENTIFYING AND CHARACTERIZING ENVIRONMENTAL FACTORS

Indirect Evidence from Human Studies

In human populations, it has been difficult to identify and characterize the environmental factors involved in type 1 diabetes. The well-known studies of identical twins have provided data suggesting that the environment influences expression of type 1 diabetes. For example, Olmos et al. (40) reported that about 34% of unaffected identical twins in twin pairs with one type 1 diabetic patient become concordant for the disease. Also, the rate of disease appearance decreased rapidly in co-twins after diagnosis in the index twin, suggesting a process that occurred over a defined period of time. The incidence of the disease peaks at about 15 years in the general population and then declines sharply. Olmos et al. suggested that this pattern, combined with a lack of clustering or outbreaks, is consistent with a period of susceptibility to an environmental agent rather than exposure to the agent(s). It has been proposed that the generation of diversity whereby T cell receptor genes and immunoglobulin genes undergo random recombination, making each individual unique with respect to the immune system, could account for the results seen in the twin studies. Indeed, this might be part of the explanation. This same argument might be invoked to justify the involvement of environmental factors (infections, toxins, dietary chemicals) early in life when twins would be likely to be exposed to similar environmental influences. Alternatively, the discordance may reflect different environmental exposures later in life.

Evidence from the twin studies was the seed for epidemiologists to examine the
question further. The Diabetes Epidemiology Research International (DERI) group adopted a strategy used in cancer research and found several indications that environmental factors are involved in the etiology of type 1 diabetes (9). The gist of the approach was to look at the following five questions. (i) Cancer can be produced in animals by external agents. Type 1 diabetes can also be induced chemically in animals using direct β-cell cytotoxic agents (alloxan, a single high dose of streptozotocin or other toxin) or multiple low doses of streptozotocin. Oral ingestion of the rodenticide, Vacor®, in humans also produced type 1 diabetes. (ii) There are geographic differences in cancer incidence. There is also marked geographic variation in type 1 diabetes incidence ranging from 0.5/100,000 in China to >35/100,000 in Finland. (iii) Rapid changes in cancer incidence have occurred that are not due to genetic change. For type 1 diabetes, the incidence has also increased in several countries, epidemics have been reported, and hot (Sardinia) and cold (Macedonia) spots have been identified. (iv) Migrants adopt the cancer risk of their new home. There is some limited evidence to suggest that this also occurs in the development of type 1 diabetes in Japanese moving to Hawaii as well as in Canadians of Jewish and French descent living in Montreal. (v) Environmental agents such as smoking, alcohol consumption, and asbestos cause cancers. Reliable epidemiologic data are difficult to obtain because, if available at all, they are mostly retrospective, and long-term prospective data on individuals who become diabetic are simply not available. Ideally, a sufficiently large prospective study would be carried out in individuals from birth to about 20 years of age in various populations with different risk levels to begin to ascertain the true interaction of genetic and environmental factors.

It is likely that the next few years will see even greater advances in characterizing the diabetes genes and the natural history of the disease. However, prevention is always preferable to cure, and the area with greatest potential for safe and cost-effective prevention is the modification of exposure to environmental factors.

Trying to identify nongenetic factors that might trigger or promote the process that leads to diabetes is exceedingly difficult in humans. Therefore information on environmental agents is very often indirect or comes from studies in susceptible animals. The main environmental factors considered in the etiology of type 1 diabetes have been chemical toxins, stress, diet, and infectious agents, mostly viruses.

**Chemical Toxins and Stress**

There are many chemical agents that can cause cytotoxic effects in β cells, and some also have effects on the immune system: alloxan, streptozotocin, Vacor® (N-3-pyridylmethyl-N’-P-nitrophenyl urea), chlorozotocin, cyproheptadine, pentamidine, cyclosporin, and others. These have been reviewed in detail elsewhere (41,42) and will not be considered further here. There is at least one study in BBdp rats that showed that diabetes-prone animals submitted to “multiple, concurrent and unpredictable” environmental stresses such as restraint, rotation, crowding, and random
cage reassignment developed diabetes sooner than animals kept under normal conditions; the final diabetes incidence was not significantly different from the control group (43). The link between the neurochemical, endocrine and immune networks is only just beginning to be understood and may yet prove to be important in the development of type 1 diabetes.

Viruses

Viruses have been implicated in diabetes pathogenesis since the report by Harris nearly 100 years ago of a case in which mumps preceded the onset of type 1 diabetes [cited in (44)]. Since that time there have been many anecdotal reports of viral infection preceding the onset of diabetes. Mumps has often been implicated, as have rubella, cytomegalovirus, polio, measles, influenza, and tick-borne encephalitis (44). The viruses most often implicated are rubella and coxsackie B4. Congenital rubella syndrome is a unique and important example of an association between a viral infection followed by development of autoimmune diabetes in humans (25,45). Approximately 20% of these patients developed diabetes, with a latency period of between 5 and 20 years. The patients who became diabetic were more likely to be HLA-DR3, and DR2 was less frequent in this group. The mechanism of this interaction is not known.

Epidemiological studies have implicated coxsackie B4 virus in some cases of type 1 diabetes. It has been shown that this virus can infect and destroy β cells in Patas monkeys. A variant of coxsackie virus isolated from a young diabetic patient who died produced diabetes when inoculated into SJL/J mice but not in CBA/J, C57BL/6j or BALB/c mice (25).

More recently, Yoon has categorized the diabetogenic potential of viruses as follows: (i) triggering agents for β cell autoimmunity: retrovirus, rubella, cytomegalovirus, reovirus, mumps virus, and parvovirus (Kilham’s rat virus, KRV); (ii) cytolytic infection of β cells: encephalomyocarditis (EMC) virus, mengovirus, and coxsackie B viruses; and (iii) viruses protective against development of type 1 diabetes: both NOD mice and BBdp rats can be protected from developing autoimmune diabetes by inoculation early in life with lymphocytic choriomeningitis virus (LCMV). Also, the diabetes that follows injection of diabetogenic EMC virus in susceptible mice can be prevented by live attenuated vaccine or immunization with a nondiabetogenic EMC virus.

It was pointed out that it is exceedingly difficult to show in vivo that viruses infect and destroy β cells, producing diabetes in humans. Nonetheless, there is indirect evidence in humans (congenital rubella syndrome) and direct evidence in animals (coxsackie B4 inoculation into susceptible SJL/J mice and Patas monkeys) that viruses can in some circumstances result in diabetes. Viruses such as the Kilham rat virus can cause diabetes in BBdr rats, but there is no evidence that viruses play a role in autoimmune-mediated diabetes in the BBdp rat (23). By contrast, the NOD mouse may contain an endogenous retrovirus, and there is speculation that this could
lead to expression of viral antigen on the β-cell surface or alter expression of cellular genes (25). The role of viruses in the etiology of type 1 diabetes and the proposed mechanisms of action have been reviewed in detail recently (1,2,25).

Dietary Factors

The concept that diet might affect development of diabetes can be traced at least as far back as the 1930s to the studies of Himsworth and colleagues (46). Unfortunately, the problem with studies up until about 1981 was that individuals under 16 years in the case of Himsworth’s study and those under 30 years of age in West’s study (47) were excluded and adequate differentiation between type 1 and type 2 diabetes was lacking. These caveats meant that the early work was of little value in looking at diet and the development of type 1 diabetes.

Willett has pointed out that nutritional epidemiology is anything but simple because diet is not a single variable and very few dietary components can be described as simply present or absent (48). Obtaining representative food intake values for individual children is difficult, and several studies have shown that information provided by children is often less reliable than that from adults. Most often data are collected retrospectively and are not representative of the individual’s usual intake. The quality of data obtained retrospectively is always open to question. An example was recently given by Kostraba (49) to illustrate the pitfalls of relying solely on retrospective case-control studies. In the long debate over the role of cow’s milk in atopic allergy, case-control studies were able to detect the protective effect of exclusive breastfeeding, but it was not recognized until prospectively collected data were analyzed that the benefits of breastfeeding were attributable to avoidance of food antigens rather than benefits due to breast milk per se. This example highlights only a few of the difficulties in linking dietary intake to disease development (48).

For these reasons, much of the work linking diet and type 1 diabetes has been carried out in the BBdp rat and NOD mouse. In fact it was not until the availability of these spontaneous models that the first experimental evidence of an effect of diet on autoimmune type 1 diabetes was shown (50). Interest in dietary modification of autoimmunity dates from the work in the early 1970s on energy restriction in the systemic lupus erythematosus (SLE)-prone, (NZB × NZW)F1 mouse. Most of the diet manipulations tried in this model of multisystem autoimmune disease have involved single nutrient and energy modification. By contrast, the work in the models of organ-specific autoimmunity, the BBdp rat and NOD mouse, has focused mainly on foods and food components (5).

The initial findings in the BBdp rat showed that semipurified AIN-76A diets based on cow’s milk casein as the protein source, starch or starch/sucrose as the carbohydrate, corn oil as the fat source, plus cellulose as a source of “fiber,” and supplemented with micronutrients, completely inhibited the expression of diabetes. The phenomenon is quite marked. Laboratory rodent diets such as Purina 5001 or NIH-07 contain a kaleidoscope of chemicals derived mainly from plant protein sources,
and feeding these mixtures routinely results in a diabetes frequency of about 68% (n = 7 experiments) in BBdp rats by 130-150 days of age. However, several protein sources are not diabetogenic. Feeding diets containing casein, hydrolyzed casein, fish meal, canola flour, corn (or others) as the protein sources markedly inhibits the development of diabetes. We use two negative control, diabetes retardant, diets based on casein or hydrolyzed casein as the amino acid source. The diabetes frequency in BBdp rats fed casein-based diets is about 9% (n = 7 experiments) and hydrolyzed casein diets produced approximately 12% diabetes frequency (n = 7 experiments). The marked effect of various diets on insulitis and diabetes frequency is shown in Fig. 1.

The protective effect of semipurified diets containing diabetes-retardant protein sources is equally apparent if the test animals are fed from weaning at about 23 days or if the dams are also fed the diets when the pups are suckling. In fact, the diabetogenic effect of NIH-07 is still apparent if feeding this diet is delayed to 50 days and a non-diabetogenic diet is fed from weaning to 50 days. The only effect is to delay age at onset by 3–4 weeks; the rate of disease appearance and the final diabetes incidence remain unchanged. This suggests that the timing of the diet-diabetes interaction extends over a long period, reaching into what would be early puberty for the rat.

Other studies showed that semipurified (casein-based) diets high in sucrose or starch (β-cell stimulation as opposed to β-cell rest) were diabetes retardant, and changing the fat source from 5–20% corn oil or 20% lard did not increase diabetes frequency above background. Qualitative and quantitative studies of numerous protein sources confirmed that the protein source determined the diabetes outcome (31,51). When all the major components of the diabetogenic NIH-07 diet were tested individually, it was clear that wheat gluten and soybean meal were diabetogenic. Tests of cow’s milk protein diets containing various amounts of casein and whey proteins showed that whey protein diets had a variable diabetes-inducing potential and were, with two or three exceptions, diabetes retardant or only mildly diabetogenic. A diet with large amounts of alfalfa seeds was moderately diabetogenic.

Others have also shown that casein (35) and hydrolyzed casein-based diets (33–35) inhibit diabetes development in NOD mice, but skim-milk-based diets were not diabetogenic (35). A recent study confirmed the observation that hydrolyzed casein inhibits the development of diabetes in NOD mice and also showed that a wheat flour diet produced a diabetes frequency of 60%, while soybean meal diets resulted in a 45% diabetes frequency (33). These results support our findings in the BBdp rat and suggest that characterizing plant food diabetogens may provide a way of modifying some of the key environmental factors in the etiology of type 1 diabetes. We believe that it should be feasible to identify and characterize foods that contain autoimmunogens (agents that trigger a process resulting in inappropriate immune response against one’s own tissues) with the aim of expanding food-oriented interventions along with studies of individual diet components. The identification and characterization of food
FIG. 1. Modification of insulitis and diabetes frequency by different foods and food components. BBdp rats were fed a standard laboratory rodent diet, NIH-07, or modified AIN-76A diets as described previously (31) from weaning usually to ~150 days of age and sometimes up to 240 days. Animals were considered diabetic if urine glucose was >2+ using Testape, fasting blood glucose was >200 mg/dl (11.1 mmol/liter), or if weight loss or failure to gain weight was observed. These rats were killed within 24 hours of diagnosis by exsanguination while under light anesthesia (3–5% halothane or isoflurane in O₂); degree of islet inflammation and damage was assessed as previously described. From Hoorfar J et al. (31) Each symbol represents a separate group of rats (n = 10–60/group). Various diets were assigned to Food Groups as follows: NIH = NIH-07 [N], an open formula diet (known % composition) that is highly diabetogenic and served as positive control; SOY, soybean meal, flakes, or flour [S]-based diets in which all amino acids were supplied from these materials; WHEAT, wheat gluten [W], the protein concentrate from wheat flour following water extraction, 80% protein, supplied all protein and was supplemented with certain amino acids; SMP, skim milk powder or other cow milk protein containing diet [M, SMP-based, MW, milk whey (10%) + casein (10%), MB, casein + 0.1% BSA (equivalent to amounts found in normal laboratory diets), MC, cow colostrum (8%) + hydrolysed casein, MF, cow’s milk-based infant formula]; CASEIN, 20% casein based AIN-76A diet [C] which was consistently diabetes retardant; HC, hydrolyzed casein based [HC] diabetes-retardant diet.
autoimmunogens or immunomodulatory foods, as distinct from single nutrient modifications, is a relatively recent phenomenon and may prove useful in the prevention of diabetes and other autoimmune diseases.

If the food diabetogens can be characterized, it might even be possible to provide advice about diabetogenic or protective foods without the necessity of screening the general population. Conceivably, it should be possible to engineer diabetogen-free plant foods.

Diet is a major factor in the etiology of autoimmune diabetes in the BBdp rat and NOD mouse. The nature of the protein source is important: laboratory rodent diets, which are commonly plant based (for example, NIH-07 has 83% plant material), usually result in a diabetes incidence of about 68% in BBdp rats and 70–100% in female NOD mice. We see a dose response to NIH-07 diet, suggesting that there are food diabetogens in this diet mixture.

In our BBdp rats, nutritionally adequate diets, in which wheat gluten (80% protein) or soybean meal (52% protein) are the sole dietary protein sources, result in a diabetes frequency of around 50%. This level of diabetes is significantly higher than either of our negative control diets that are casein or hydrolyzed casein based and produce a diabetes incidence of 9–12%.

The low rates of diabetes observed in animals fed the control diets may be the true baseline or natural rates of the disease in these animals. Recall that when the BBdp rat was first discovered in 1974, the rate of diabetes was 10% (21). The difference between these rates and the diabetes frequency of 68% seen in BBdp rats fed NIH-07 (or other plant-based laboratory diets such as Purina 5001) represents diet-inducible diabetes in the BBdp rat, that is, about 86% of their diabetes is due to dietary factors.

The Cow Milk Protein Hypothesis

Much excitement has been generated concerning the possibility that a 17 amino acid peptide called ABBOS (a peptide sequence from bovine albumin that differed from the corresponding region of human, rat, and mouse albumin) might trigger the autoimmune process that causes type 1 diabetes (28). The basic hypothesis suggests that there could be an immune response against the ABBOS peptide resulting in cross reaction to a 69 kDa protein (ICA69, purported to contain a peptide fragment homologous to part of ABBOS) on the β-cell surface. Karjalainen et al. (28) reported that 100% of newly diagnosed children with diabetes had raised levels of IgG bovine serum albumin (BSA) antibodies. A polyclonal rat anti-ABBOS serum reacted against the ICA69 islet protein and other smaller proteins in liver, muscle, and heart. The authors concluded that their finding of the ubiquitous presence of anti-BSA antibodies in newly diagnosed type 1 diabetic children and the cross reactivity with ICA69 might somehow, in concert with recurring infections in the prediabetic period, be part of the process that destroys β cells.

This intriguing study caused much heightened interest and concern that there might
be a link between dietary bovine serum albumin and type 1 diabetes. Atkinson et al. (29), using some of the same techniques as the previous group, measured BSA antibodies and the response of peripheral blood mononuclear cells from newly diagnosed type 1 diabetic patients, people at varying degrees of risk for diabetes (islet cell antibody positive or negative first-degree relatives), and people with other autoimmune diseases such as thyroiditis, rheumatoid arthritis, or systemic lupus erythematosus. They found only 10% of newly diagnosed diabetic patients were BSA antibody positive and this was not significantly different from control subjects. Hardly any of their subjects showed increased incorporation of labeled thymidine in the presence of ABBOS or BSA. Findings were similar in islet cell antibody positive or negative first-degree relatives, showing no association between islet cell antibody positivity and cellular response to BSA or BSA antibodies. However, they did find that BSA antibodies were more common in relatives of type 1 diabetic patients (nine out of 42 were BSA antibody positive) and in patients with thyroiditis. They concluded that BSA antibodies may reflect a defect in immunological tolerance associated with susceptibility to autoimmune disease. That only 10% of their newly diagnosed type 1 diabetic patients were BSA antibody positive compared with the 100% found by Karjalainen et al. and the absence of a cellular immune response to BSA or ABBOS, does not support a role for BSA in the development of type 1 diabetes.

Another group proposed a similar hypothesis in rheumatoid arthritis, invoking reactivity against an overlapping but nonidentical BSA peptide close to ABBOS (52). This peptide showed high homology with vitamin D binding protein, human collagen type I, and complement component C1q. They reported that some rheumatoid arthritis patients have high titers of BSA antibodies. This is in keeping with the finding of Atkinson et al. suggesting that oral tolerance may be abnormal in people prone to autoimmune diseases. It also suggests, perhaps not surprisingly, that protein homologies are either more common than previously thought or that there is greater access to protein databases.

In NOD mice, Coleman et al. found that an AIN-76A diet with an additional 10% skim milk powder was not diabetogenic (35). Leiter reported that anti-BSA antibodies were not present in NOD mice fed a highly diabetogenic diet based largely on wheat, and there was no correlation between an immune response to BSA and diabetogenesis (53). Our data in the BBdp rat suggest that BSA antibodies are not predictive of diabetes and the antibodies do not occur in all rats. Similarly, feeding studies with semipurified diets indicate that various BSA-containing diets are only occasionally diabetogenic and there is no correlation between daily dose of dietary BSA and diabetes frequency. It is not clear why milk protein diets should produce this variability.

These data suggest that a role for BSA in the pathogenesis of diabetes is at the very least controversial. Considering these concerns and the complete absence of data in humans on the required time of introduction, duration, and chemical identity of food diabetogens, it is premature to consider using the cow’s milk hypothesis as the basis of an intervention trial. It is clear, however, that diet has major effects in the development of diabetes in the BBdp rat and NOD mouse. Identifying and
characterizing food diabetogens could lead to safe and effective means of primary prevention of this disease in susceptible humans.

REFERENCES

THE ETIOLOGY OF TYPE 1 DIABETES


DISCUSSION

Dr. Dakou: The child who is breastfed has a different environment in an early and very critical period. Do you think this may be important?
Dr. Scott: Of the 16 or so published case-controlled studies, about half find a link between breastfeeding and type 1 diabetes. Some find no link. In two of the studies in which there are what we might call prospective data, nurses went into the home and wrote down how the infant was being fed. From those studies there was no relationship between early exposure to cow’s milk or breastfeeding and the development of diabetes. I think one of the difficulties here is that we are trying to simplify what is really a very complex relationship. The infant is also weaned onto different foods in different cultures and at different ages and these weaning foods could also be important.

Dr. Otten: I am one of those who found a protective effect of breastfeeding. Could you tell us what you fed your rats before you did your nutritional studies.

Dr. Scott: We fed the animals protective casein or hydrolyzed casein-based diets or the diabetogenic, cereal-based laboratory diets (1). The nature of the pre-weaning diet did not change diabetes outcome. We can expose animals as late as 50 days and they still get diabetes. This is a key point. If we find the effect this late in animals, it suggests that the exposure need not occur only in early infancy; later sustained exposure may also be important.

Dr. Guesry: I completely agree with Dr. Scott when he says that the issue about the importance of breastfeeding is very complex. If we draw a parallel between the development of IDDM and the development of cow’s milk allergy in infants at risk of developing allergy, we know that breastfeeding can partially suppress sensitization to cow’s milk in the infant. There is a reduction of about 50%, but not a total suppression. We also know that lactating mothers secrete heterologous proteins in their milk, I mean protein from other origins, either animal or vegetal. For example in Finland, I am sure that mothers secrete reindeer milk protein in their milk, and in Sardinia they probably secrete goat’s milk protein. Since we also know that goat’s milk and cow’s milk share about half the same antigens, I am very surprised when I hear that there is a direct correlation between switching from goat’s milk to cow’s milk and the likelihood of developing IDDM.

Dr. Assan: How early after birth did you give the bovine protein? Many things are currently said about induction of oral immune tolerance, but I understand the earlier the better.

Dr. Scott: In the experiment that I showed you, the animals were tolerized at around 40–45 days. The point you raise is important. In an attempt at earlier exposure to dietary constituents, we orally dosed control and diabetes-prone animals between days 4 and 7 with Pregestimil®, which is based on hydrolyzed casein as a protein source (in other words it is not diabetogenic), and with the complex diabetogenic mixture (NIH-07), and then, at weaning, the animals were weaned to either the hydrolyzed casein diet or the NIH-07 diet—that is, the nondiabetogenic or the diabetogenic diet. When we first exposed the animals to a nondiabetogenic hydrolyzed protein material for 4–7 days in infancy and then weaned them to a nondiabetogenic diet, none of the seven animals became diabetic up to 140 days. In the positive control, the animals were dosed with hydrolyzed casein and then weaned to NIH-07; in this case 60% of them became diabetic. However, when we exposed the animals early to NIH-07 orally and then weaned them to the same material, 12.5% of them became diabetic. This experiment indicates that there could be a possibility of tolerizing animals early on to this dietary mixture and preventing disease, but it needs to be repeated.

Dr. Beauvais: So far as breastfeeding is concerned, we have many people in our population who come from Algeria and Morocco—they represent 15% of our population, and 50% of our cases of diabetes. However, all these patients have been breastfed until the age of 9 months.

Dr. Drash: Is it the absence of human breast milk or the negative effect of the cow’s milk protein that is harmful? As pediatricians, we praise the mother for all the wonderful things
she gives her baby through breastfeeding, but my reading of the literature suggests that it is not the positive effect of human breast milk but the presence of a negative factor in cow's milk protein that may be diabetogenic.

*Dr. Scott:* I have some difficulty with the human literature. I think the studies are very difficult to interpret. As I said, the only two where there are prospective data do not really support the hypothesis well. One of the key things that has not been addressed is the timing of the interaction between diet and genetic susceptibility. We also do not know whether it is only the first 6 or 9 months of life that are important or whether it could be the first 10 years.

*Dr. Drash:* But isn't it truly amazing that those studies suggest that cow's milk protein is a factor when we are dealing with a population in which only a small number of children are genetically susceptible? The only way to get the answer, it seems to me, is to focus a careful study on individuals who are identified as being at genetic risk.

*Dr. Scott:* As I said, the difficulty I have is that most of the data are retrospective and contradictory. The milk hypothesis based on BSA is controversial. If the evidence were more convincing, one would be more encouraged to follow it up. It is also a problem that we know so little about the timing.

*Dr. Carrascosa:* Overproduction of free radicals has been implicated in the damage to the \( \beta \) cells. What do you think about this possibility? Do you think that overproduction of free radicals could be a common type of nutritional and environmental influence in the progressive destruction of the \( \beta \) cell?

*Dr. Scott:* We get back to the comment I have been making about nutritional epidemiology. I have some experience with this because colleagues have been doing dietary surveys in Canada. The available information is not extensive. It gives no real indication of free radical content and indeed it would be virtually impossible to obtain this. Not only that, but if you are looking at antioxidants in the diet you are only looking at one side of the story because we are dealing with a very complex system of protective and damaging components.

*Dr. Crofford:* Most of us here understand the difference between epidemiologic studies in humans that can point out the associations between variables (and are so important in the formulation of hypothesis) and the randomized intervention trials that are necessary in order to establish the validity of these hypotheses. Unfortunately this distinction is not well understood by the general population and, even worse, it is not well understood by the people who write for the general population by interpreting scientific literature. Often this leads to overinterpretation of these epidemiologic experiments, and the public believes that causality has been established. This can result in panic or in people making unwise health decisions. So I think it is always worth pointing out that we, as scientists, have a very profound responsibility to emphasize over and over again the fact that the associative studies do not necessarily establish causality, and to try to minimize the overinterpretation of such studies, as happens so often in the press and on television.

*Dr. Bergman:* I am curious to know what one can do to protect against the possible effect of changes in diet that may lead to changes in the need for insulin secretion, thus possibly contributing to diabetes as an end point. If you change the composition of the diet, how do you control for the possibility that the animal is more or less sensitive to insulin, and therefore that there is more or less stress on the pancreas, leading to earlier or later onset of diabetes?

*Dr. Scott:* This is a difficult problem to approach. What I can say is that we have tested dozens of diets and have categorized them as to their diabetogenic potential; in many cases what we have done is to substitute what we consider to be very similar protein sources, and when we measure the insulin levels we find they are similar. I think what you are talking
about relates also to the concept of β-cell rest or stressing the β cell, which then produces more antigens on the surface.

_Dr. Marliss_: Several people have tried more or less successfully to bridge the metabolism-immunology gap. Buschard _et al._ very successfully demonstrated a decrease in diabetes incidence with exogenous insulin treatment (2), and this has been confirmed by others (3). This has led to current trials that may run into problems because of the difficulty in predicting who is actually going to get type 1 diabetes among humans. However, from the results of various other experiments, it is entirely predictable that if, for one reason or another, individuals susceptible to type 1 diabetes have a need to secrete more insulin to achieve their metabolic goals, these individuals will ultimately be more susceptible to the development of diabetes; this is likely to be one of those extremely difficult variables to control in any dietary type of study. There are several ways of approaching the problem, but if one uses either C-peptide secretion or excretion of C-peptide in urine it would appear that as much as 80% of the total amount of insulin that will be secreted in a day is secreted in relation to meals; thus one would anticipate that anything that is ingested in a meal that is capable of being an insulin secretogogue may well play a role in the etiology of diabetes, and it may play this role over a very prolonged period of time.

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