Perspectives in Diabetes
Speculations on Etiology of Diabetes Mellitus
Tumbler Hypothesis
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Although clinically useful, the conventional partition of diabetes mellitus into two major classes, insulin dependent and non–insulin dependent, has become obsolete from an etiological standpoint. Contemporary research, particularly that with advanced cellular and molecular methodologies, suggests that the expression of diabetes depends on a wide range of factors. We suggest that the etiology of diabetes has become analogous to the cylinder of a lock containing many tumblers. Each tumbler, e.g., environment, genetics, or cellular interactions, must be aligned before the key can be turned and an understanding of the etiologic process claimed. Diabetes 37:257–61, 1988

Diabetes mellitus is not a single disease but a group of disorders characterized by hyperglycemia that can be due to either absolute or relative deficiency of insulin. To bring order to this complex biological problem, diabetes has been segregated into two major categories: insulin-dependent (IDDM) and non-insulin-dependent (NIDDM) diabetes mellitus (1).

Complete understanding of the pathogenesis of both forms of diabetes continues to elude researchers. The reasons are many, beginning with the perplexing mode of inheritance of diabetes. Neel (2), asserting that it does not fulfill the criteria of a classic model, has described diabetes as a “geneticist’s nightmare.” Attempts to understand environmental factors involved in its pathogenesis have also proven difficult. Diet, viruses, and toxins have all been associated with the disease, and some cases of diabetes are ascribable to a single factor. Classic examples include pancreatectomy, pancreatic agenesis, and exposure to certain β-cell cytotoxins. Few cases of human diabetes, however, are associated with such a clear-cut etiological mechanism.

The probability that a single genetic or epidemiological factor will “explain” any of the common forms of diabetes is small. It is far more likely that an array of factors are responsible, acting together in a cumulative or synergistic fashion.

Our view of the etiology of diabetes uses the analogy of a lock and tumblers (3). A lock turns only when all its tumblers are in proper alignment. Designing a key de novo to do this requires knowledge of each tumbler. Diabetes researchers are only beginning to appreciate how many tumblers there are, to say nothing of their design. Nonetheless, if rational preventive and curative therapy of diabetes is to be achieved, turning rather than forcing the key must be our goal.

Understanding the structure of the lock and designing the keys that may eventually turn the cylinder within are the achievable, if daunting, goals of contemporary diabetology. Central to this effort is the explosion of new technology, particularly in cellular and molecular biology, that has provided extraordinary new insights into the pathogenesis of diabetes. Scientists using these new technologies have, for example, identified the relationship of IDDM to the major histocompatibility complex (MHC), collateral a worldwide epidemiology of diabetes, uncovered new chemical and molecular markers of the disease, performed sophisticated testing of insulin reserve, and provided immunological tools of breathtaking sophistication. Together, these advances provide a new perspective on the diabetic condition.

The first requirement of this perspective is reconsideration of the dichotomous conventional phenotypes of diabetes. What environmental, molecular, and cellular factors might both individually and in concert determine whether a given individual becomes diabetic can now be considered. These factors are symbolically represented in Fig. 1. The combinations and interactions of these factors are the determinants of diabetes in both humans and animals.
ENVIRONMENTAL PERTURBANTS

Many environmental factors have been associated with diabetes; the clearest association is that of dietary abundance with NIDDM. Clinicians know that in this situation, dietary restriction dramatically reverses as many as 75% of cases of hyperglycemia. This is a clear example of environmental impact on the diabetic phenotype; but how does this occur, and why does it not work in 25% of cases? Perhaps the change in diet is more than a metabolic effect; it may in fact alter gene expression or cellular function. Should we neglect the observation that dietary constituents also influence IDDM-like syndromes such as that of the BB rat (3)?

Another well-investigated environmental perturbant is infection. Mumps virus as a cause of pancreatitis and IDDM has been recognized for >60 yr. More recently, Coxsackie B4 virus infection as a cause of IDDM has been documented (4). Encephalomyocarditis virus infection in mice is an animal analogue of the same phenomenon.

The importance of viruses in the pathogenesis of diabetes and the mechanisms by which they might act are controversial issues. The simplest hypothesis holds that viruses infect β-cells and destroy them directly. Alternatively, they might alter β-cell surface antigens, marking the cell as foreign tissue and targeting it for immunological destruction. Virus particles could also share antigens in common with β-cells; immune system activity against the virus might then inadvertently destroy the “innocent bystander” β-cell. Viruses could also induce the expression of class II antigens on the surface of β-cells, thereby initiating their destruction, or affect the immune system by altering regulatory- or effector-cell populations. Another possibility is that viruses may incorporate into the genome of β-cells and act as persistent viruses (5). In this way, viruses may play a pathogenetic role in IDDM as well as NIDDM.

Toxins can also kill β-cells. Pyrimidinil and alloxan are well-studied examples. Streptozocin is of particular interest because it is directly ß-cytotoxic at high doses but seems to induce an autoimmune type of diabetes when given as multiple small doses to certain strains of mice (6).

The potential importance of environmental factors is obvious, but they probably seldom act alone. They act within constraints imposed by cellular activities and genetic elements and affect systems as varied as the immune response, food-intake regulation, and perhaps gene expression itself.

GENETIC ELEMENTS

Understanding diabetes requires an understanding of the diverse genes relevant to the disease: genes that establish susceptibility, that initiate the process, and that prevent restoration of homeostasis. Not only are regulatory and structural genes involved but also “families” of genes that act in concert to regulate poorly understood processes like aging and the development of obesity.

Gene activation. The expression of many genes is influenced by promoters and enhancers. Located proximal to sequences coding structural elements, they regulate the rate of downstream transcription. Other regulatory gene sequences code for proteins that act on other parts of the genome, i.e., transactivators. The importance of these regulatory elements and their responsiveness to the local environment has been a theme in molecular biology since the time of Jacob and Monod (7) who demonstrated in seminal studies that an environmental factor could activate a complex genetic unit, an operon, in prokaryotic organisms.
Contemporary studies suggest that insulin itself is a direct activator of gene expression (8). Gene regulation as a function of environmental interaction has come of age and is certain to play a critical role in future understanding of diabetes.

**Structural genes.** The expression of genes coding for proteins is obviously relevant to the development of diabetes. Such a protein might, for example, be a lymphocyte receptor. Such receptors are expressed in the course of IDDM and are influenced by genes not within the MHC. An example is found in the BB rat, in which expression of the RT6 lymphocyte differentiation alloantigen is influenced by a non-MHC gene (9). Other genes relevant to the production of cytokines (or to key receptors such as the insulin receptor) or to the production of insulin are probably important. With respect to the latter possibility, abnormal sequences flanking the insulin gene have been observed in both IDDM and NIDDM.

**Gene families.** It is unlikely that one gene regulates body weight or aging; families of genes regulate these processes, and these genes in turn influence the appearance of diabetes. Hayflick’s classic experiments suggest that the genome of an organism contains information that limits the number of divisions of a cell. The younger the cell, the more divisions remain possible. With aging, certain genes can no longer be expressed. Loss of melanin in hair follicles and loss of the ability to produce the enzyme lactase are examples. How these time-locked phenomena relate to diabetes is still to be discerned, but surely it is no accident that IDDM occurs at ~13 yr of age (as the thymus involutes?) and that NIDDM is largely a disorder of middle age (as certain varieties of β-cells become senescent?).

The association of obesity and mellitus has been recognized since the time of Avicenna, ca. 1000 A.D. However, the presence of obesity does not imply the inevitability of diabetes. Why this should be is not clear, despite the fact that the inheritance of obesity is better understood than that of diabetes. When both parents are obese, the probability of diabetes in their offspring is >90%. The concordance rate of NIDDM in monozygotic twins is ~100%, even when such twins are separated in childhood and raised in families with disparate nutritional habits. Even here, however, the role of the environment cannot be ignored. Social class, social mobility, education, and immigrant status all influence the obesity gene in epidemiologically unequivocal but biologically obscure ways.

Animal studies lend further complexity to the issue. Zucker fatty (fa/fa) rats, for example, are grossly obese but not hyperglycemic. Obese (ob/ob) and diabetic (db/db) mice, in contrast, are proportionally as obese, but both are diabetic. Furthermore, the severity of diabetes associated with these two single gene mutations is strongly influenced by genetic background. In the C57BL/6 mouse, these genes cause mild diabetes; in the C57BL/Ks mouse, the disorder is severe, even fatal. The algebra of obesity and diabetes is complex but becomes even more so when a third factor, immune dysfunction, is added to the equation. Crossing the Zucker rat with the spontaneously diabetic BB rat yields progeny with diabetes syndromes that range from obesity with hyperglycemia to lean ketosis-prone diabetes.

The key point to observe is the way seemingly unrelated issues, i.e., obesity, general genetic background, environment, MHC, and autoimmunity, interact to create a novel biological system that may yield, or prevent, diabetes mellitus.

**MHC: genes that determine susceptibility?** The extraordinarily allelic MHC is thought to have evolved from a single ancestral locus that mutated and reduplicated countless times. Its primordial importance can be inferred from the observation that even earthworms are capable of allograft rejection. Macrophage-like coelomocytes mediate the process. With evolution, more sophisticated defense mechanisms were acquired and with them, a complex histocompatibility system to distinguish self from nonself. Genes of this system code for molecules that specify rejection (class I) and immune system activation (class II). The class II region regulates responses to foreign antigens. Animal studies show that the immune response to infectious agents is associated with the ability of these immune regulatory genes to respond appropriately to external stimuli.

Remarkably, however, research has uncovered an association of the MHC with various heretofore perplexing disease states—an astonishing encryption of self-destruction within self-protection. In the case of IDDM, the disease was first correlated with the HLA-B8 and -B15 haplotypes. A stronger association in White people was later observed with HLA-DR3 and -DR4. More recently, a still stronger association was made with the DQ locus, and in an exciting development it has been announced that one amino acid residue at position 57 of the HLA-DQα gene may determine susceptibility to IDDM (10). Clearly, in humans the MHC region must be important in determining susceptibility or permissiveness. However, in and of itself it does not lead to diabetes and may not be linked to any other diabetogenic gene in the MHC region (11). In the end, the permissive but probably not causative role of the MHC in diabetes is still left to ponder. The question invites speculation.

It is apparent that as species evolved, continued survival depended not only on the acquisition of food and shelter but also on resistance to whatever pathogenic organisms were encountered. The immune response to foreign organisms depends on the responsiveness of the MHC to a given stimulus. MHC haplotypes were undoubtedly selected for according to the degree to which they conferred a survival advantage on their bearer. With each epidemic, survival enriched the MHC repertoire. Just as likely, survival and the selection for a robust response to a given stimulus may also have conferred a subtle susceptibility to other disorders—those now known as the autoimmune disease states.

Studies in both the NOD mouse and the BB rat have confirmed the importance of the MHC in autoimmune diabetes, but studies in humans are by far the most intriguing.

As humans migrated, gradually occupying all habitable niches on the planet, survival depended on the ability to generate diverse but appropriate immune responses. We speculate that the MHC haplotypes associated with many autoimmune diseases in White populations originated from selection pressures brought to bear in northern Europe. The MHC haplotypes of the people who settled these regions are those that are most strongly associated with autoimmunity in general. Furthermore, anthropologists suggest that migrating Scandinavian explorers influenced the gene pool in many areas worldwide. This is exemplified by the fre-
frequency of northern European MHC haplotypes in the United Kingdom.

We speculate that these northern European immune mechanisms most effective in protecting against foreign antigens were those that engendered an exceptional immune response that concurrently led to hyperresponsivity and impaired self-recognition. Did a viral epidemic afflict northern Europeans millennia ago, leaving behind only those who mounted an appropriate immune response? Were those who survived left hyperresponsive to autoantigens?

Two clinical examples illustrate how MHC genes might predispose to diabetes. The first is that of American Blacks with IDDM. Their disease is strongly associated with the HLA-B8 and -B15 MHC haplotypes, precisely those that are also risk factors in northern European populations. African and American Blacks who do not share this allele have a very low prevalence of IDDM. Furthermore, some non-DR3 or non-DR4 Blacks who do develop IDDM appear to undergo spontaneous remission, suggesting the possibility of both immune system modulation and β-cell regenerative capability (12).

The second example is that of the Jewish ethnic group (13). Ashley Montague defines an ethnic group as a population that maintains physical and cultural distinctness by means of isolation mechanisms such as geography and social barriers. While clearly an identifiable ethnic group, the Jews have mixed with non-Jewish populations since their earliest days. Through both Diaspora and captivity, the discernible subdivisions of the Jews have multiplied. Yemenite Jews settled in and never left the Near East. The Sephardim migrated west, mixed with Arab and southern European populations, and settled in Spain. The German- and Yiddish-speaking Ashkenazim moved north and east, settling in central and eastern Europe. What is fascinating to the diabetologist in this history is that among all these groups of common heritage, it is the Ashkenazi Jews who have a high prevalence of both HLA-B8 and IDDM. Much of the migration occurred when, according to speculation, enrichment for MHC susceptibility alleles occurred in northern Europe.

But the Jewish story extends beyond immunology and the MHC, of course. Yemenite Jews have a very low incidence of diabetes of any kind in their Arabian homeland, but when immersed in a Western life-style after migration to Israel, many have developed NIDDM.

**CELLULAR RESPONSES AND DIABETES**

**β-Cells.** Genes controlling β-cell structure and function may also influence the expression of diabetes. In animals, there is considerable interspecies and even interstrain variation in the sensitivity of β-cells to toxins such as alloxan and streptozocin. The variable ability of β-cells to replicate is even more intriguing. Some animals have significant regenerative capability; others do not. Nesidioblastosis is a reflection of the incompletely understood generative capacity of β-cells. Whatever processes lead to β-cell injury or dysfunction, it must be remembered that the ability of β-cells to proliferate will greatly influence the clinical phenotype.

**Insulitis.** Insulitis is a hallmark of autoimmune diabetes and is regarded as evidence of autoimmune cell-mediated β-cell cytotoxicity. However, it is easy to overlook the possibility that not all the inflammatory cells present are involved in tissue destruction. Some, even most, of the immunocytes present may actually be attempting to downregulate the cytotoxic immune process. Although this is a speculative proposition, it is intriguing to note that investigators working with the experimental allergic encephalomyelitis model of autoimmunity suggest that tissue destruction requires very few of the inflammatory cells present (14). What proportion of the cells comprising insulitis are β-cell cytotoxic and what proportion may be attempting to modulate the process remains to be determined.

**Endothelium.** How lymphocytes migrate from the circulation and find target β-cells in autoimmune diabetes is an intriguing but poorly defined problem. There is activation of pancreatic endothelial class II antigens during the acute diabetic state, but the importance of this phenomenon is unknown. Recent studies have demonstrated that the pancreas may be uniquely susceptible to venular leakage. Perhaps activation of the endothelium with lymphokines induces intercellular gaps and the migration of immunocytes toward the islet β-cells.

**ETIOLOGY OF DIABETES: THE TUMBLER HYPOTHESIS**

In this article, we have adduced several factors that may eventually align to produce diabetes. We liken these factors to the tumblers of a lock. Genetic susceptibility to IDDM-like syndromes is encoded in the MHC. Cellular immunologic responsiveness dictated by other genetic determinants influences an effector lymphocyte population directed against the β-cell. This effector cell, normally downregulated, is freed only in the absence of a regulatory mechanism. The entire process is superregulated by other factors, including the aging-gene family, perhaps as it affects the involution of the thymus. Additional factors, e.g., β-cell sensitivity to cytotoxicity and the regenerative capacity of the β-cell, determine the ultimate clinical phenotype of the diabetic condition.

Different factors determine susceptibility to many cases of NIDDM. The obesity-gene family is paramount and exceeds the influence of the MHC in importance. Other factors, equally critical, include loss of β-cell responsiveness to perturbants like glucose, the presence or absence of β-cell regenerative capability, the loss of insulin receptors on target cells, and postreceptor abnormalities.

We have attempted to dissect the lock that restricts access to an understanding of diabetes mellitus. We have highlighted some of the tumblers that influence the expression of diabetes and have speculated on the many ways in which they might align to lead to what is phenotypically seen as IDDM or NIDDM. There will be simple keys, master keys, and eventually grandmaster keys. Unexpected interactions will play key roles in the design, and tumblers as diverse as diet, immunology, virology, MHC, and gene regulation (occurring in unforeseen combinations and only with the right genetic background) will lead to diabetes.

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