# Diabetes—A Possible Autoimmune Disease

## *Insulin-dependent diabetes may be caused by an immune attack on islet cells, but efforts to prevent it with immunosuppression are still controversial*

When diabetes afflicts the young, its impact can be devastating. The juvenile-onset form of the disease almost always leaves its victims totally dependent on insulin. Not only does this encumber them with a daily routine of insulin injections and dietary restrictions but many patients also eventually develop serious complications, often involving the circulatory system and culminating in blindness, limb amputations, kidney failure, or stroke. The life-span of the average insulin-dependent diabetic may be shortened by as much as 50 percent.

The cause of insulin-dependent diabetes, which affects some half million individuals in this country,* is not completely understood, but there is growing evidence that it is an autoimmune disease, one in which the immune system somehow goes awry and attacks the body's own tissues, in this case the insulin-secreting cells located in the islets of Langerhans of the pancreas. "A few years ago diabetes just didn't fit into this category at all," notes Abner Notkins of the National Institutes of Health.

The evidence also shows that, contrary to previous views, the disease develops slowly over a period of years. "Diabetes is a disease of acute onset," says G. Franco Bottazzo of Middlesex Hospital in London, England, "but it is preceded by a long latent period." During this time, the insulin-secreting cells are lost slowly and insidiously until a critical point is reached at which the amount of hormone produced becomes insufficient to support life and then is lost completely. Nevertheless, the early period of the disease is marked by immunological and metabolic abnormalities that may make it possible to identify the victims before they show more dramatic symptoms.

These new insights have already led to attempts to treat newly diagnosed patients with drugs that suppress the immune system in the hopes that this will prevent them from completely losing their insulin-secreting ability. If this should prove possible it could produce a major reduction in the patients' morbidity and mortality. Uneven control of blood glucose concentrations by insulin injections, as compared to that maintained by the endogenous hormone, apparently contributes to the complications of diabetes. Although the early trials of immunosuppression have met with some success, they have also engendered some controversy over the advisability of treating young patients—usually adolescents and children—with powerful drugs that have serious side effects of their own and may have to be administered for long periods, even for life.

The largest study to date of immunosuppressive drugs for diabetes therapy has been conducted by Calvin Stiller, John Dupré and their colleagues at University Hospital in London, Ontario.† They have now treated more than 40 patients, all of whom had been diagnosed as having insulin-dependent diabetes within a year of entering the study, with the immunosuppressive drug cyclosporine. The treated patients experienced, Dupré says, "an unexpectedly high reduction in insulin requirements."

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The reduction was most marked in patients who began taking the drug within 6 weeks of their original diagnosis. About half of this group lost their dependency on insulin injections during the year-long course of the treatment. Although newly diagnosed diabetes patients often experience a period of remission, lasting a few months to a year before completely losing their insulin-secreting ability, only about 3 percent become insulin-independent during this time. The University Hospital group now plans to participate in a larger, controlled study of cyclosporine therapy that will include four additional medical centers in Canada plus four more in Europe. A number of medical centers in this country are also beginning or contemplating similar trials.

Presumably cyclosporine acts to prevent the destruction by the immune system of the patients' insulin-secreting islet cells. Exactly how it might do this is unclear, at least partly because the immune defects that cause the destruction are themselves incompletely understood. Cyclosporine is thought to work by suppressing the division of activated T cells, which have among their many functions both direct cell-killing and the regulation of immune responses.

Researchers have found evidence of T-cell abnormalities both in human patients and in animals with diabetes. In a particularly pertinent example, George Eisenbarth of the Joslin Diabetes Center of Harvard Medical School and his colleagues observed that, compared to normal individuals, newly diagnosed diabetes patients have greatly increased numbers of activated T cells that might contribute to the immune attack on islet cells. They also showed that low doses of prednisone, another immunosuppressive drug, can lower the activated T-cell count in the patients. The investigators are currently beginning a clinical trial to determine whether this drug might suppress the development of the disease.

Despite the success of the cyclosporine study, questions have been raised about giving immunosuppressive drugs to insulin-dependent diabetics at this time. Aldo Rossini of the University of Massachusettas Medical School in Worcester says, "I feel that the potential ill effects of the immunosuppression outweigh the disease." The potential ill effects depend on the particular immunosuppressive regimen used but among the more common and serious are increased susceptibility to infections and to cancer.

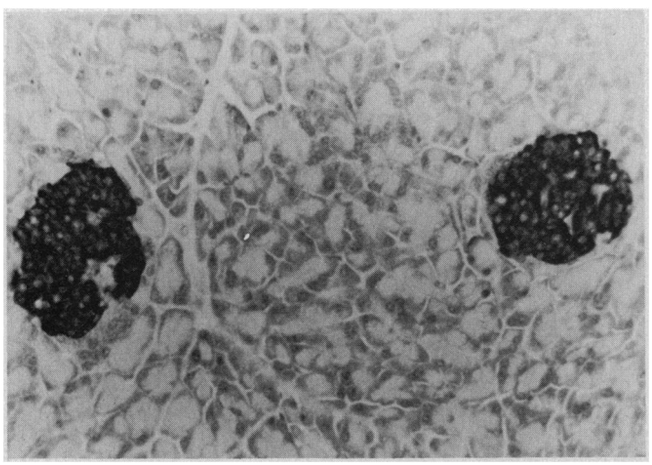
So far clinical experience with cyclosporine, a relatively new drug which has been used mainly for organ transplant patients, indicates that it is less likely to lead to cancer than older regimens. Dupré points out that there has been only one case of cancer in approximately 2000 patients. Still, as Rossini notes, the drug has been in widespread use for only a few years and it may be too early for its full effects to have emerged.

In addition, cyclosporine has been linked to kidney toxicity. According to Dupré this was not especially severe in the diabetes patients and the changes that were seen were reversible when the drug was stopped. In Dupré's view, the long-range effects of diabetes are worse than those of immunosuppression.

*An additional 9 to 10 million people have the insulin-independent form of diabetes, which usually develops later in life and is apparently a different disease.

†C. R. Stiller *et al.*, *Science* **223**, 1362 (1984).

Some of the reservations about exposing young diabetics to immunosuppression might be resolved if it could be shown that treatment for some relatively short critical period, say a year or two, is sufficient to permanently interrupt the disease process. According to Arthur Like of the Massachusetts group, there is evidence for such a critical period in the development of diabetes in the BB rat, an animal model with many features in common with the human disease.



J. Srinivasappa and Abner Notkins, NIH

***Normal Islets of Langerhans***

*The two islets have reacted with a monoclonal antibody to a virus, an example of molecular mimicry.*

At present, however, there are indications that the autoimmunity of human patients, once it is established, may be irreversible. Early results from the Canadian group suggest that cessation of cyclosporine therapy leads to a relapse. "A few months after stopping, a few patients who appeared to benefit appeared to lose that benefit," Dupré explains. Moreover, David Sutherland and his colleagues at the University of Minnesota School of Medicine have preliminary evidence that the immune systems of diabetic patients may maintain the ability to mount an attack on islet cells for many years after the disease is diagnosed.

The Minnesota group has under way a large study of pancreas transplantation in advanced diabetic patients during the course of which they have performed transplants on four individuals for whom the donors were their nondiabetic but otherwise identical twins. (Although susceptibility to diabetes is hereditary, the disease is not wholly genetic; both members of pairs of identical twins become diabetic only about 40 to 50 percent of the time.)

The great advantage of doing transplants between identical twins is that immunosuppression can be avoided because the donor tissue is not recognized as foreign by the recipient's immune system and rejected. However, as Sutherland reported at this year's meeting of the American Association of Physicians, immunosuppression appears to be necessary for successful pancreas transplants between twins. The first three twins to receive the transplants were not immunosuppressed. Although they became insulin-independent after the operation, a few weeks later their transplants gradually stopped producing the hormone.

Biopsies of the transplants did not show the typical signs of rejection, but instead revealed "insulitis," an invasion by inflammatory white cells of the islets of Langerhans. The discovery some years ago that insulitis is commonly found in newly diagnosed diabetics was one of the early clues that the disease might have an autoimmune origin.

Because all the twins in the Minnesota study had had their diabetes for at least 15 years, the presence of insulitis in the transplants suggests that the autoimmunity that initiated the disease is still active many years later. The fourth twin was immunosuppressed and has not experienced the same loss of insulin secretion by the transplanted pancreas. Still to be determined is whether there is a critical period for permanently blocking autoimmunity and the loss of insulin-secreting cells in that long latent period before overt diabetes symptoms occur.

The Minnesota findings also have potential implications for another experimental approach to diabetes therapy, one aimed at transplanting islet cells that have been treated to make them resistant to graft rejection. The idea is that the recipients would then not require immunosuppression. Such transplants work in—and cure—diabetic animals, according to Paul Lacy of Washington University School of Medicine and Kevin Lafferty of the University of Colorado Health Sciences Center in Denver.

Islet cells will not be rejected even when transplanted into animals with a different histocompatibility composition, these investigators have found, if they are first cultured under conditions that destroy their passenger lymphocytes, the cells that apparently trigger the rejection attack. (The histocompatibility molecules, which are located on cell surfaces, are the markers that enable the immune system to distinguish self from nonself.)

Both Lacy and Lafferty plan to begin islet cell transplants in human diabetes patients. The first to receive them will be individuals who will be immunosuppressed because they also are having kidney transplants. These early trials are aimed at determining whether the transplanted cells will actually secrete insulin and how many cells will be needed to make the recipients independent of injections of the hormone. Then the investigators will move on to the next stage, using the treated islet cells without immunosuppressing the patients.

But the question remains. Will the transplanted islet cells escape rejection only to be destroyed by an autoimmune attack? If they are, Lacy says, the next step might be to use islet cells as mismatched as possible to the histocompatibility makeup of the recipient, in other words to do just the opposite of what is usually done for transplants. This strategy is based on the assumption that the histocompatibility molecules of the patients' islet cells are needed to evoke the autoimmunity. If this is not the case, then the approach is unlikely to work. Meanwhile, as Lacy notes, "No one will know until the studies are done."

Another clinically significant outgrowth of the research into the autoimmune origins of insulin-dependent diabetes is the growing ability to predict who will get the disease before overt symptoms develop. Over the past few years, investigators have identified a variety of immune abnormalities in the patients. An abnormality that may be especially useful for its predictive value is the presence in the blood of antibodies against islet cell antigens.

Close relatives of diabetes patients, because they have a high risk of also developing the disease, have proved to be an especially useful group for identifying prognostic indicators. For example, about 8 to 10 percent of diabetics' siblings get diabetes, whereas only about 0.2 percent of the general population does. Prospective studies of family members, which have been conducted by a number of groups, including those of Bottazzo, Eisenbarth, and Fredda Ginsberg-Fellner at Mount Sinai School of

Medicine in New York, have shown that antibodies to islet cell antigens appear in the blood several years before an individual develops the disease.

It is not clear whether the antibodies help to initiate the immune attack on islet cells or result from it, but they do help to identify future patients especially when combined with other prognostic indicators. One of these is the histocompatibility makeup of the individual. Among the early evidence for an immune system involvement in diabetes was the discovery several years ago of an association between certain histocompatibility molecules and diabetes susceptibility. In particular, investigators including Jorn Nerup of Steno Memorial Hospital and the late Andrew Cudworth and his colleagues noted a link between the disease and the HLA-DR3 and 4 molecules. Some 90 percent of the patients have either or both of these.

Investigators are now finding in the family studies that those members who get diabetes usually have the same HLA-DR composition as their diabetic relatives. For example, Ginsberg-Fellner, who is collaborating with the Notkins group, has found this to be true for seven of the nine individuals who developed the disease since their trial began.

A third predictor of diabetes is a progressive decline in the ability to secrete insulin in response to administration of the sugar glucose, which apparently results from the slow loss of islet cells. Taken together, the three indicators, HLA-DR type, the presence of antibodies against islet cells, and declining insulin responses, are enabling accurate prediction of who will get diabetes in high-risk families. The same indicators may also work for the members of the general population although the logistical problems will be more severe for them.

The ability to predict diabetes will be invaluable should it ever prove possible to prevent the disease by immunosuppression or to cure it with islet transplants. Meanwhile it can at least help by enabling physicians to initiate insulin-replacement therapy before the child becomes very ill and suffers weight loss or the potentially fatal condition of ketoacidosis. "We have some evidence that if we treat early, there is better control," Ginsberg-Fellner says.

At present no one knows exactly why the immune system launches its abnormal attack on islet cells. A better understanding might help to design a more specific immunosuppressive therapy, one that could knock out the aberrant reaction causing the destruction without producing a general loss of the body's immune defenses.

For example, it might be possible to block production of antibodies to the islet cells, if one or another of these is shown to trigger the immune attack. Or, if some subpopulation of immune cells can be identified as at fault, it might be possible to destroy it, perhaps with a monoclonal antibody. In the BB rat at least there is evidence that a lymphocyte population mediates islet cell destruction. The Rossini group has found that they can produce the disease in rats by injecting them with activated lymphocytes from diabetic BB rats. In addition, transfer of T cells from a strain of BB rats that were bred to be resistant to the disease prevented diabetes development in susceptible animals. The resistant animals appear to have a T-cell population that suppresses the effector cells in susceptible animals.

**There is a growing ability to predict who will get the disease before symptoms develop.**

The HLA-DR molecules are important regulators of immune responses and the presence of the DR3 or 4 variants no doubt contributes to diabetes susceptibility, although the way in which they do this is not understood. Moreover, having either molecule does not guarantee that an individual will get the disease. Roughly 40 to 50 percent of the population has at least one.

However, new results suggest that diabetes susceptibility may be linked to the presence of variants of the DR3 and 4 antigens that are not detected by the serological tests ordinarily used for classifying histocompatibility molecules. Åke Lernmark and David Owerbach of the Hagedorn Research Laboratory in Gentofte, Denmark, and their colleagues have directly examined the HLA-DR genes of diabetics and found that they are not the same as those of normal individuals. In addition, the Eisenbarth group has observed structural variations (polymorphisms) in the equivalent genes of the BB rat. Diabetic animals have one particular variant. "The polymorphism can distinguish those susceptible from those that are not," Eisenbarth says. The variants possessed by the diabetics might produce altered immune responses in these individuals.

One way in which the HLA-DR molecules might contribute to an immune attack on islet cells has recently been suggested by Bottazzo and his colleagues. The molecules are normally found only on immune cells where they are needed for antigen presentation and the activation of immune responses. However, the Bottazzo group has detected them on normal endocrine cells that have been stimulated to divide and also on islet cells from a diabetes patient. They propose that the aberrant presence of the HLA-DR antigens helps to activate the autoimmunity that leads to islet cell destruction. Additional defects, perhaps in the suppressor cells that normally keep immune responses in check, would also contribute.

Because insulin-dependent diabetes is not a completely genetic disease, environmental influences must also contribute. There is evidence, from Notkin's laboratory and others, that links infection by any of a number of viruses to the development of the disease. At present, the viral contributions are not fully understood, although there are a number of ways in which they might act. For example, Bottazzo suggests that viral infections might act by eliciting HLA-DR expression on islet cells. A virus may rarely destroy islet cells directly, but, because of the long latent period of diabetes and the evidence for involvement of the immune system, other mechanisms are more likely.

One virus that has been specifically linked to diabetes is rubella. Studies by researchers in Australia and by Ginsberg-Fellner and her colleagues have shown that about 20 percent of children who have been infected with this virus while still in the womb eventually develop the disease. Rubella virus may work by establishing a persistent infection in islet cells, although this has not yet been proven. A persistent infection, by eliciting an immune attack directed at viral antigens, could also cause the destruction of the infected cells.

Viruses might trigger an attack on islet cells, even without infecting them directly, Notkins suggests, if antibodies to the virus are also capable of recognizing islet cell antigens. There is evidence for this type of molecular mimicry. Notkins and his colleagues have identified monoclonal antibodies to viruses that can react with cellular antigens, including those of islet cells. In any event, whatever initiates the autoimmunity of insulin-dependent diabetes the research is producing new hope that the disease may one day be conquered.—**JEAN L. MARX**