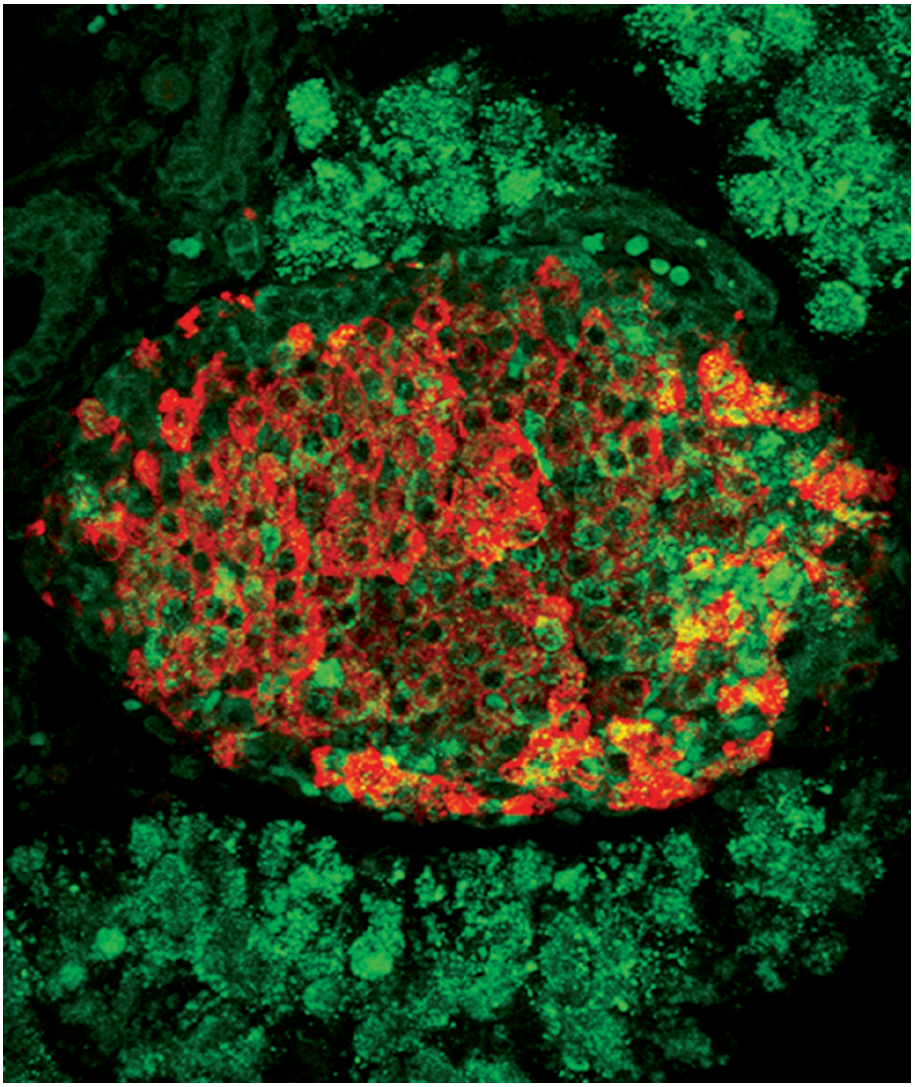

The Coxsackie virus has been linked to diabetes, but do viral infections trigger or stave off diabetes?

FLODSTROM, M. *ET AL. NAT. IMMUNOL.* **3**, 373–382 (2002).

PATHOLOGY

Cause and effect

Decades of study into the causes of diabetes have produced no definitive answers.

BY ERIKA JONIETZ

Type 1 and type 2 diabetes have long been viewed as two diseases, the first auto-immune with a large genetic component, the second metabolic, linked to obesity and a sedentary lifestyle. "These are two very different disorders," says C. Ronald Kahn, a senior investigator at Joslin Diabetes Center and professor of medicine at Harvard Medical School in Boston, Massachusetts. "They lead to similar metabolic problems and similar long-term complications, but they have two very different pathogenic routes."

Researchers, however, are showing that each type has more in common with the other than once believed: both involve a faulty immune system and share some mechanisms that ultimately kill the insulin-producing beta cells in the pancreatic islets. Yet in neither type 1 diabetes (T1D) nor type 2 diabetes (T2D) does genetics or behaviour fully explain why some people get the disease and others don't.

Recent findings show that despite some common risk factors, the two are indeed separate conditions. In addition to the many genetic factors involved, scientists have implicated epigenetic and environmental influence in each type of diabetes. Researchers continue to search for certain causes in an effort to prevent both.

ALL IN THE FAMILY?

Type 1 diabetes is an autoimmune disease in which the immune system kills insulin-producing beta cells. It runs in families, the hallmark of any genetic disease. About 60% of the genetic risk comes from a few specific variants in the human leukocyte antigen (HLA) genes. These genes encode the proteins that present antigens to immune cells and are involved in the misguided immune response in T1D.

Better understanding HLA, therefore, could help unravel the origins of T1D. Over the past five years, George Eisenbarth, an endocrinologist at University of Colorado Medical School in Denver, along with immunologist John Kappler at National Jewish Health, has been working out the structure of a three-protein complex he believes is the crux of the disease. The complex consists of an antigen-presenting HLA molecule, the antigen itself (a specific insulin peptide) and a T-cell receptor that recognizes the HLA–antigen combination.

T cells are central to all autoimmune diseases, including type 1 diabetes. Normally, cytotoxic T cells destroy only infected cells; T cells that react to molecules native to the body are eliminated before they mature, thus endowing the immune system with tolerance to 'self'. In type 1 diabetes, however, things go awry: T cells primed to recognize beta cells enter circulation and go on to attack the cells. How these T cells escape destruction and reach maturity isn't clear. A number of factors appear to be involved, including variations in the gene encoding insulin, diet, and the presence or absence of certain bacteria in the gut flora (see 'The critters within', page S12).

Eisenbarth was involved in much of the early work that identified the antigens that prime T cells against beta cells; besides insulin, the major autoantigens are ZnT8, GAD65 and IA-2. "By following the development of antibodies to these four antigens," Eisenbarth says, "we can now predict diabetes." He adds: "Whoever has two of those, they almost all get diabetes."

Further insight into the origin of diabetes could come from a new technology that can track the development of the disease in humans and mice. Researchers at Harvard Medical School and Massachusetts General Hospital in Boston have used magnetic resonance imaging (MRI) of magnetic nanoparticles to visualize insulitis, the inflamed pancreatic tissue that is the earliest clinical manifestation of diabetes. They also used MRI to distinguish at just 6–10 weeks of age which non-obese diabetic (NOD) mice — a model of T1D — will develop full-blown diabetes; mice with the highest pancreatic accumulation of the magnetic nanoparticles, used as a probe, got diabetes.

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"We now have a way to know very early whether [mice] will or won't get diabetes and then compare them at the molecular level," says Diane Mathis, an immunologist at Harvard Medical School. Performing these comparisons has enabled her group to identify several previously unknown molecular and cellular elements associated with a lower chance of the mice developing diabetes.

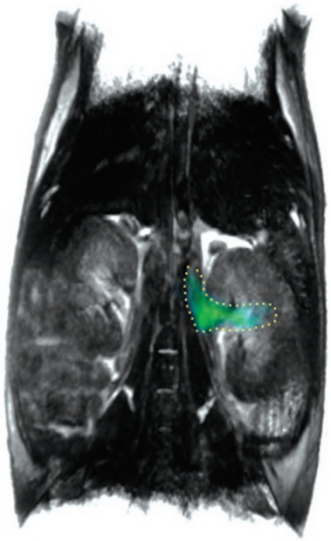
As not everyone with a genetic susceptibility to T1D actually develops the disease, some sort of trigger might be involved. Evidence suggests that a viral infection — possibly by enteroviruses such as the Coxsackie virus — causes the immune system to misbehave. There are two theories about viral exposure: one suggests that viruses and other microorganisms improve tolerance and may thus protect against T1D. The presence of such pathogens might help overcome a sort of "boredom of the immune system" resulting from fewer childhood infections, says Matthias von Herrath, director of the Type 1 Diabetes Center at La Jolla Institute for Allergy and Immunology in California. The other theory is that a virus somehow exposes antigens on beta cells, causing the immune system to attack them.

To determine whether viruses or anything else in the environment trigger type 1 diabetes, the Diabetes Auto Immunity Study in the Young (DAISY) began in July 1993. DAISY HLA-typed about 30,000 newborns and enrolled children with a parent or sibling with T1D or children in the general population with genetic markers that indicated they were at moderate or high risk for the disease. Researchers collected blood samples and interviewed parents about diet, health and other aspects of their children's lives; as of February 2007, 61 of the children were diagnosed with type 1 diabetes. According to the DAISY organizers, the team identified several autoantigens and genes associated with T1D over 15 years. They also linked diet to the onset or delay of diabetes, and disproved any association between type 1 diabetes and the age of childhood vaccinations. And although a small prospective study found no link between enterovirus infection and T1Ds, the team noted the need for more studies.

IT'S COMPLICATED

The search for the triggers of type 2 diabetes is not any easier. This condition occurs when muscle and fat tissue respond abnormally to insulin, together with a failure of beta cells to compensate by pumping out more insulin. The statistical connection between T2D and a high-calorie diet and sedentary lifestyle is well established, but researchers still debate how — or whether —these factors cause the initial resistance to insulin. After all, 75-80% of obese people never develop type 2 diabetes. Moreover, as with type 1 diabetes, type 2 diabetes seems to run in families. Together these data suggest genetic elements.

The data from genome-wide association studies (GWAS) are far from clear, however. Studies so far have identified more than 40 genes associated with T2D, most of them having to do with beta-cell function[1]. But added together, they account for only about 10% of the apparent genetic causes. To find the missing heritability, biochemist Alan Attie at the University of Wisconsin-Madison has crossbred two strains of mice used as models — one obese but non-diabetic, the other obese and prone to diabetes — to hunt down genes linked to intermediate processes involved in diabetes, such as those that govern beta-cell regeneration, insulin degradation and insulin secretion.



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MRI of a mouse pancreas (colour) tracks disease progression at the cellular level.

Some genes implicated by GWAS are expressed only in adipocytes (fat cells), which might help explain how overeating can lead to diabetes. Adipose tissue stores excess lipids, which are otherwise toxic to the body. When fat cells malfunction and aren't able to store away the extra lipids generated by overeating, lipids begin to accumulate in muscle tissue and in the liver. Philipp Scherer, a diabetes researcher at the University of Texas Southwestern Medical Center in Dallas, believes this aberrant build-up triggers insulin resistance. When adipose tissue expands in a healthy way there is no insulin resistance — which for Scherer explains why some obese people never develop type 2 diabetes.

"Researchers in the field are confused and have different opinions."

Another consequence of abnormal adipose growth is inflammation. Expanding fat mass produces proteins called cytokines and other substances that promote inflammation and recruit macrophages (killer immune cells). As macrophages accumulate in adipose tissue, they change and secrete even more cytokines and other inflammatory factors into the bloodstream. This promotes inflammation in other tissues, including pancreatic islets. Researchers agree almost unanimously that insulitis plays a role in type 2 diabetes; the nature of that role, however, is still a matter of debate.

While Scherer sees the inflammation as a result of insulin resistance, other biologists believe inflammation is a primary cause of diabetes. Steven Shoelson, a doctor and structural biologist at Joslin Diabetes Center and Harvard Medical School, sees things the latter way: he believes that cytokines released in response to metabolic stress may directly lead to insulin resistance. Shoelson is involved in trials to assess whether salsalate, a non-steroidal anti-inflammatory drug, can lower levels of sugar and lipids in the blood of patients with T2D, and plans to present the results of the latest large-scale trial of salsalate at the American Diabetes Association meeting in June 2012 in Philadelphia, Pennsylvania.

But even genetics, diet and activity levels combined don't completely explain the origins of type 2 diabetes. Other factors that might contribute include environment toxins and the gut microbiome. Another influence may be maternal diet: research in both mice and humans has shown that maternal caloric restriction during gestation increases the risk of T2D in offspring[2]. The mechanism might involve strong epigenetic programming, Kahn says. Rat and human studies, for example, show that poor diet during pregnancy may affect the expression of genes that influence fetal fat-cell development, making it harder for adipocytes to effectively store excess lipids.

In a novel effort to identify specific environmental factors associated with T2D, Atul Butte, a paediatric endocrinologist and medical informaticist at Stanford University in California, created an environmental-wide association study analogous to GWAS[3]. A pilot study found significant links between T2D and the pesticide derivative heptachlor epoxide, vitamin E and polychlorinated biphenyls (PCBs).

Despite experts' increasing knowledge about both types of diabetes, much about the pathologies of both diseases and virtually everything about their aetiologies remains a mystery. Most researchers acknowledge that it's unlikely there is a single trigger; some even suggest that different genes and environmental factors may lead to disease processes that differ from person to person. "Researchers in the field are confused and have different opinions," says Shoelson. Whether scientists ultimately find the factors that cause diabetes or not, Scherer agrees, "the bottom line is, it's complicated". ■

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