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Innovations

New Approaches to Diabetes Disease Control, Insulin Delivery, and Monitoring

In the United States, 18.2 million people have the endocrine disease diabetes, but as many as one-third do not even know it. About 5%–10% have type 1 diabetes, whereby the body fails to make sufficient insulin and the person requires exogenous insulin supplementation. Most Americans who are diagnosed with the disease have type 2 diabetes, which usually develops later in life. This type of diabetes is often successfully managed through lifestyle modifications and possibly non-insulin medications, although some people with type 2 diabetes require insulin support.

Because diabetes is a medical condition without any cure at this point—and its incidence is on the rise—researchers are actively seeking advances targeting the source or triggers of the disease, delivery of therapeutics, and tighter management of its long-term complications.

Anti-CD3 Antibodies May Delay Disease Progression

Researchers in the United States and Europe are enthusiastically pursuing therapies designed to halt or slow the progression of type 1 diabetes, even if it may not yet be possible to stop it from developing in the first place. Type 1 diabetes mellitus is a chronic autoimmune disease that results when T lymphocytes cause the destruction of insulin-producing beta cells inside pancreatic islet cells. One such therapy involves treatment with anti-CD3 monoclonal antibodies (Mabs), the focus of which is two-fold: to directly inhibit the action of destructive T cells and simultaneously induce the action of protective T regulatory cells. Researchers involved in anti-CD3 antibody research believe the antibody reestablishes a balance between pathogenic and regulatory T cells such that diabetes disease progression may be significantly slowed.

The original anti-CD3 monoclonal antibody, known as OKT3, was derived from murine sources and has been used extensively in transplantation medicine. In 1994, when research into anti-CD3 Mabs began in earnest in animal studies, a humanized version was not available. "The antibodies used then in clinic for transplantation induced side effects because they can also activate T cells, which in turn induces cytokine release," says long-term anti-CD3 antibody researcher Lucienne Chatenoud, M.D., from the Laboratoire d'Immunologie at Hôpital Necker in Paris. "When these antibodies were injected in vivo to transplant patients, they induced the flu-like syndrome. This was something affordable in the transplant setting because [transplant rejection] is a life-threatening situation, but it was not the kind of side effect that could be afforded in the context of a disease like type 1 diabetes, where there is an alternative treatment." Humanized anti-CD3 monoclonal antibodies, such as hOKT3gamma1(Ala-Ala), are mouse-human hybrid antibodies that selectively bind to beta-cell-specific T phocytes. Humanized versions do not activate harmful inflammatory cytokines that cause nausea, fever, headache, hypertension, and even cardiac problems, commonly seen in the transplant setting with murine-based versions. In May 2002, researchers published the first human clinical studies of hOKT3gamma1(Ala-Ala) in people who had been diagnosed within 6 weeks with type 1 diabetes. Although the study was small—24 volunteers in total—the results are encouraging. After only a single 14 day course of antibody treatment, 9 of 12 patients had maintained or improved insulin production as far out as one year. Responses were seen as soon as 30 to 90 days after treatment. In contrast, only 2 of 12 control volunteers had a sustained response. Very recently, the researchers followed the subjects for another year—without retreatment—and found a sustained effect. "It does not permanently prevent diabetes from progressing, but it at least seems to stabilize things for quite a while. It kept them from progressing with just 10–14 days of treatment," says lead scientist for this study, Kevan C. Herold, M.D., who is an associate professor at Columbia University College of Physicians and Surgeons in New York. Herold found that the antibody reduces glycosylated hemoglobin levels as well as necessary insulin doses. Non-severe side effects were limited mainly to fever, rash, and anemia. Drs. Chatenoud and Herold will publish results of other phase II human studies with anti-CD3 monoclonal antibodies in 2005.

Although it may not yet be possible to prevent diabetes—which affects 18.2 million people in the United States—in the first place, researchers are pursuing several promising leads on ways to slow or halt the disease.

Inhaled Insulin Delivery

Once type 1 diabetes (or occasionally type 2) progresses to the point at which insulin is required, treatment with one of a number of needle-based injection devices is fairly standard. However, several companies are pursuing insulin systems based on inhaled delivery. One such company, Hayward, California-based Aradigm Corporation, partnered with diabetes care giant Novo-Nordisk in 1998 to develop the AERx Diabetes Management System (AERx iDMS), an aerosolized inhaled insulin system. "The foundation of Aradigm, and AERx iDMS in particular, is to use microprocessors within a medical device to enable precisely controlled delivery of an aerosolized drug from that device," explains Steven Farr, Ph.D., Chief Scientific Officer at Aradigm. "The company

was founded on the theory of using the lung as a portal of entry for drugs that are currently given by injection, of which insulin is a great example."

Dr. Farr explains that the greatest difference between AERx iDMS and other inhaled insulin systems in development is that Aradigm has developed a liquid formulation of insulin that becomes aerosolized into the lungs of patients when they inhale it. "All of the other players are developing dry powders of insulin," says Dr. Farr. "We selected an aqueous system because insulin has been in a liquid formulation in excess of 70 years. We leveraged all that was known about an injectable form of insulin to create a specific formulation incorporated into our device for inhalation. The excipients we use to stabilize the insulin in our aerosolized formulation are the same as those in the injectable formulations. Our testing to date shows this approach to be safe."

The AERx iDMS system, currently in phase III clinical testing with partner Novo-Nordisk, was designed to offer the major conveniences and requirements of today's pen injectors. "This device allows patients to adjust their doses with the same degree of accuracy and precision they demand from pen injection systems," says Dr. Farr. To create the aerosol in the AERx device, a small aliquot of liquid insulin, according to the required dose, passes through a plastic film manufactured with 1 μm-size holes. "That was a significant technological challenge we had to resolve in order to ensure we had the right-sized aerosol particles for deep lung delivery," recalls Dr. Farr.

Other inhaled insulin products—though all based on dry-powder insulins—are in the development pipeline. These include the Exubera product, which Pfizer, Aventis, and Nektar Therapeutics now have in phase III testing and for which they are awaiting European market approval. Eli Lilly, another major player in the diabetes market, has joined ranks with drug delivery manufacturer Alkermes to produce the inhaled AIR delivery system. The product is now in phase II testing for diabetes. Additionally, Mannkind Corporation just completed phase II testing with its Technosphere Insulin System, although no commercial development or marketing partner has yet been identified.

Monitoring for Kidney Complications

Some of the most dangerous outcomes of either form of diabetes are those related to long-term complications, including kidney damage, or diabetic nephropathy. Over time, small blood vessels inside the kidneys can become damaged and reduce the body's ability to filter toxins from the blood. Damaged kidneys leak a protein, albumin, into the urine. Detection of even small albumin levels, microalbuminuria, is one of the earliest signs of kidney problems, and also one that is not always noticeable in the earliest stages of the disease. Left untreated, the damage worsens until the kidneys fail, a condition called end-stage renal disease (ESRD). Because of the serious long-term consequences of kidney damage, in mid-November, 2004 the International Society of Nephrology urged national health bodies around the world to proactively implement albuminuria screening with the goal of preventing further kidney function deterioration in diabetics and others with kidney disease. Currently, the American Diabetes Association recommends that people diagnosed with type 2 diabetes be tested for microalbuminuria at the time they are diagnosed and every year thereafter. Those with type 1 diabetes should be tested 5 years after diagnosis and every year thereafter.

New York, NY-based AusAm Biotechnologies, founded in 2000, has developed a new type of diagnostic test to better detect extremely low levels of albuminuria. The FDA approved the company's Accumin diagnostic test in August 2003 as the first test to measure total intact urinary albumin. The technology is based on the work of Wayne Comper, Ph.D., D.Sc., of Monash University, Australia. "The problem with conventional tests for urine albumin is that they can miss intact albumin that does not react with the antibodies used in making these tests. As a result, conventional urine testing is subject to a potentially large false negative rate for microalbuminuria," says Mr. James McCullough, AusAm's CEO. "It was derived from raising antibodies to serum albumin. Dr. Comper recognized that as the serum albumin passes through the kidney and out through the urine, it can undergo a slight conformational change rendering it unreactive to conventional serum-derived albumin antibodies. In other words, intact albumin that is invisible to conventional antibodies—which is in all the major manufacturers' platforms and dipsticks—ends up unrecognized in the urine." In the clinical tests that Dr. Comper and colleagues performed, conventional tests generated a 33% false negative rate for microalbuminuria when they were compared with Accumin.

"In May 2004, we published that we can detect microalbuminuria up to 12 years earlier than radioimmunoassay, which is the most sensitive of the conventional urine tests," says McCullough, "But on average, it is really about 3 years earlier." The key, claims McCullough, is that the antibody used in the test was raised to urine albumin. "After doing 2D electrophoresis and HPLC, we discovered this whole population of intact albumin which was unrecognizable by the conventional antibodies in all the major immunoassays around the world," says McCullough.

The test is done in combination with a test for creatinine, a protein excreted by the muscles. The results are reported as a ratio of microalbumin/creatinine. "If you are above 30 mg of albumin per gram of creatinine, you are said to be microalbuminuria positive," explains McCullough. "We can pick up as low as 3–4 mg albumin per gram of creatinine."

"We are platform agnostic," says McCullough. "The FDA-approved Accumin quantitative test is currently done with chromatography. But we are in the process now of working with several analyzer companies to place the technology in high-throughput immunochemical devices." In 2005, AusAm hopes to roll out a dipstick version of the technology to bring it even closer to the point of care.

***Chemistry & Biology* invites your comments on this topic. Please write to the editors at chembiol@cellpress.com**

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