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- A link between diabetes prevention and the hygiene hypothesis
- The protein interferon-alpha appears to trigger type 1 diabetes
- Transplanted islets survive in mice without the use of immunosuppressive drugs

## JDRFI-Funded Study Links “Hygiene Hypothesis” to Diabetes Prevention

A research study funded by JDRFI suggests that common intestinal bacteria may provide some protection from the development of type 1 diabetes. The findings provide an important clue toward understanding how and why type 1 diabetes develops and may lead to potential treatments and cures. The study, reported this past October in the journal *Nature*, lends further support to the “hygiene hypothesis,” which postulates that exposure to an appropriate amount and variety of bacteria, viruses, and other microorganisms may be important to living a healthy life, and that susceptibility to type 1 diabetes and other autoimmune disorders may actually be caused by a lack of exposure to these agents.

In this research, teams led by Li Wen at Yale University School of Medicine and Alexander V. Chervonsky at the University of Chicago studied mice that under normal conditions would not develop diabetes. When raised in a germ-free environment, however, these mice developed severe diabetes. The researchers were able to establish that the microbial environment was responsible for this difference because mice that were exposed to common intestinal bacteria maintained a lower risk for the disease. (The bacteria the mice were exposed to were actually harmless microbes that are often found in the human intestine.)

The study’s findings suggest that the interaction between intestinal microbes and the early immune system can somehow modify the risk for type 1 diabetes. In these

experiments, exposure to certain bacteria triggered an immune system response in the mice. That response, although not fully understood, is believed to be what prevents autoimmune disorders. The scientists thus suggest that safe, measured exposure to certain bacteria may indeed lower the risk of immune disorders.

“This study outcome gives us a new avenue to explore,” said JDRFI executive vice president of research Richard A. Insel, M.D. “With type 1 diabetes in the U.S. and in many countries around the world at about a three per cent annual rate, every lead is significant. The research gives impetus to better understanding how the bacterial flora in our body influences host immune defenses and responses that provide resistance to the development of type 1 diabetes. This understanding may suggest new therapeutic approaches to prevention.”

## Key Point:

Common inteastinal bacteria may somehow interact with the early (innate) immune system to modify type 1 diabetes risk, a finding that supports the hygiene hypothesis.

## Diabetes Researchers Discover an Early Trigger of Autoimmunity

JDRFI-funded researchers from Stanford University School of Medicine have shown that the protein interferon-alpha (IFN- $\alpha$ ), an immune signal molecule, is an early initiator of the autoimmune attack that leads to type 1 diabetes. The finding, in mice, sheds important new light on how type 1 diabetes first develops and may point to new prevention and treatment targets.

According to the researchers, it was somewhat surprising to identify IFN- $\alpha$  as a major player in early type 1 diabetes. Dr. Hugh McDevitt, the study’s senior scientist, explained that the main function of IFN- $\alpha$  in the body is to fight viral infections,

both acute and chronic, and that synthetic forms of the protein are used clinically to treat viral diseases such as hepatitis C and the flu.

“IFN- $\alpha$  is generally not thought of as a pro-inflammatory agent,” he added, alluding to a behaviour seen in many of the other molecules that are involved in the development of type 1 diabetes.

A central role for IFN- $\alpha$  was established using DNA microarrays, a molecular technique that can quickly identify expression levels of thousands of genes simultaneously. The researchers were specifically interested in identifying changes in gene expression during the development of diabetes. Knowing that CD4+ T cells are involved in the early autoimmune attack on the insulin-producing beta cells, they examined gene expression changes in these cells both before and after the onset of diabetes in mice with different susceptibilities for the disease. They performed their assays on CD4+ T cells taken from the pancreatic lymph nodes, since it is within the pancreatic lymph nodes that these cells often become self-reactive, leaving eventually to infiltrate the islets where they provoke inflammation and beta cell destruction.

### IFN- $\alpha$ activates T cell genes

Microarray analysis revealed that many of the CD4+ T cell gene that are “up-regulated,” or revved up in expression, just before these cells infiltrate the islets are genes controlled by IFN- $\alpha$ . Expression levels of several of the IFN- $\alpha$ -controlled genes were measurably higher in four- and six-week-old mice that develop an aggressive early diabetes than in two-week-old mice who were at a less advanced stage of disease—in one instance, the difference was about 150-fold.

### Other findings

Perhaps most noteworthy, the researchers were able to alter the progression of diabetes in the mice by using an antibody to block IFN- $\alpha$  activity. Two to three weeks after birth but well before developing diabetes, mice were injected with either the blocking antibody or a non-interfering control antibody. Among the control mice, diabetes first presented at 12 weeks of age. By contrast, the treated mice had (on average) a four-week delay in disease onset. Furthermore, by 45 weeks of age, all of the control mice—but only 40 per cent of the treated mice—had become diabetic.

Given the increased expression of IFN- $\alpha$ -induced genes, the researchers took the next steps of examining levels of the protein itself in the pancreatic lymph nodes as well as investigating the principal cellular source of IFN- $\alpha$ : a type of circulating immune cell known as the plasmacytoid dendritic cell.

What they found is that in three- to four-week-old mice—an

age that was identified as a critical period for the initiation of T cell autoimmunity to beta cell antigens—both IFN- $\alpha$  levels and the number of IFN- $\alpha$ -producing dendritic cells were markedly increased relative to that in younger mice. Moreover, in the pancreatic lymph nodes of the two- to three-week-old mice who were given the neutralizing antibody, and whose diabetes was delayed or prevented, the researchers observed a notable increase in the number of *immature* dendritic cells. When dendritic cells are in an immature form, the authors explained, they are somehow able to dampen misdirected T cell responses.

### What may be happening

Taken together, these findings paint a compelling picture of the early molecular events that may trigger type 1 diabetes. Within the pancreatic lymph nodes and in normal circumstances, IFN- $\alpha$  is produced by dendritic cells that encounter an invading virus; in this situation, IFN- $\alpha$  draws attention to cells that should be cleared from the body and also initiates steps that prevent additional cells from becoming infected. In the earliest stages of diabetes, the authors suggest, it is debris from dying beta cells that activates the dendritic cells to produce greater amounts of IFN- $\alpha$ . While this mechanism may be more complex in humans, the consequence in mice is increased expression of those CD4+ T cell genes under IFN- $\alpha$  control, CD4+ T cell infiltration into the pancreatic islets, and ultimately, the onset of diabetes.

An interesting sidebar to the Stanford research is that the findings support the idea that viral infection by itself may be an independent trigger of the diabetic process.

“About 15 to 18 per cent of children exposed to rubella virus in the womb develop type 1 diabetes by age 18,” a strikingly high number, Dr. McDevitt said, adding that these individuals often have the same increased frequency of diabetes susceptibility genes that is seen in type 1 children not exposed to rubella during gestation. “Since the advent of rubella vaccination, it has not been possible, however, to study this striking phenomenon as it develops,” he noted. “But the data do suggest that rubella exposure *in utero* potentiates an underlying partial, and possibly insufficient, genetic susceptibility for type 1 diabetes.”

Important next steps will be to establish which of the many IFN- $\alpha$  genes are actually involved in initiating the diabetic process—there are 14 known IFN- $\alpha$  genes in the mouse and 13 in humans. Once this has been determined, researchers can then begin to develop more refined strategies that block the actions of these molecules.

The study was published this past summer in the journal *Proceedings of the National Academy of Sciences*. Collaborating with Dr. McDevitt were Qing Li, Baohui Xu, and Sara Michie from Stanford University School of Medicine in

California; Kathleen Rubins, a former Stanford scientist who is now a research fellow at the Massachusetts Institute of Technology; and Robert Schreiber of the Washington University School of Medicine in St. Louis, Missouri.

## Key Point:

The protein interferon-alpha, which is not typically associated with autoimmune disease, appears to be an essential initiator of the cascade of events that lead to type 1 diabetes.

## A New Antigen Therapy Brings Long-Term Survival of Transplanted Islets without Immunosuppression

Human islet transplantation is a promising therapy for type 1 patients. After receiving infusions of healthy islets from a cadaver donor pancreas, most islet recipients not only experience a period of complete insulin independence, but also have longterm improvement in blood sugar control and are at significantly reduced risk for life-threatening episodes of hypoglycemia. However, patients who undergo the procedure must continually take immunosuppressive drugs to keep their bodies from rejecting the new islets, drugs that can cause side effects and be toxic to the insulin-producing beta cells.

To circumvent this need for life-long immunosuppression, diabetes researchers have been investigating ways to make an islet recipient's immune system fully accepting of the donor tissue, achieving what is called "donor-specific tolerance." As reported in the journal *Proceedings of the National Academy of Sciences*, JDRFI-funded researchers are a step closer to reaching this important milestone.

Scientists Stephen D. Miller, Xunrong Luo, and colleagues from Northwestern University Feinberg School of Medicine in Chicago have developed an efficient way to prevent islet rejection in diabetic mice without using toxic immunosuppression. "The lack of a need for any immunosuppression and the avoidance of associated toxicities make this protocol a highly attractive potential therapy for human islet cell transplantation," the authors stated.

"Currently, transplantation of islets harvested from deceased individuals is the only practical solution for islet cell replacement," but the procedure is associated with robust donor-directed immune responses and also puts patients at risk for both opportunistic infections and possibly cancer, explained Dr. Miller, the study's principal investigator.

"Therefore, the establishment of a donor-specific tolerance protocol without a requirement for immunosuppression is highly desirable within the broader goal of finding a safe and effective cure for type 1 diabetes in humans."

To induce tolerance, Dr. Miller and his colleagues used a unique antigen-specific treatment (antigens are substances that can trigger an immune response). The antigens of interest to them were proteins known as MHC molecules—and specifically those from the islet donor. MHC proteins, which play a central role in tissue rejection, are expressed on the surface of cells and help to distinguish self antigens from foreign ones.

## Delivering spleen cells, then the islets

As a first step, the researchers isolated a type of antigen-presenting white blood cell from the islet donor's spleen, called splenocytes, and treated these cells with a chemical known to interfere with cell-to-cell communication. The chemically treated splenocytes, which remain intact but exhibit an altered immune profile, were then injected into diabetic mice seven days before and one day after the mice underwent an islet cell transplantation.

Providing just the two infusions of treated donor splenocytes led to prolonged survival of the donor islets—even though no immunosuppressive drugs were added to the protocol to curtail the immune response. A full 70 per cent of the islet recipients achieved so-called "indefinite graft survival," meaning the transplanted islets survived for more than 100 days.

"The large percentage of islet recipients displaying indefinite graft survival was somewhat surprising to us," Dr. Miller said, "given the knowledge that a substantially large percentage of T cells in the mouse, about five per cent, are known to react against any given whole MHC mismatch. We feel that our success reflects the high potency of this method for inducing tolerance."

## Additional outcomes

The surviving islets also produced substantial amounts of insulin within the first two weeks following transplantation, and robust insulin secretion continued long-term.

In contrast, giving the mice untreated splenocytes or splenocytes from a mouse other than the donor did not bring these benefits, indicating that protection is donor specific and highly dependent on the chemical treatment that was used. Ultimately, the researchers showed that their antigen-based treatment led to a sustained immune unresponsiveness in the islet recipients—that is, once tolerance to the transplant was established, it was permanent. As confirmation, the investigators showed that when a second same-donor islet graft was given to the mice with long-term islet survival, the mice accepted the graft without further therapeutic intervention.

## Key Point:

In diabetic mice, researchers have developed an effective new way to prevent the rejection of transplanted islets without using immunosuppressive drugs. The protocol has strong therapeutic potential for human islet cell transplantation..

## The findings explained

Additional experiments suggested that the treated splenocytes may have induced tolerance by independently initiating a decrease in the activity of effector T cells and an increase in the activity of regulatory T cells. According to the investigators, these two cell types—the first orchestrates immune responses and the second suppresses them—likely interacted synergistically to establish tolerance to the donor MHC molecules.

“It is possible,” they explained, “that donor-specific T effector cells are kept effectively in a state of anergy [or unresponsiveness] by continuous interaction with the tolerated graft.”

Next steps for the researchers will be to try the tolerance-induction strategy in mice with autoimmune diabetes. (In the current study, diabetes was chemically induced; the mice were otherwise healthy.) Dr. Miller said they will attempt to

cure overt diabetes not only by inducing tolerance to the transplanted islet cells but also by overcoming the recipient's inherent autoimmunity. Their approach will involve the use of treated splenocytes that display both the donor antigens (to prevent rejection of the transplanted islets by T cells directed against donor MHC) and beta cell antigens (to suppress the autoreactive T cell response that destroyed the beta cells originally). The researchers decided to use a combined approach because initial experiments showed that induction of tolerance to the MHC antigens alone will not lead to a reversal of diabetes.

## Related research

Dr. Miller's team is also interested in determining if chemically treated immune cells can be used to induce tolerance to xenogeneic islet transplants, which are transplants between different species. In his words, “Can we tolerize mice to islets from another species, such as rat islets transplanted into mice?” If so, this would support “the eventual possibility of using transgenic pigs as a source of islets for transplant to humans.”

The tolerance-induction strategy described in this report is being evaluated for the treatment of other autoimmune diseases as well. In the spring of 2009, there are plans to launch a phase I/IIa clinical trial in patients with multiple sclerosis; the study will test tolerance induction to brain autoantigens using Dr. Miller's protocol. Success here, Dr. Miller said, will also set the stage for tests of the tolerance induction protocol in type 1 patients who undergo islet transplantation. ■



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