

# Immunotherapy for type 1 diabetes

Type 1 diabetes is an autoimmune disorder in which the immune system attacks the body's own insulin-producing pancreatic beta cells. When the body targets and destroys its own cells, it is often called a failure of "tolerance." Making the body "tolerate" its "self" is a promising strategy in preventing autoimmune attack in those who are:

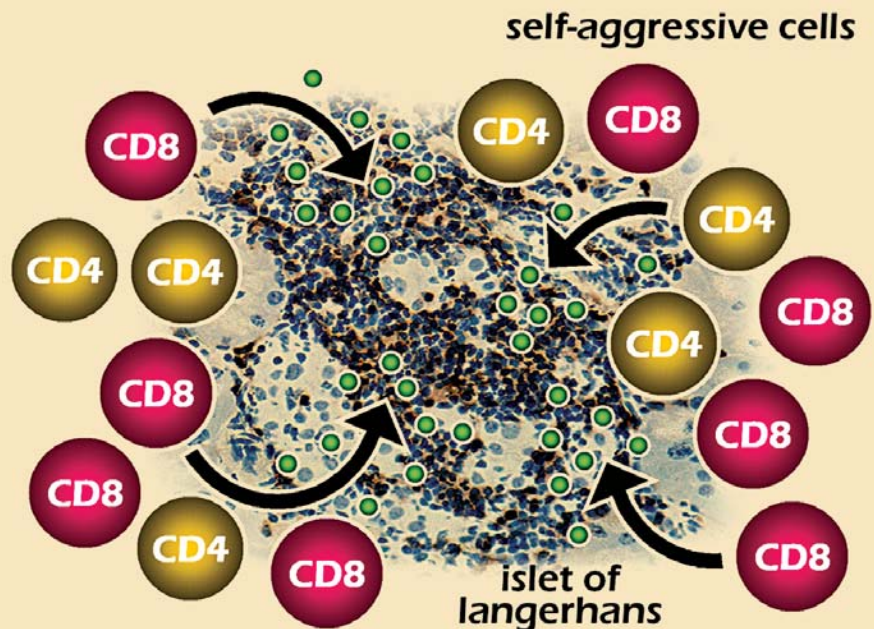
- at risk for type 1 diabetes;
- recently diagnosed with type 1 diabetes and may still have some functioning beta cells; or
- rejecting transplanted islet cells.

Research into immune tolerance is a rapidly exploding field of research. Also called "immunotherapy," there are several avenues being pursued to re-train the immune system in people with type 1 diabetes.

In the past, drugs that dampen the immune system have been used to prevent the immune cells from attacking the pancreatic beta cells and destroying their ability to produce insulin. However, as Dr. Åke Lernmark explained in a presentation at the 67th Annual Scientific Sessions in Chicago last year, immunosuppressive agents can increase the risk of infection and dangerous or unwanted side-

effects are common. Immune tolerance induction is a more promising area of research, because it allows scientists to "redirect the immune system to establish antigen-specific tolerance, and restore normal self-tolerance in autoimmune diseases."

## TYPE 1 DIABETES MELLITUS



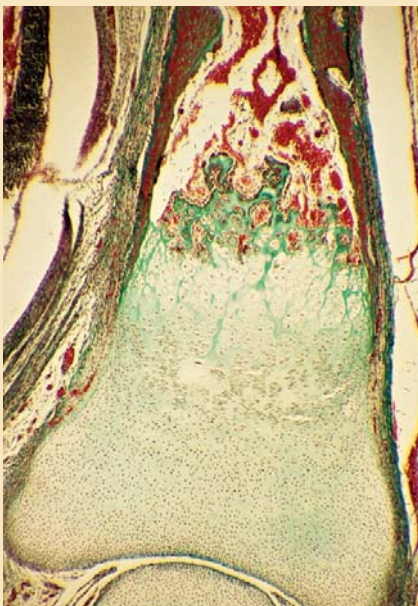
*A model of immune cells (CD4 and CD8 T cells) attacking the islet of a mouse with type 1 diabetes. Submitted by Matthias von Herrath, MD, and Urs Christen, MD, at the La Jolla Institute for Allergy and Immunology.*

## Bone Marrow Transplantation

An editorial by Jay Skyler, MD, in an April 2007 issue of the *Journal of the American Medical Association* provides a brief overview of the issue of immunotherapy and focuses specifically on a study published by Júlio Voltarelli, MD, PhD, from the University of São Paulo, Brazil, and his colleagues.

The study in question reports on a small group of people who underwent a procedure known as autologous hematopoietic stem cell transplantation (AHSCT) within 6 weeks of type 1 diabetes diagnosis. Hematopoietic stem cells are stem cells which give rise to all types of blood cells, and can be found in bone marrow,

umbilical cord blood, and even in the blood stream after a special “pre-treatment” to allow the stem cells to be released from the bone marrow. In this study, the hematopoietic stem cells were obtained from the study participant, the individual was treated to then eliminate all of their immune-destroying cells, and finally they received a transplant of their own hematopoietic stem cells.



Bone marrow

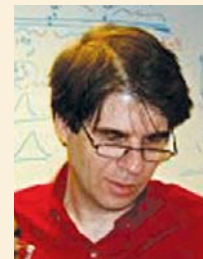
Researchers discovered that after AHSCT the study participants had increased beta-cell function. This was determined by observing an increase in levels of C-peptide. C-peptide is made when proinsulin (a precursor to insulin) is released from the pancreas and is

split into insulin and C-peptide. There is one C-peptide for each insulin molecule, so researchers know that when C-peptide goes up, it means insulin levels are also on the rise. They also noted low A1C levels despite very low doses of insulin or, in some cases, no insulin therapy at all.

Dr. Skyler writes that, although the goal of AHSCT is to eliminate the “self-

reactive” T cells (immune system cells that destroy the “self”) and allow the patient to generate new T cells that are self-tolerant, scientists are really not sure if this why the procedure works. It could be that after AHSCT the patient is able to make larger numbers of regulatory T cells (cells in the immune system that actually prevent the immune system from attacking). Or, it could be that the patients own stem cells are

*The American Diabetes Association funds several research grants in immunotherapy. This is an exciting area of research that could lead to new breakthroughs in type 1 diabetes prevention and treatment*



**Christophe Benoist, MD, PhD**

*Joslin Diabetes Center  
ADA-TPNA Mentor-Based  
Minority Postdoctoral  
Fellowship Award*

Dr. Benoist is studying signals occurring within the T cell to try to determine whether signaling mechanisms are involved in T cell tolerance. Funded by Takeda Pharmaceuticals North America, Inc.



**Jeffrey Bluestone, PhD**

*University of California,  
San Francisco  
Mentor-Based Postdoctoral  
Fellowship Award*

Dr. Bluestone is studying the FoxP3 gene. The FoxP3 gene is a master switch of the regulatory T cells, turning them on and off. Identifying FoxP3 targets may help us figure out how regulatory T cells suppress autoimmune attack.

now differentiating into new healthy cells within the pancreas.

Dr. Skyler concludes that, “Research in this field is likely to explode in the next few years...” and that, “Other approaches [to immunotherapy] under consideration include infusion of dendritic cells [immune cells], T-regulatory lymphocytes, umbilical cord cells, embryonic or adult stem cells, and allogenic bone marrow transplant, in addition to further studies with AHSCT.”

## Umbilical Cord Blood Transplantation

At the 67th Annual ADA Scientific Sessions meeting, scientists reported a breakthrough in type 1 diabetes immunotherapy using umbilical cord blood. Seven young children recently diagnosed with type 1 diabetes received a transfusion of their own umbilical cord blood, resulting in a decrease in their disease severity. Researchers at the University of Florida College of Medicine compared the seven children (ages 2-7) who received an infusion of cord blood with 13 children of similar age and diabetes duration who were treated with intensive insulin therapy and served as a control group. Children who received cord blood had lower average A1C levels than the control group, and required lower amounts of daily insulin on average than the control group. The children who received the cord blood may retain the ability to make their own insulin for a longer period of time, which may reduce their overall risk for diabetes complications.

Because cord blood storage is expensive, researchers are trying to identify the component(s) within cord blood that resulted in a lower A1C. Desmond A. Schatz, MD, senior author of the study, stated, “While cord blood contains stem cells capable of differentiating into

insulin-producing cells, and infused cells could have stimulated islets to regenerate, it is most likely that infused regulatory T cells, known to be able to induce autoimmune tolerance, may have prompted a type of immune regulation.” Indeed, researchers noted an increase in the number of regulatory T cells in the patients' blood up to six months after the infusion.

Discussing future research avenues, Michael J. Haller, MD, lead author of the study, explained, “Eventually we might be able to take Tregs [regulatory T cells] out of cord blood in order to have a source from which to grow more. The type 1 diabetes process may be altered with Tregs plus a mild immunosuppressive or other immunomodulating drug or additional cell therapy, which is called 'cocktail therapy,' in a manner similar to the breakthroughs made in treating HIV and cancer.”

Bruce Blazar, MD, from the University of Minnesota, is also studying umbilical cord blood as a source of regulatory T cells, and has used cord blood to prevent rejection of donor tissue in transplant patients. Dr. Blazar and his colleagues have developed new approaches to expand regulatory T cells for use in transplantation.

## Drug Studies

There are also several drug studies taking place where researchers are attempting to re-educate the immune system to prevent autoimmune attack.

### Daclizumab

Henry Rodriguez, MD, from Indiana University, recently conducted a pilot study using the drug daclizumab to prevent progression of type 1 diabetes in recently diagnosed patients. Daclizumab (DZB) is an antibody which blocks the interleukin-2 receptor, or IL-2, and is

already used to prevent kidney transplant rejection. Scientists know that overexpression of IL-2 in mice leads to diabetes.

Dr. Rodriguez hypothesized that by blocking the IL-2 receptor, one can prevent destructive T cells from being activated and therefore prevent destruction of the beta cells. In summary, his research showed that DZB therapy in newly diagnosed type 1 diabetes was safe, preserves C-peptide, decreases insulin requirements and leads to improved A1C. Dr. Rodriguez suggested in his presentation at the 67th Annual Scientific Sessions that DZB could be even more effective in combination with other drugs, and may be valuable in preventing type 1 diabetes in high-risk individuals.

TrialNet is performing just such a combination therapy study with DZB, which has recently completed enrollment and is undergoing follow-up. TrialNet is a group of studies funded by the American Diabetes Association, the National Institutes of Health, and the Juvenile Diabetes Research Foundation, designed to look at ways to prevent or reverse type 1 diabetes and to understand how type 1 diabetes develops in the first place. Two TrialNet studies currently underway involve testing drugs to slow down or stop the destruction of beta cells in people newly diagnosed with type 1 diabetes. *The Rituximab Study* (Anti-CD20) aims to preserve some of the body's insulin

production by preventing further destruction of the beta cells. The *MMF/DZB Study* has the same goal, but uses a combination of two medicines [Mycophenolate mofetil (MMF/CellCept®) and Daclizumab (DZB/Zenapax®)] to stop the immune system from destroying beta cells. Rituximab, MMF and DZB have all been studied for use in treating other illnesses, and researchers think there is a good possibility they will be beneficial in type 1 diabetes.

Both studies are being completed in people newly diagnosed with type 1 diabetes. In both cases, even though a person already diagnosed with type 1 diabetes would need to keep taking insulin, his or her blood glucose would be easier to control. If rituximab or the MMF/DZB combination works, individuals taking it will be at less risk for severe hypoglycemia and major complications of diabetes such as blindness, heart disease and stroke.

You can read more about the TrialNet studies by going to the TrialNet web site at [www.diabetestrialnet.org](http://www.diabetestrialnet.org).

#### **GAD65**

Åke Lernmark, PhD, at the University of Washington, found that a low dose of human recombinant GAD65 (Diamyd™) has preserved insulin secretion in people recently diagnosed with type 1 diabetes.

GAD, or glutamate decarboxylase, is an important enzyme involved in cellular communication in the brain and



**Teresa DiLorenzo, PhD**  
*Albert Einstein College  
of Medicine  
Innovation Award*

Dr. DiLorenzo is working to prevent the protein IGRP from leading to the destruction of beta cells. She is analyzing hundreds of drugs that are already approved for use in humans for other conditions and testing them for their ability to block the production of IGRP. Funded by the Estate of Gail Patrick.



**George Eisenbarth, MD, PhD**  
*University of Colorado  
Health Sciences Center  
Mentor-Based Postdoctoral  
Fellowship Award*

Dr. Eisenbarth's study focuses on a specific portion of the T cell receptor (called the "alpha chain") to see whether its deletion can prevent autoimmune attack of the beta cells.

pancreas. The immune system's attack on GAD triggers a progressive autoimmune response that leads to diabetes. The frequency of GAD antibodies is high at diabetes onset.

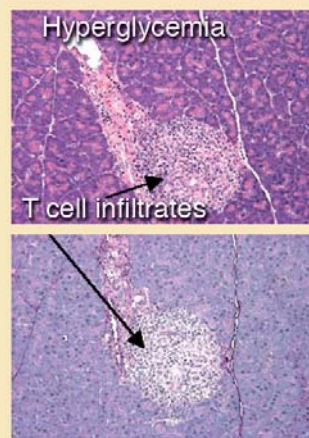
The purpose of Dr. Lernmark's study was to determine if Diamyd™ was safe and if it prevented diabetes. His study involved patients aged 30-70 years who were diagnosed with diabetes within the past five years and had antibodies to GAD. A 20ug (20 microgram) dose of Diamyd™ showed a significant rise in C-peptide levels, meaning that insulin secretion was taking place in the pancreas. There were no serious adverse events among any of the patients.

This study led to a Phase II GAD65 trial conducted in children with type 1 diabetes in Sweden.

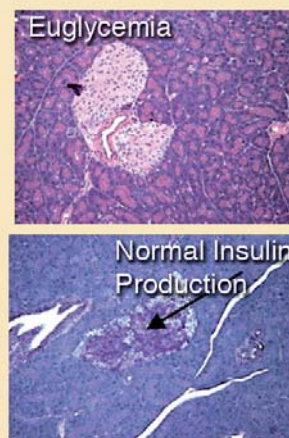
Children in the study had GAD antibodies and were given the 20ug dosage of GAD65. Based on C-peptide levels, GAD65 was shown to have a protective effect on the ability of the children to secrete insulin. According to Dr. Lernmark, the study concluded that, "GAD65-alum has a clear and statistically significant protective effect on residual insulin secretion. GAD65-alum intervention represents an efficacious, safe, and easily administered treatment in patients with newly diagnosed type 1 diabetes." Phase III studies are planned in the U.S. (led by Jerry Palmer, MD) and Europe (led by Johnny Ludvigsson,

MD, PhD). In both studies, researchers will recruit 300 recently-diagnosed type 1 diabetes patients: 200 patients will receive 20 micrograms of GAD65, and 100 will receive a placebo. Researchers will look for an increase in C-peptide levels for up to 15 months after treatment.

### CD4<sup>lo</sup> CD40<sup>+</sup> Recipients



### CD4<sup>hi</sup> CD40<sup>-</sup> Recipients



*Images courtesy of David Wagner, PhD, of the Webb-Waring Institute of the University of Colorado Health Sciences Center, demonstrate that CD4<sup>lo</sup> CD40<sup>+</sup> T cells severely infiltrate the pancreas and destroy insulin production while CD4<sup>hi</sup> CD40<sup>-</sup> T cells do not.*

### hOKT3γ1(Ala-Ala)

The Immune Tolerance Network (ITN), a consortium of international research investigators studying immune tolerance mechanisms in autoimmune diseases, allergies, and organ transplantation, is currently enrolling study participants into a trial to test the ability of a monoclonal antibody to preserve beta-cell function in people newly diagnosed with type 1 diabetes.

The ITN-sponsored clinical trial, known

as AbATE (*Autoimmunity-blocking antibody for tolerance in early type 1 diabetes*), will test the drug hOKT3γ1(Ala-Ala) for its ability to induce immune tolerance in those with type 1 diabetes, thus preventing the ongoing destruction of beta cells. The drug hOKT3γ1(Ala-Ala) has been shown in smaller studies to preserve insulin secretion for up to two years in patients who only received one, two-week treatment regime. Led by principal investigator Kevan Herold, MD, at Yale University, the AbATE study proposes to administer a second treatment of hOKT3γ1(Ala-Ala)

one year after the first to determine if beta-cell function can be preserved even longer.

The antibody hOKT3 $\gamma$ 1(Ala-Ala) works by binding itself to a portion of the T cell known as “CD3.” It is called a monoclonal antibody because it only binds to one receptor site on the T-cell—the CD3 site. By binding to this spot, it prevents the T cell from attacking and destroying the beta cell. Readers can find more information on the AbATE trial at [www.abatetrials.org](http://www.abatetrials.org).

Bernhard Hering, MD, at the University of Minnesota, is also studying hOKT3 $\gamma$ 1 (Ala-Ala). He is leading an ITN-sponsored study which utilizes a combination of hOKT3 $\gamma$ 1(Ala-Ala) and the immunosuppressant drug sirolimus to prevent rejection of transplanted islet cells. Dr. Hering will study 12 patients with type 1 diabetes who have undergone islet cell transplantation. Each study participant will receive both hOKT3 $\gamma$ 1(Ala-Ala) and sirolimus for the first 12 months following the transplant. After the first 12 months, sirolimus will be slowly withdrawn over a four-month period.

By studying this unique drug combination, and monitoring study participants during the slow withdrawal of immunosuppressant, Dr. Hering and his colleagues hope to determine whether the two drugs can help the recipient achieve tolerance to both the donor islet cells and the recipient’s own remaining islet cells.

## Thymoglobulin

The ITN is also conducting a study to test the drug Thymoglobulin. While the monoclonal antibody hOKT3 $\gamma$ 1(Ala-Ala) attaches only to the CD3 portion of the T cell, Thymoglobulin is a much broader polyclonal drug that binds to several areas of the T cell. Researchers leading this study feel that this may be a more effective way to block the T cells that destroy insulin-producing beta cells in the pancreas. Thymoglobulin is already used as an immunosuppressant drug in people who have undergone organ transplants.

This study, led by Stephen Gitelman, MD, at the University of California, San Francisco, will enroll 78 participants with newly diagnosed type 1 diabetes at five clinical trial locations around the U.S. Half of the patients will be given Thymoglobulin over a four-day period, and half will enroll into a control group. C-peptide levels will be measured after 12 months, and the investigators anticipate that the study participants who received Thymoglobulin will be able to produce more of their own insulin and will need fewer insulin injections after 12 months than those in the control group.

## Antigen-Presenting Cells

Stephen Miller, PhD, from Northwestern University, was able to prevent autoimmune attack on cells by injecting antigen-presenting cells into people affected with autoimmune diseases.



**C. Garrison Fathman, MD**

*Stanford University  
Mentor-Based Postdoctoral  
Fellowship Award*

Dr. Fathman’s goal is to effectively administer anti-CD3 antibody to prevent the immune system from destroying pancreatic beta cells. He will then try to reestablish proper immune system control and cure diabetes.

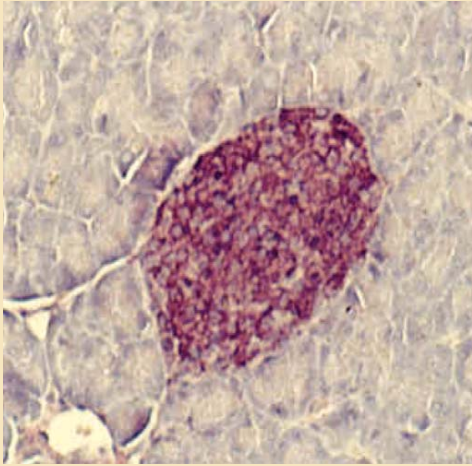


**Pamela Fink, PhD**

*University of Washington  
Innovation Award*

Dr. Fink is investigating why the T cells are not tolerant by studying T cells as they emerge from the thymus, the organ in which they are created. Funded by the Estate of Gail Patrick.

### Intact islet from normal mice

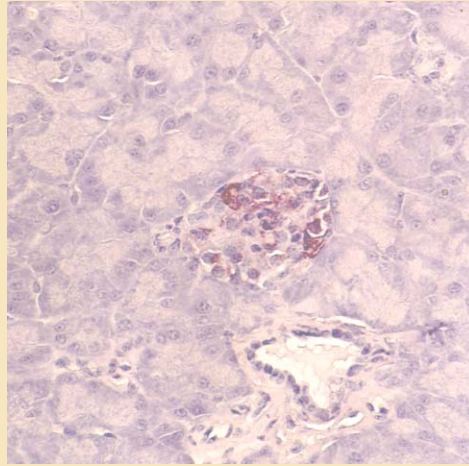


Images of mouse islets courtesy of Ji-Won Yoon, PhD, of Rosalind Franklin University of Medicine and Science.

The bulk of Dr. Miller's research has been in multiple sclerosis (MS), an autoimmune disease of the central nervous system. His research has shown that the substances responsible for causing an autoimmune response in MS change during the course of the disease. He has found that T cell responses in MS are dynamic and evolve over time due to the release of antigens from the target organ of the autoimmune disease. Therefore, he explains, "If you are going to prevent development of disease using antigen-specific therapies, you need to have some indication of what the autoantigens are you need to target and a way to induce effective immune tolerance."

Dr. Miller and his colleagues showed that antigen-specific tolerance, or, helping the animal tolerate the specific antigens responsible for starting and

### Destroyed islet from diabetic mice



spreading the disease, is extremely effective in treating multiple sclerosis.

He then turned his success with MS to studying type 1 diabetes. Using a mouse model of type 1 diabetes (the NOD mouse), Dr. Miller tolerized individual groups of animals with one of five purported diabetes-causing epitopes. Epitopes are the portion of the antigen that the antibody attaches itself to.

Dr. Miller found that only the insulin B-chain protected the animals and the longer chain, B9-23, worked the best. This is consistent with the hypothesis that the insulin B9-23 epitope is the initiating epitope in the NOD mouse. Dr. Miller explained that one could also use a cocktail and tolerize against all five epitopes and achieve protection against type 1 diabetes.



### Richard Flavell, PhD

*Yale University School of Medicine*

*Research Award and Mentor-Based Postdoctoral Award*

Dr. Flavell will study how regulatory T cells function and are regulated by the cytokine TGF beta as type 1 diabetes develops. He is also studying T cell anergy—a process by which T cells stop their destructive tendencies towards the beta cells. This process is most likely defective in people with type 1 diabetes. Funded by the Order of the Amaranth.



### Zhiguang Guo, MD, PhD

*University of Minnesota*

*Research Award*

Dr. Guo found that after islet-cell transplant, infusing the patient with lymphocytes (immune system cells) from the donor helps them tolerate the transplant and avoid rejection. This project will study that phenomenon and why it occurs in mice.

Dr. Miller and his colleagues are currently testing a combination therapy for type 1 diabetes. They are transplanting islets from one NOD mouse into another, non-related, NOD mouse to induce tolerance to multiple islet antigens to imitate what happens when human cadaver islets are transplanted into living human patients.

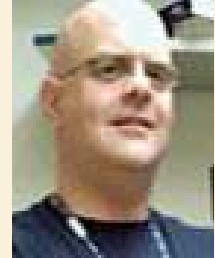
The ITN is also sponsoring a Phase I clinical trial to test the safety and effectiveness of an insulin B-chain vaccine in preventing further loss of beta cells in newly diagnosed type 1 diabetes patients.

The study, led by Tihamer Orban, MD, at Joslin Diabetes Center, involves injecting the human insulin B-chain vaccine once, within a month of type 1 diabetes diagnosis. Researchers will then test study participants for C-peptide levels to monitor their beta-cell function up to 24 months after the vaccine is given. If successful, it is possible that this vaccine could be given to those at high risk for type 1 diabetes in hopes of preventing the disease.

## Next Steps

As you can see, there are many research opportunities in immunotherapy that are being pursued. There is no one avenue, thus far, that has been thought of as more promising than another, and researchers continue to work together to share their findings and collaborate on different projects. The good news is that many of the areas being pursued are already being studied in human clinical trials, which means we are that much closer to finding effective ways to prevent, and possibly even cure, type 1 diabetes.

The American Diabetes Association is also funding research projects in the area of immune tolerance, and will continue to partner with groups such as TrialNet to ensure that immunotherapy research to prevent and treat type 1 diabetes continues to move forward. ■



**John Iacomini, PhD**

*Brigham and Women's Hospital  
ADA-TPNA Mentor-Based  
Minority Postdoctoral  
Fellowship Award*

Dr. Iacomini is evaluating the use of genetic manipulation of the cells of the thymus to prevent autoimmune attack of the beta cells. Funded by Takeda Pharmaceuticals North America, Inc.



**Mark Rigby, MD, PhD**

*Emory University School of  
Medicine  
Junior Faculty Award*

Dr. Rigby is using a treatment known as “costimulation blockers” to prevent T cells from destroying the pancreas. Read more about Dr. Rigby’s research on page 6.