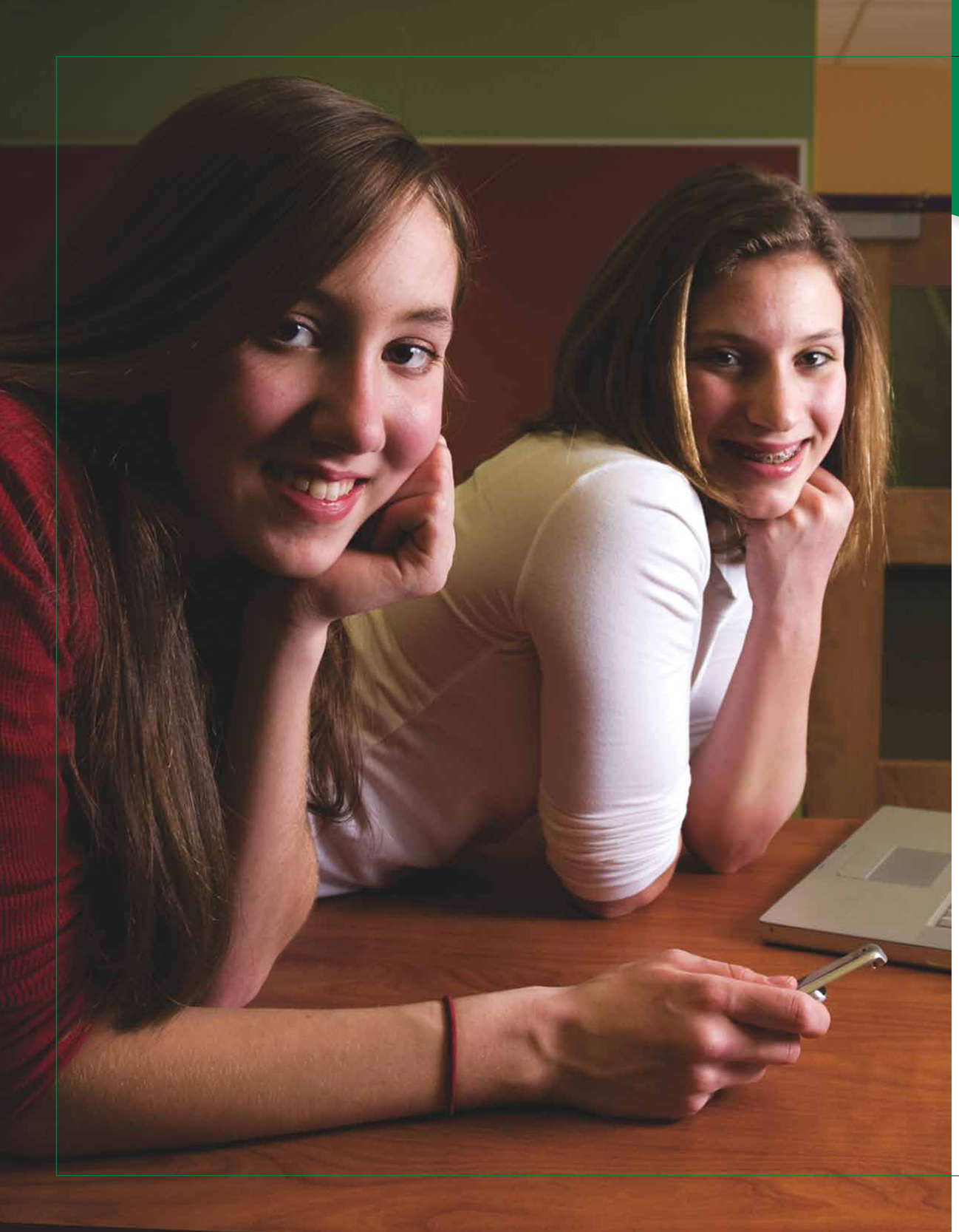


DIVISION OF IMMUNOGENETICS

Mission

The Division of Immunogenetics is committed to providing basic science support aimed at better understanding the etio-pathogenesis of pediatric diseases to more efficiently and correctly diagnose, prevent, and possibly better treat them.

FACULTY AND STAFF

Massimo Trucco, MD
Hillman Professor of
Pediatric Immunology

Suzanne Bertera, PhD
Research Assistant Professor

Rita Bottino, PhD
Research Associate Professor

H. Henry Dong, PhD
Associate Professor

Yong Fan, PhD
Research Assistant Professor

Nick Giannoukakis, PhD
Associate Professor

Jing He, MD, PhD
Research Assistant Professor

Jonathan Piganelli, PhD
Associate Professor

Steven Ringquist, PhD
Research Assistant Professor

William Rudert, MD, PhD
Research Associate Professor

Tatyana Votyakova, MD, PhD
Research Assistant Professor

OVERVIEW OF DIVISION

The Division of Immunogenetics is committed to providing basic science support aimed at better understanding the etio-pathogenesis of pediatric diseases to more efficiently and correctly diagnose, prevent, and possibly treat them. In particular, the Pediatric Research Section of the Diabetes Institute of the University of Pittsburgh, which is administratively considered a component of the division, is the forum created to foster studies on type 1 diabetes (T1D), with the specific goal of finding new avenues for more efficiently predicting who will eventually convert to the disease, for preventing the disease in genetically predisposed children, and for finding therapeutic approaches more efficient than daily injections of recombinant insulin. Toward these aims, members of the division are currently exploring different aspects of diabetes etio-pathogenesis.

T1D is a disease in which mechanisms common to the majority of autoimmune diseases contribute to its pathological process. What seems certain is that, at some point postnatally, environmental triggers activate the immune system of an individual who is genetically predisposed to autoimmunity. Once silent, auto-reactive T cells, which have somehow escaped thymic censorship, are then activated and become able to reach and kill their targets (i.e., the insulin-producing beta cells of the pancreas) by recognizing self-markers on them. Resident antigen presenting cells, like the dendritic cells (DCs), respond to the micro-environmental anomaly (e.g., cell death) and initiate the autoimmune process by phagocytizing dead beta cell debris and then migrating out of the tissue and into the proximal lymph nodes. Through presentation of the newly acquired self-antigens, the DCs trigger the activation and proliferation of new naïve T lymphocytes. The recurrent activation of additional T cells against once "ignored" self-antigens, or other epitopes of the same antigen, constitutes a vicious circle, known as the "epitope spreading" phenomenon. In fact, once activated, new T cells leave the lymph node and extravasate into the target tissues, where they destroy additional target cells recognizing new antigens. Once a large enough portion of insulin-producing cells is lost, the clinical onset of the disease presents.

To date, there are no cures for autoimmune disorders in general and for T1D in particular. In T1D, significant tissue destruction has already occurred at the moment of the clinical onset, and there is very little that can be done to restore function of the target organ other than hormone replacement or transplantation of the lost cells, which in itself is immunologically challenging. It would be far easier to manipulate either the immune system of the recipient patients or transplant the cells. Even better would be to intervene in genetically at-risk individuals, in a manner by which tolerance to tissue-restricted antigens of the self could be restored well in advance of disease onset.

To block auto-immunity, without the use of immunosuppressant, Nick Giannoukakis and Massimo Trucco have successfully completed a National Institutes of Health (NIH)-funded, U.S. Food and Drug Administration (FDA)-approved phase I clinical trial that was designed to test the safety of a treatment in which autologous DC—rendered functionally immature by exposing them to CD40, CD80, and CD86 antisense oligodeoxyribonucleotides (AS-ODN) to reduce costimulatory molecule levels at their surface—were administered to T1D patients with established disease. Leukocytes of the patient were obtained by apheresis, and DCs were then isolated and expanded. DC engineered in GMP facilities to express low levels of CD40, CD80, and CD86 were then injected into the patient by intradermal administration at an anatomical site proximal to the pancreas. DCs then migrate to the nearest (i.e., pancreatic) lymph nodes, where they are able to interrupt the vicious circle that maintains islet-specific inflammation (i.e., insulitis). In the pancreas, DCs acquire new beta cell-specific antigens from apoptotic cells, leading to the eventual display of these antigens to naïve T cells in the pancreas-draining lymph nodes. The lack of costimulatory molecules will result in an anergizing signal to the T cells, induce regulatory immune T and B cells, and limit the T-cell mediated anti-beta cell epitope-spreading phenomenon. In particular, a role for tolerogenic dendritic cell-induced B-regulatory cells in T1D was formally ascertained. The abrogation of the autoimmune diabetogenic insult should be sufficient to promote the rescue of any remaining insulin-producing beta cells and/or neogenesis of other insulin-producing cells in the host endocrine pancreas, even after the onset of the disease. This possibility waits to be tested in a phase II (efficacy) trial involving new-onset diabetic patients. This therapeutic approach should, in fact, be more successful when DC injections start close to the clinical onset of the disease or even before the clinical onset in patients at high risk. To help promote islet transplantation as a more successful and easily approachable alternative to insulin administrations, making available a virtually endless source of functional islets, Bottino and Bertera tested the possibility of using pigs as islet donors. An alpha 1-3 galactosyltransferase knockout pig was used as the recipient of additional transgenes useful to reduce complement activation and blood coagulation in the recipient. Our ability to isolate still functional islets for performing auto-transplants in chronic pancreatitis patients who underwent total pancreatectomy to reduce their excruciating pains helped to successfully test the pig to nonhuman primate recipient islet xenotranplants. The extremely promising results were recently reported. Also, Yong Fan and William Rudert's efforts were focused on characterizing factors able to break immune central and peripheral tolerance, thus promoting autoimmunity, and in conceptualizing clinically useful contra-measures. Under the supervision of Steven Ringquist, next-generation sequencing (using the Roche/454 instrument) genotyping of human leukocytle antigen (HLA) loci, critical to define susceptibility to T1D and determinant to achieving long-term survival of allogeneic bone marrow transplants, was compared with more classical typing approaches, like the ones which use sequence specific oligonucleotide (SSO) probes, or the imputation of genotypes from high-density SNP analysis. Jon Piganelli continued to pursue the goal of better defining the effects of inflammation and ROS production on the demise of pancreatic beta cells to provide new approaches to alleviate their effects not only in the animal model but also in humans. Tatyana Votyakova is determining the role of mitochondrial performance and level of mitochondrial reactive oxygen species in islet viability prior to their transplantation, focusing on this aspect of potential injury from hypoxia and reperfusion inevitably arising in the course of pancreas harvesting and islet isolation. All these aspects have the major target of better defining the outcome of transplantable isolated islets of the pancreas. Jing He supports the other faculty efforts by characterizing the morphological changes in immune cells and different organs to provide structural evidences. Finally, Henry Dong was analyzing the new phenomenon of type 2 diabetes (T2D) in children, focusing on the role of factors, like FoxO1 and O6, as the possibly acting genes in this pathology.

DIVISION OF IMMUNOGENETICS

RESEARCH AND OTHER SCHOLARLY ACTIVITIES

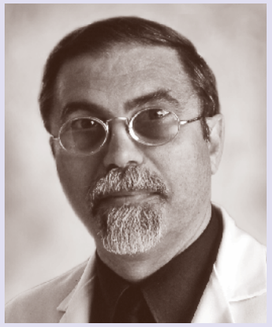
Massimo Trucco, MD

RESEARCH

Currently, the most actively pursued therapeutic approaches to avoid the daunting complications of T1D are islet allo-transplantation, stem cell-based islet replacement, beta cell regeneration attempts, and viral vector-based gene therapies. Although significant advances have been made in all of these areas, and an enormous number of therapeutic approaches to cure diabetes has been successfully tested in the NOD mouse—the genetically diabetes-prone non-obese diabetic mouse strain, whose etiopathogenesis is widely held to parallel the one that occurs in humans—the majority of them simply did not work in humans. The gap between mice and humans seems to be too large to justify the translation of therapies efficacious in mice directly to human individuals. But, even if the nonhuman primate (NHP) seem to be the best animal model available for testing new therapeutics on the basis of the phylogenetic similarities between monkeys and humans, it has to be considered that NHP do not spontaneously develop autoimmune diabetes.

Given these considerations, Massimo Trucco's research team thought it useful to find the means to promote autoimmune diabetes in NHP. Toward this objective, the team was motivated by the evidence proving that insulin expression in the thymus can regulate the negative selection of auto-reactive T cells, being the first self-antigen whose lack of expression in the thymus is sufficient to break central immune tolerance toward pancreatic beta cells. To gain the functional insights of this ectopic insulin expression, the team took advantage of the Cre-lox system to knockout the mouse *Ins2* gene specifically in *Aire*-expressing medullary thymic epithelial cells (mTECs), without affecting its production in the pancreatic beta cells. These ID-TEC (i.e., carrying insulin-deficient mTECs) mice—previously also crossed to an *Ins1* knockout background—spontaneously developed diabetes around three weeks after birth, even in a strain's background expressing diabetes-resistant H-2b MHC molecules. Beta cell-specific, autoimmune destruction was observed and documented by the presence in the islets of effector T cells, directed specifically against insulin epitopes. Even more pertinent to the new aim were the results obtained from ID-TEC-thymus transplantation experiments in a nude (thymus-deprived) mouse, which proved how the *Ins2*-depleted thymus was sufficient, to: 1) successfully reconstitute the animal's T cell repertoire, 2) break its central tolerance, and 3) induce anti-insulin autoimmunity.

This study suggested that in a monkey, like in the mouse, a drastic reduction of expression of self-antigens (e.g., insulin) in the mTECs might favor the generation of an autoimmune reaction specifically directed against the insulin-producing cells of the pancreas, eventually bringing the animal to clinically overt T1D.



Massimo Trucco, MD
Division Chief, Immunogenetics

On this basis, the team intended to engineer autoimmune diabetes in monkeys by safely removing the thymus from Cynomolgus monkeys (i.e., *Macaca Fascicularis*), transducing it *in vitro* with a lentivirus expressing monkey-insulin-specific short-hairpin RNA (shRNA) to knock down insulin expression, and then to return the transduced thymus back into the donor monkey (whose mature B and T cells had been concomitantly depleted), to facilitate the onset of autoimmunity against pancreatic beta cells. Even if the virus might not have transfected all the cells of the thymic medulla, a drastic reduction in insulin expression/presentation was expected to be sufficient to favor the development of a specific autoimmune reaction. Following depletion of all immuno-competent cells in the thymectomized monkey and transplantation of the transduced thymus back to it, the team observed a successful repopulation of its immune cells (mature and functional T and B lymphocytes) after roughly three months from transplantation. However, no more than ~5% of insulin reduction in the transplanted thymus was achieved, by the process of transducing *ex vivo* the sliced thymic tissue, exposed *in vitro* to the monkey insulin-specific shRNA expressing vector. This reduction was insufficient to break central tolerance and failed to promote clinically overt diabetes in the recipient monkey. It was then concluded that the only way to achieve better transduction with the vector was to decompose the thymic tissue so to obtain a single cell population, which could be more efficiently exposed to the shRNA-carrying vector. The problem remained then how to reconstruct the thymus in order to transplant it back to the donor monkey. In the mouse, this final problem was solved utilizing a process that successfully repopulates a thymic scaffold with enriched mTECs. Transplanting the reconstructed thymus, the team successfully reconstituted mature and functional T and B cells in nude mice. Utilizing this approach, the researchers should become able to not only create autoimmunity in the monkey, but also generate the basis for an efficient, safe, new therapeutic approach to cure T1D.

STUDY SECTIONS

- Member, TrialNet Operations Strategic Review Group
- Speaker, Health Research Advisory Committee meeting, Commonwealth Universal Enhancement (CURE) Program, Harrisburg, Pa., September 2011
- Member, Scientific Advisory Board, Diabetes Research Institute, University of Miami, Miami, Fla., November 2011
- Member, Special Emphasis Panel for the review of R43 applications submitted in response to RFA-DK-11-024, "Small Business Innovation Research to Develop New Method and Technologies Able to Identify Individuals at Risk of Developing T1D," National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), Bethesda, Md., March 2012
- T1D TrialNet Steering Committee Meeting, Reston, Va., April 2012
- Beta-cell Biology Consortium (BCBC) Investigator Retreat 2012, Chantilly, Va., May 2012
- Sanofi T1D Mellitus Advisory Board Meeting, Bridgewater, N.J., June 2012
- Takeda T1D Advisory Board Meeting, Philadelphia, Pa., June 2012

ADVISORY COMMITTEE MEMBERSHIPS

- Research Advisory Committee, Children's Hospital of Pittsburgh of UPMC
- Cochrane-Weber Endowed Fund Advisory Committee
- Rangos Research Center Steering Committee, Children's Hospital

EDITORSHIPS

- Editor, *Review of Diabetic Studies*
- Associate editor, *Pediatric Diabetes*
- Reviewer, *Advances in Experimental Biology and Medicine*
- Reviewer, *Biotechniques*
- Reviewer, *Diabetologia*
- Reviewer, *Diabetes*
- Reviewer, *Diabetes Care*
- Reviewer, *Endocrinology*
- Reviewer, *Human Immunology*
- Reviewer, *Journal of Autoimmunity*
- Reviewer, *Journal of Clinical Endocrinology and Metabolism*
- Reviewer, *Journal of Immunology*
- Reviewer (on reviewers panel), *Medical Science Monitor International*
- Reviewer, *Transplantation*

MAJOR LECTURESHIPS AND SEMINARS

- "Practical Ways to Achieve Targets in Diabetes Care," University of Colorado School of Medicine Office of Continuing Medical Education and Children's Diabetes Foundation, Denver, Colo., July 2011
- "Can We Really Prevent and/or Cure Diabetes?: Cellular Therapy for Diabetes Prevention," Keystone, Colo., July 2011
- Giornate Diabetologiche Di Palazzo Ducale, Genova, Palazzo Ducale, November 2011
- "Tavola Rotonda, I protagonisti della ricerca scientifica: Testimonianze di ricercatori italiani che lavorano all'Estero e quelli rimasti in Italia," November 2011
- Diabete … Un'Iniezione Di Fiducia: "Combattere il diabete, con mezzi fisiologici," A.G.D. Sicilia Convegno Socio/Scientifico, Catania, Italy, May 2012
- "Cellular Treatment of Autoimmunity in T1D," "Microsphere-based treatment of autoimmunity in T1D," Sanofi Advisory Board Meeting, Bridgewater, N.J., June 2012
- "Cellular Therapy for Diabetes Treatment and Prevention," Third Annual Sanford T1D Symposium—Recent Advance in the Immunology of T1D, Sioux Falls, S.D., July 2012

PROFESSIONAL AFFILIATIONS/SOCIETY MEMBERSHIPS

- Italian Society of Medical Genetics
- Italian Society of Immunology and Immunopathology
- American Society for Histocompatibility and Immunogenetics
- American Association for the Advancement of Science
- American Diabetes Association, Inc.
- New York Academy of Science
- American Society of Clinical Investigation
- Society for Pediatric Research
- Società Italiana di Diabetologia
- American Association of Immunologists

Suzanne Bertera, PhD

RESEARCH

Suzanne Bertera focuses her research on genetic immunomodulation to improve allo- and xeno-islet transplantation. She is testing several therapeutic genes to prolong function of allogeneic and xenogeneic islets in an autoimmune mouse model. Other projects include quality-control testing of the isolated islets *in vivo* with immunocompromised mouse models. In a related project, Bertera is studying the differences in the immunologic responses of mice to genetically altered pig islets. She also provides isolated mouse islets for the Diabetes Institute and other related researchers.

She also is investigating the biological properties of C-peptide, a small peptide generated in the pancreatic beta cells as part of the normal insulin production and secreted in the bloodstream in healthy individuals. This study combines C-peptide with insulin therapy in diabetic mice on a high-fat diet to reduce the amount of atherosclerosis formed compared to insulin therapy alone.

Bertera is a member of the Islet Isolation Core team with Rita Bottino. She participates in pancreatic islet isolation from human, porcine, and nonhuman primates (NHP) donors. The human islets are distributed to JDRF-funded investigators (supported by the recently concluded funding, JDRF #31-2008-381). The porcine and NHP islets are used in basic research within and outside the division as well as for primate xenotransplantation studies. Bertera also is a member of the autotransplant islet isolation team, which is vital for patients with chronic pancreatitis requiring surgical removal of the pancreas. The islets are isolated from the excised pancreas and then infused back to the patient as an autologous cell transplant. This is being done in collaboration with the Department of Surgery of the Cleveland Clinic and more recently with UPMC in Pittsburgh. To date, there have been more than 40 patients, including pediatric cases, receiving this therapy, and more are being scheduled.

She is continuing to collaborate with several members of the Department of Surgery and Thomas E. Starzl Transplantation Institute as head of the Rodent Islet Core, a subdivision of the Islet Isolation Core, providing mouse islets, transplanting mice with islets under the kidney capsule, and helping to plan these projects and analyze the generated data.

EDITORSHIPS

- Editorial Board, *ISRN Endocrinology*
- Reviewer, *Xenotransplantation*
- Reviewer, *Transplant Immunology*
- Reviewer, *Diabetes*
- Reviewer, *ISRN Endocrinology*

PROFESSIONAL AFFILIATIONS/SOCIETY MEMBERSHIPS

- International Pancreas and Islet Transplant Association
- International Xenotransplantation Association
- American Association of Laboratory Animal Science
- American Diabetes Association

Rita Bottino, PhD

RESEARCH

Rita Bottino is responsible for the isolation and provision of pancreatic islets obtained from adult and pediatric patients affected by chronic pancreatitis and undergoing total or partial pancreatectomy at UPMC and the Cleveland Clinic. Following surgical removal of the pancreas, a necessary treatment for untreatable pain, autologous islets transplantation is a safe and unique way to prevent or reduce the onset of surgical diabetes. This procedure is highly specialized and is carried out in a very limited number of centers worldwide. Stemming from the experience of this successful program, Bottino, in collaboration with the Department of Surgery, is working on the implementation of a program of allogeneic islet transplantation. This cell therapeutic approach involves the purification of viable human islets from deceased organ donors and their transplantation in patients affected by T1D.

Bottino's research interests focus on developing preclinical experimental models of diabetes and islet xenogeneic transplantation. One of the main goals is to develop a model of autoimmune diabetes, similar to human T1D, in Cynomolgous monkeys. The approach involves ex vivo knocking down of key genes in the thymus and thymus transplantation. The project stems from a successful murine model developed by Massimo Trucco and Yong Fan.

Genetic manipulation of pig donor tissue is also the basis for rendering porcine cells more compatible to humans and monkeys in transplantation settings. In collaboration with the Thomas E. Starzl Transplantation Institute and Revivicor, Bottino is testing the effect of porcine islets expressing xenogeneic molecules aimed at reducing islet graft loss following transplantation in diabetic monkey recipient. The long-term goal is to identify an appropriate porcine source of islets as an alternative to cadaveric donor islets for clinical transplantation.

The islet lab directed by Bottino is also a reference center for isolating human islets from deceased donors for the purpose of distribution to international investigators carrying out diabetes research.

EDITORSHIPS

- Managing editor, *Journal of Pancreas*
- Guest editor, *Anatomy Research International*
- Reviewer, *Diabetes*
- Reviewer, *Diabetologia*
- Reviewer, *Cell Transplantation*
- Reviewer, *Transplantation*
- Reviewer, *American Journal of Transplantation*
- Reviewer, *Pediatric Diabetes*
- Reviewer, *Endocrine*
- Reviewer, *Hepatobiliary and Pancreatic Diseases International*
- Reviewer, *Xenotransplantation*
- Reviewer, *Journal of Transplantation*
- Reviewer, *World Journal of Gastroenterology*
- Reviewer, *PLoS One*

MAJOR LECTURESHIPS AND SEMINARS

- "Pig to Monkey Islet Xenotransplantation," grand rounds, Thomas E. Starzl Transplantation Institute, Pittsburgh, Pa., December 2011
- "Outcome of the First Xenotransplantations of 4-5 GE Pig Islets in Diabetic Monkey Recipients," Thomas Starzl Transplantation Institute, Pittsburgh, Pa., January 2012
- "Autoimmune Diabetes in Nonhuman Primates: A Model," Division of Immunogenetics Seminars, February 2012
- "The Formation of a Collaborative Consortium to Address Islet Encapsulation," Division of Immunogenetics Seminars, May 2012
- "Porcine Islet Xenotransplantation Using Genetically Engineered Islets–An Update," Thomas E. Starzl Transplantation Institute, Pittsburgh, Pa., May 2012
- "Pancreatic Islet Transplantation. Clinical Experience," Cell Therapy Course (A. Soto-Gutierrez), University of Pittsburgh, July 2012

PROFESSIONAL AFFILIATIONS/SOCIETY MEMBERSHIPS

- American Diabetes Association
- Cell Transplantation Society
- Transplantation Society
- International Pancreas and Islet Transplant Association
- International Xenotransplantation Society

Henry Dong, PhD

RESEARCH

Henry Dong focuses on studies of the molecular basis that links insulin resistance to diabetic dyslipidemia in subjects with morbid obesity and T2D. Insulin resistance is defined as inept responsiveness of tissues to insulin. To overcome insulin resistance in peripheral tissues, the pancreatic beta cells are called upon to produce more insulin via a compensatory mechanism, which over time can lead to beta-cell failure and overt diabetes. Despite tremendous efforts to elucidate the molecular basis of insulin resistance, a unifying idea to account for the pathophysiology of insulin resistance in obesity and T2D is still lacking. Using transgenic, gene knockout, and gene transfer approaches, Dong's lab focuses on the characterization of forkhead transcription factors in glucose and lipid metabolism to understand how insulin resistance perturbs carbohydrate metabolism, contributing to the development of diabetic dyslipidemia. His research team identified the forkhead transcription factor FoxO1 as an important regulator of lipid metabolism, providing a scientific rationale that targeted FoxO1 inhibition by small-molecule drugs would improve lipid metabolism for treating diabetic dyslipidemia. Dong published these studies in the *Journal of Clinical Investigation*, *Diabetes*, and *Endocrinology*. The significance of his studies was reported by *Science Daily*, *University Times* (University of Pittsburgh), and *Scientific American*.

Dong also focuses on the development of insulin replacement therapy for T1D. T1D is caused by the lack of insulin production in the pancreas. Insulin gene therapy is being developed as an alternative insulin replacement therapy because it offers great potential for achieving long-term blood glucose control without eliciting immune rejection. The insulin gene is delivered through a gene vehicle to the liver, such that insulin will be produced in liver cells and released into the blood stream. His research has provided proof-of-principle that sustained insulin production at a basal level in the liver is sufficient to prevent urine ketone and relieve diabetes symptoms in diabetic animals. To improve this procedure, his research team has designed an autoregulated system to control insulin production to achieve insulin release in a glucose-dependent manner. In this autoregulated system, insulin production will be stimulated when blood sugar is high and suppressed when blood sugar is low. His research is in preclinical studies to validate this highly regulated system for improving blood sugar control without the need of daily insulin injection in animal models of T1D. Dong has received research grants and a career development award from the American Diabetes Association for this study.

STUDY SECTIONS

- Ad hoc member, NIH National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), Integrative Physiology of Obesity and Diabetes Study Section, 2011
- Ad hoc member, NIH NIDDK, Cellular Aspects of Diabetes and Obesity Study Section, 2011 and 2012

EDITORSHIPS

- Editorial Board, *World Journal of Gastrointestinal Pathophysiology*
- Editorial Board, *Immunology, Endocrinology, and Metabolic Agents*
- Editorial Board, *Journal of Geriatric Cardiology*
- Editorial Board, *Open Endocrinology Journal*
- Reviewer, *Journal of Clinical Investigation*
- Reviewer, *Cell Metabolism*
- Reviewer, *American Journal of Transplantation*
- Reviewer, *Journal of Applied Physiology*
- Reviewer, *Diabetes*
- Reviewer, *Hepatology*
- Reviewer, *Diabetologia*
- Reviewer, *Diabetes Care*
- Reviewer, *Metabolism*
- Reviewer, *Proteomics*
- Reviewer, *Trends in Endocrinology*
- Reviewer, *Medical Science Monitor*
- Reviewer, *Journal of Molecular Medicine*
- Reviewer, *Molecular Therapy*
- Reviewer, *International Liver*
- Reviewer, *Metabolic Syndrome and Related Disorders*
- Reviewer, *International Journal of Obesity*
- Reviewer, *European Journal of Pharmacology*

MAJOR LECTURESHIPS AND SEMINARS

- "FoxO1 in Lipid Metabolism," University of Alabama Birmingham, Department of Medicine, Comprehensive Diabetes Center, Birmingham, Ala., September 2011
- "FoxO1 in Insulin Action and Lipid Metabolism," Cincinnati Diabetes and Obesity Center, University of Cincinnati College of Medicine, Cincinnati, Ohio, May 2011

PROFESSIONAL AFFILIATIONS/SOCIETY MEMBERSHIPS

- American Heart Association
- American Diabetes Association
- New York Academy of Sciences

HONORS

- Career Development Award, American Diabetes Association
- Patent Pending, Ref No.: 01812, "Targeting FoxO1 for Treating Hypertriglyceridemia," Innovator(s): H. Henry Dong

Yong Fan, PhD

RESEARCH

The major focus of Yong Fan's group is to understand the roles of tissue-specific autoantigen (TSA) expression in both primary and secondary lymphoid organs in establishing and maintaining immune tolerance. The goals are to develop better therapeutics for the prevention and treatment of autoimmune diseases, such as T1D.

The success of adaptive immunity depends on its capability to effectively distinguish self from nonself and take actions accordingly. Both central and peripheral tolerogenic mechanisms are essential to maintain such immune tolerance. Developing thymocytes with high affinities to autoantigens of specific tissues and organs are negatively selected within the thymic medulla, whereas T cells escaped the central selection and are inactivated by various peripheral tolerogenic mechanisms, including induced anergy, ignorance, and active suppression mediated by T regulatory cells. Recent studies highlighted the importance of TSA ectopic expression in the stroma of immune organs in regulating self-tolerance. Using gene targeting and transgenic technique, Fan's research team previously demonstrated that insulin, the primary islet autoantigen in T1D etiopathogenesis, is expressed in Aire+ medullary thymic epithelial cells (mTECs), and such thymic ectopic expression is essential to establish central self-tolerance toward pancreatic beta cells. In addition, the team identified a population of insulin-expressing, Aire+MHCII+CD11c+ antigen-presenting cells of bone marrow origin in the spleen, which might play essential maintenance roles in regulating peripheral tolerance of beta cells under defective central selection conditions. To further investigate the roles of Aire+, self-antigen-expressing cells in tolerance induction, Fan's team has developed a number of animal models in which it was able to achieve either targeted deletion of specific islet autoantigens or depletion of Aire-expressing cells. Autoimmunity affecting multiple organs and tissues was observed in these animal models, implying that defective TSA expression in immune organs might contribute to etiology of multiple autoimmune diseases.

EDITORSHIPS

- Editorial Board, *World Journal of Biological Chemistry*
- Reviewer, *Pediatrics Diabetes*
- Reviewer, *Cellular Immunology*
- Reviewer, *PLosOne*

PROFESSIONAL AFFILIATIONS/SOCIETY MEMBERSHIPS

- American Diabetes Association

Nick Giannoukakis, PhD

RESEARCH

Cell and Particle Vaccines to Prevent T1D. The objective of this study is to develop immunoregulatory dendritic cells and microparticle carriers of dendritic cell-modifying agents toward prevention and reversal of T1D. Autologous dendritic cells are engineered *ex vivo* with nucleotides that downregulate co-stimulation. Microparticle carriers of such nucleotides are used *in vivo* to achieve downregulation of co-stimulation. Both methods successfully prevent T1D in mouse models and reverse new-onset disease. A phase I trial demonstrated that the dendritic cells were safe and well tolerated, and intriguing data suggested that they may be beneficial in new-onset diabetic patients. The microparticles are in the preclinical phase of safety testing and are poised to surmount critical studies enabling eventual phase I trials.

Replication-defective Viruses as Vehicles with Which Insulin Cell Transplants Can Be Engineered to Resist Immune Rejection in Diabetic Recipients. The objective of this study is to determine whether intact allogeneic islets of Langerhans engineered to express immunosuppressive soluble proteins following transduction with fourth-generation lentiviral and adenoviral gene vectors exhibit prolonged survival after transplantation. Intact murine islets are transduced *in vitro* with fourth-generation lentiviral and adenoviral gene vectors encoding a variety of soluble immunoregulatory proteins and cytokines and transplanted into diabetic and autoimmune diabetic allogeneic murine recipients. Transplants expressing inhibitors of CD8 cytotoxic T-cell-mediated cytolysis and cytotoxicity exhibit prolonged survival. Improvements in vector delivery as well as transplant site conditioning improve the outcome of survival. This approach will be translated to facilitate the transplantation and survival of porcine xenogeneic islets in nonhuman primates prior to human safety trials.

Molecular Mechanisms and Pathways of Immunoregulation in Regulatory Leukocyte Populations Induced by the Diabetes-therapeutic Dendritic Cells and Particles. The objective of this study is to identify immune cells with immunoregulatory/immunosuppressive activity that are upregulated in successfully treated diabetic recipients of the autologous dendritic cells and microparticle vaccines. FACS, microscopic, whole-body imaging, conventional biochemistry, and a variety of immune assays are used to identify the leukocyte subpopulations that are upregulated as well as to ascertain the possible intracellular mechanisms by which the nucleic acids in the cell and particle vaccines activate immunoregulation pathways. TLR-mediated signaling appears to be involved in the mechanism by which some of the nucleic acids induce suppressive capacity in dendritic cells. Successfully treated recipients of the dendritic cells and the particles exhibit increased prevalence of Foxp3+ regulatory T cells as well as a novel population of B cells with immunosuppressive activity. Further identification of the upregulated cell populations may offer an additional cell type(s) that can be harnessed as alternative T1D cell therapeutics.

STUDY SECTIONS

- Biological and Chemical Technologies, SBIR Study section, National Science Foundation, 2012–sent
- Reviewer, NIDDK: F30, F31 and F32 NRSA Fellowship Grants, 2009–present
- Reviewer, NIDDK: ZDK-GRB-R-J2, K and T32 Awards, 2011
- Grant reviewer, Regular and Innovative Grants Study Section, JDFR
- Grant reviewer, Regular Grants Study Section, American Diabetes Association
- Grant reviewer, Regular Grants Study Section, Canadian Diabetes Association
- Grant reviewer, Regular Grants Study Section, Diabetes UK

- Reviewer, Thrasher Foundation, 2010–present
- Program reviewer, Government of Belgium, Belgian Science Policy Office, Interuniversity Attraction Poles (IAP) Phase VII
- Reviewer, Framework Consortium Projects, Government of Chile (CONFICYT), 2009–present
- Reviewer, Hellenic Republic (Government of Greece Post-graduate State Scholarships; Medicine Committee), 2011–present

EDITORSHIPS

- Editor, *Clinical and Developmental Immunology*, special issue on T1D Immunological Tolerance and Immunotherapy, 2012
- Reviewer, *Gene Therapy*
- Reviewer, *Cell Transplantation*
- Reviewer, *Immunology*
- Reviewer, *Diabetes*
- Reviewer, *Molecular Therapy*
- Reviewer, *Clinical and Experimental Immunology*
- Reviewer, *Journal of Immunology*
- Ad hoc reviewer, *Transplantation*
- Ad hoc reviewer, *Nature Genetics*
- Reviewer, *American Journal of Transplantation*
- Reviewer, *Pharmacoeconomics*
- Reviewer, *Human Gene Therapy*
- Reviewer, *Pediatric Diabetes*
- Reviewer, *Journal of Leukocyte Biology*
- Reviewer, *Diabetes Technology and Therapeutics*

PROFESSIONAL AFFILIATIONS/SOCIETY MEMBERSHIPS

- American Society of Gene Therapy
- American Diabetes Association
- European Society of Gene and Cell Therapy
- Federation of American Societies for Experimental Biology

Jing He, MD, PhD

RESEARCH

Jing He's expertise is in morphology of tissue and organs. She is interested in reorganizing and analyzing three-dimensional images, using both conventional and fluorescent confocal microscopy. Her three-dimensional image study on the mouse islet vascular structure has been published. He is focused on projects related to structures and functions in various species islets and the morphological changes of NOD mouse islets, including the support of capillaries, different kinds of immune antigens, and the communication between islet cells. He also works as an advisor on morphological and immuno-histochemical studies in the Division of Immunogenetics, and she has been involved with numerous projects. Collaborating with Rita Bottino, He has tested the regeneration of transplanted porcine islets obtained from genetically manipulated pigs in diabetic nonhuman primates. Collaborating with Yong Fan, He has studied immunocytochemical changes in different organs (thymus, spleen, lymph nodes, pancreas, and liver). These organs were from different genetic background mice. In order to demonstrate the balance between autoimmunity and self-tolerance in T1D pivots on the antigenic presentation of peptides derived from the thymus epithelial expression of a single tissue-specific gene, He studied the infiltration and location of lymphocytes of both CD4+ and CD8+ subsets in the mouse different organs. He also is involved in the morphological study of reconstructed thymus transplanted to mouse kidney. Collaborating with Suzanne Bertera, He has studied the immunocytochemistry on the differentiation of umbilical cord blood–derived stem cells into islet cell. Collaborating with Jon Piganelli, He is involved in the study of immune reaction on islets transplanted to mouse kidneys.

PROFESSIONAL AFFILIATIONS/SOCIETY MEMBERSHIPS

- American Diabetes Association

Jon Piganelli, PhD

RESEARCH

Jon Piganelli's research team has identified Chromogranin A as the antigen for the well-characterized diabetogenic T cell clone BDC-2.5, which the team has studied for a number of years. Team members are now poised to assess the interaction of endoplasmic reticulum (ER)-stress induced posttranslational modification of Chromogranin A in beta cells to determine if ER-stress facilitates the interaction and the exposure of this protein to the immune system. Also, they are assessing whether the ER stress leads to a situation of posttranslational modification of the protein as a result of the fluctuations in calcium contact in the cytoplasm. The researchers also are expressing CHgA in prokaryotic and eukaryotic expression systems to determine whether PTM is necessary for antigenicity. They are interested in these studies as they help to better understand the question of end organ autoimmunity and why certain organs are more prone to self-reactive recognition.

Work also continues on the role of redox-dependent signaling in immune system activation. It is known that ectodomain shedding of key transmembrane proteins is involved in regulating inflammation, a primary mechanism of chronic immune-mediated diseases, such as T1D.

LAG-3, or lymphocyte activation gene 3, is upregulated on CD4 T cells early after antigen recognition, binding with high affinity to MHC class II on APCs to temper the clonal burst and control aberrant immune responses. For an efficient T-cell response to occur, LAG-3 must be extracellularly cleaved by the redox-dependent metalloproteinase TACE, allowing successful CD4-class II engagement and progression toward effector function. In T1D, reducing LAG-3 shedding to impede CD4 T-cell responses may show promise in restraining autoreactive T-cell activation/expansion and hindering disease progression. The lab has previously shown that a manganese metalloporphyrin catalytic antioxidant (MnP) can scavenge reactive oxygen species, decrease proinflammatory cytokine production, prevent diabetes transfer and impair TH1 cell function in diabetogenic models. However, the mechanism of MnP-mediated TH1 hyporesponsiveness is unknown. Therefore, redox modulation during diabetogenic TH1 cell activation can decrease LAG-3 cleavage by reducing enzymatic activity of the redox-dependent TACE and, consequently, impair the transition to autoreactive CD4 T cell effector function. Furthermore, redox-modulated LAG-3+ T cells may obtain a regulatory phenotype, inhibiting autoreactive responses and transfer of diabetes. This hypothesis is being tested by trying to further determine the direct relationship of redox-modulated, TACE-dependent LAG-3 shedding and TH1 effector function, and if redox-modulated CD4+LAG-3+ T cells can inhibit diabetic spleen and diabetogenic CD4+ T cell transfer, as well as provide peripheral regulation of autoimmune diabetes.

STUDY SECTIONS

- Grant reviewer, American Diabetes Association

ADVISORY COMMITTEE MEMBERSHIPS

- Chair, Recruiting Committee for the Interdisciplinary Biomedical Sciences Graduate Program
- Representative, Interdisciplinary Biomedical Sciences Graduate Program, University of Pittsburgh to the Annual Biomedical Research Conference for Minority Students, Berkeley, Calif.

Steven Ringquist, PhD

RESEARCH

T1D is among the most common chronic diseases of childhood. Steven Ringquist's research goal is to elucidate molecular networks affecting T1D susceptibility that are directly influenced by inherited genetic variants. Increased understanding of the gene networks underlying disease risk will aid in the development of accurate screening tools as well as creation of new therapeutic treatments. The goal will be attained through an interdisciplinary approach involving development of molecular and statistical tools to identify perturbations of expression gene networks, as well as the integration of levels of analyses ranging from mathematics, cellular, molecular, *in silico* and *in vitro* interactions, and pathway modeling to understand molecular networks.

PROFESSIONAL AFFILIATIONS/SOCIETY MEMBERSHIPS

- American Association for the Advancement of Science

William A. Rudert, MD, PhD

RESEARCH

William Rudert's experience in HLA molecular typing is facilitating the high-throughput molecular typing to characterize T1D patients relative to susceptibility to the disease or its complications.

Rudert is assisting Yong Fan in the validation of his diabetic model based on impaired thymic expression of insulin. The consistent and rapid onset of diabetes in this model makes it an ideal system for studying the T cells that have critical roles in the disease process. Recently developed methods for massive sequencing of the mRNAs that encode the T-cell receptors are being used to characterize the T cells present at the onset of islet destruction.

A parallel diabetes model system based on reduced thymic insulin expression is being developed in Cynomolgus monkeys. This is expected to be a genuine autoimmune model of diabetes in subhuman primates. If successful, this primate model will be a valuable proving ground for potential human therapies for T1D. The need to focus on eventual human applications has also led to a project to develop minicircle DNA technology as the means of safely and permanently modifying cells able to prevent or suppress the autoimmune attack that leads to diabetes.

Rudert also is investigating the molecular mechanisms that mediate C-peptide effects on vascular endothelial cells, which may be protective against the vascular complications of diabetes. Efforts are being made to identify a receptor specific for C-peptide.

Tatyana Votyakova, PhD

RESEARCH

Tatyana Votyakova's expertise is in energy metabolism, mechanisms of oxidative stress, and mitochondrial physiology. She utilizes her expertise working on the projects connected to islet biology and diabetes-related pathophysiology.

The Role of Mitochondrial Functions in Beta-cell Physiology in the Scope of a Better Preservation of Donor Islets and Their Transplantation into a Diabetic Patient. The well-being of transplanted beta cells is critical to the success of blood glucose normalization in recipient diabetic patients. The production of insulin, its storage, and timely release to maintain glucose homeostasis is a high-energy, demanding function. This notion implies an important role of mitochondria in beta cells. The aim of this project is to elucidate mitochondrial mechanisms linked to beta-cell performance *in vitro* and *in vivo* with practical issues in mind of better islet preservation and assessment of their potential to survive and function well in a recipient's body. In particular, it was established that exposure of islets to xeno plasma *in vitro* resulted in decrease of their respiratory activity, indicating once again importance of mitochondrial performance in islets survival. Tatyana Votyakova has continued the project in collaboration with Rita Bottino and Suzanne Bertera.

Study of Mitochondrial Functions in Development of Kidney Complications. About 30% of diabetic patients develop kidney complications at some point of the disease. The aim of this project is to investigate the role of oxidative stress and bioenergetic factors in the development of diabetic nephropathy using a rodent model. Two diabetic mice strains with the same mutation (the Akita mutation), but with different severity of complications, are used in this project. The Akita mutant mice on the DBA background exhibit prominent features of diabetic nephropathy, whereas in C57BL/6 (B6) background, kidney functions are less changed. At a young age, both types of diabetics demonstrate compensation in their kidney mitochondria activity. With aging, B6-Akitas that did not develop complications stay in par with their normal age-matched counterparts, whereas diabetic DBA-Akita mice, which develop complications, lose compensatory features of their kidney mitochondria. Knowledge on mechanisms underlying development of diabetic nephropathy will be instrumental in finding new therapeutic approaches for preventive treatment in patients with diabetic kidney.

Study of Protective Mechanisms of Catalytic Antioxidant in Development of Autoimmune Diabetes. Votyakova is assisting Jon Piganelli and Meghan Delmastro in their investigation of protective mechanisms of catalytic antioxidant against development of autoimmune diabetes in mice. The data have been collected, indicating involvement of mitochondrial mechanisms in this process; in particular, there is evidence that catalytic antioxidant render protection via modulation of respiratory activity of mice splenocyte. In this project, Votyakova helps with experimental design, practical issues, and interpretation of acquired data.

Study of Mechanism of Oxidative Stress Related to Metabolic Derangements of Diabetes. Increased production of reactive oxygen species (ROS) in mitochondria underlies major systemic diseases such as diabetes and pathological side effects accompanying acute conditions such as tissue transplantation and ischemia. On the model of isolated mitochondria, Votyakova's team directly demonstrated that after temporal hypoxia, resuming O2 supply leads to an increase in mitochondrial ROS generation. Using combination of experimental model of isolated mitochondria and computational approaches, new insights have been gained into mechanisms of ROS production in mitochondria, which are related to hypoxia-induced increase of ROS and different substrate supply relevant to diabetic conditions. This project was performed in collaboration with computational biologists from the University of Alabama and University of Barcelona (Spain).

EDITORSHIPS

- Reviewer, *Metabolism*
- Reviewer, *Current Medicinal Chemistry*

MAJOR LECTURESHIPS AND SEMINARS

- "Islets from Mitochondrial Point of View: Islet Respiration as a Measure of Their Well-being," invited speaker, Thomas E. Starzl Transplantation Institute, Faculty Research Meetings, Pittsburgh, Pa., May 2012
- "The Ways Mitochondria Produce Free Radicals," invited speaker, Mitochondria, Metabolism, and Disease," across-campus seminar, June 2012

PROFESSIONAL AFFILIATIONS/SOCIETY MEMBERSHIPS

- American Diabetes Association
- Biophysical Society

TEACHING ACTIVITIES

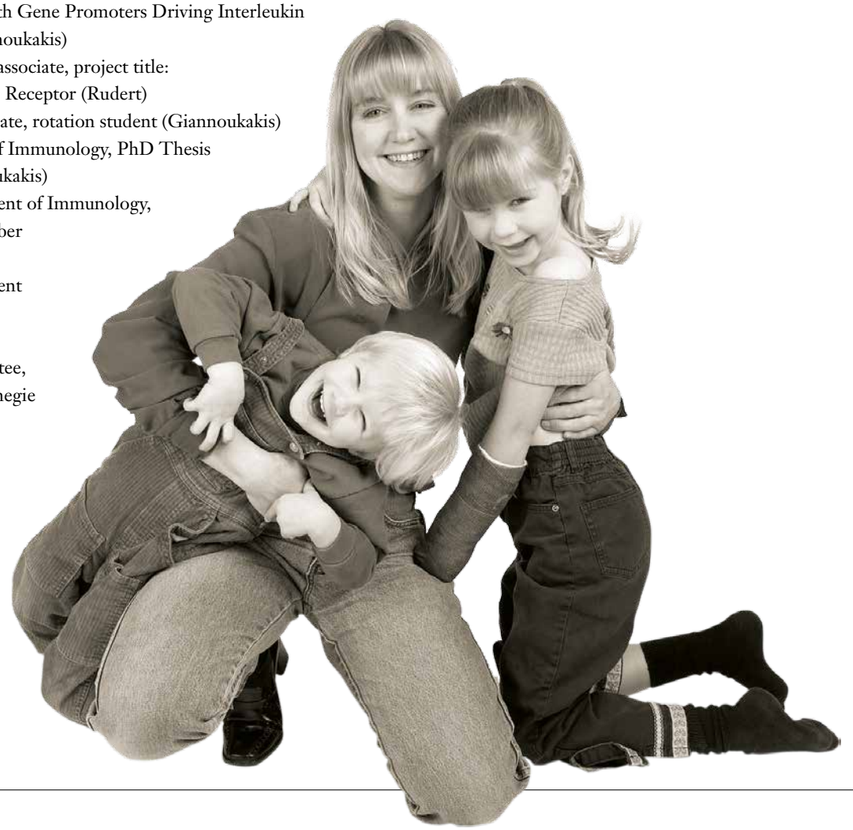
MEDICAL SCHOOL LECTURES AND COURSES

- Pancreatic Islet Transplantation: Basic Mechanisms, Cell Therapy Course, Department of Pathology (Trucco)
- Pancreatic Islet Transplantation: Clinical Experience, Cell Therapy Course, Department of Pathology (Bottino)
- Instruction for visiting scholars in rodent islet isolation techniques and murine microsurgery (Bertera)
- Training and laboratory orientation of graduate and undergraduate students rotating through the laboratory in small-animal handling and surgical techniques, separation of mononucleocytes from human blood samples, and molecular techniques including DNA extraction and PCR analysis, Department of Human Genetics, Graduate School of Public Health (Bertera)
- Chapter of Diabetic Dyslipidemia, Molecular Pathology Course (Dong)
- Introduction to Immunobiotherapeutics, course director, Department of Immunology, School of Medicine (Giannoukakis)
- Epigenetics and Malignancy, MSBMG 3510: Advanced Topics in Gene Expression, Molecular Genetics, and Biochemistry, School of Medicine, graduate students in the Molecular Biochemistry Program (Giannoukakis)

- The Thyroid, MSCMP 2730, Department of Pathology, School of Medicine, graduate students in the Molecular Pathology Program (Giannoukakis)
- Stem Cells: Endocrine Pancreas, Department of Pathology, School of Medicine (Giannoukakis)
- Neuroendocrine-immune System Interactions MSIMM 3230: Immunology and Human Disease, graduate students of the Immunology Program, Department of Immunology, University of Pittsburgh School of Medicine (Giannoukakis)
- Histology and immunohistochemistry background and techniques for graduate students and fellows; mentoring them in microscope technology (He)
- Molecular pathobiology, Module 3 section leader (Piganelli)
- Introduction to Immunobiotherapeutics (Piganelli)
- Methods in Logic and Medicine (Piganelli)
- Immunobiotherapeutics course, Department of Immunology, University of Pittsburgh School of Medicine (Votyakova)

RESEARCH MENTORSHIP

- Kritika Kachapati, PhD student, Department of Rheumatology and Clinical Immunology (Trucco)
- Terri C. Thayer, PhD student, Department of Immunology (Trucco)
- Maria Grupillo, MS, research fellow, ISMETT, Italy, project title: Regulation of Immune Self-tolerance Toward Pancreatic Beta Cells Via Islet Autoantigen Expression in Immune Systems (Trucco and Fan)
- Antonina Coppola, MS, research fellow, University of Palermo, Italy, project title: Immune Tolerogenic Roles of Aire-expressing Cells (Fan)
- Giulio Gualtierotti, MS, research fellow, University of Pittsburgh, Department of Pediatrics, Division of Immunogenetics, project title: Role of Autoimmunity against Islet Autoantigen 69 (ICA69) in T1D Development (Fan)
- Asako Tajima-Otsubo, MD, visiting scholar, Jikei University School of Medicine, Japan, project title: Islet Autoantigen 69 (ICA69) in Etiopathogenesis of T1D (Fan)
- Valentina Di Caro, PhD, international research fellow (RiMed), project title: Interactions of Foxp3 with Gene Promoters Driving Interleukin Expression (Trucco and Giannoukakis)
- Xuehui Geng, PhD, research associate, project title: Characterization of C-peptide Receptor (Rudert)
- Kristia Hamilton, MSc candidate, rotation student (Giannoukakis)
- Angela Pardee, Department of Immunology, PhD Thesis Committee member (Giannoukakis)
- Meghan Delmastro, Department of Immunology, PhD Thesis Committee member (Giannoukakis)
- Meghan Delmastro, Department of Immunology, PhD Thesis Committee chair (Piganelli)
- Gaia Bellone, Thesis Committee, Department of Statistics, Carnegie Mellon University (Trucco)
- Rachel Tobin, summer student (Bottino)
- Richard Peluso, summer student (Piganelli)
- Adam Attaar, summer student (Votyakova)



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