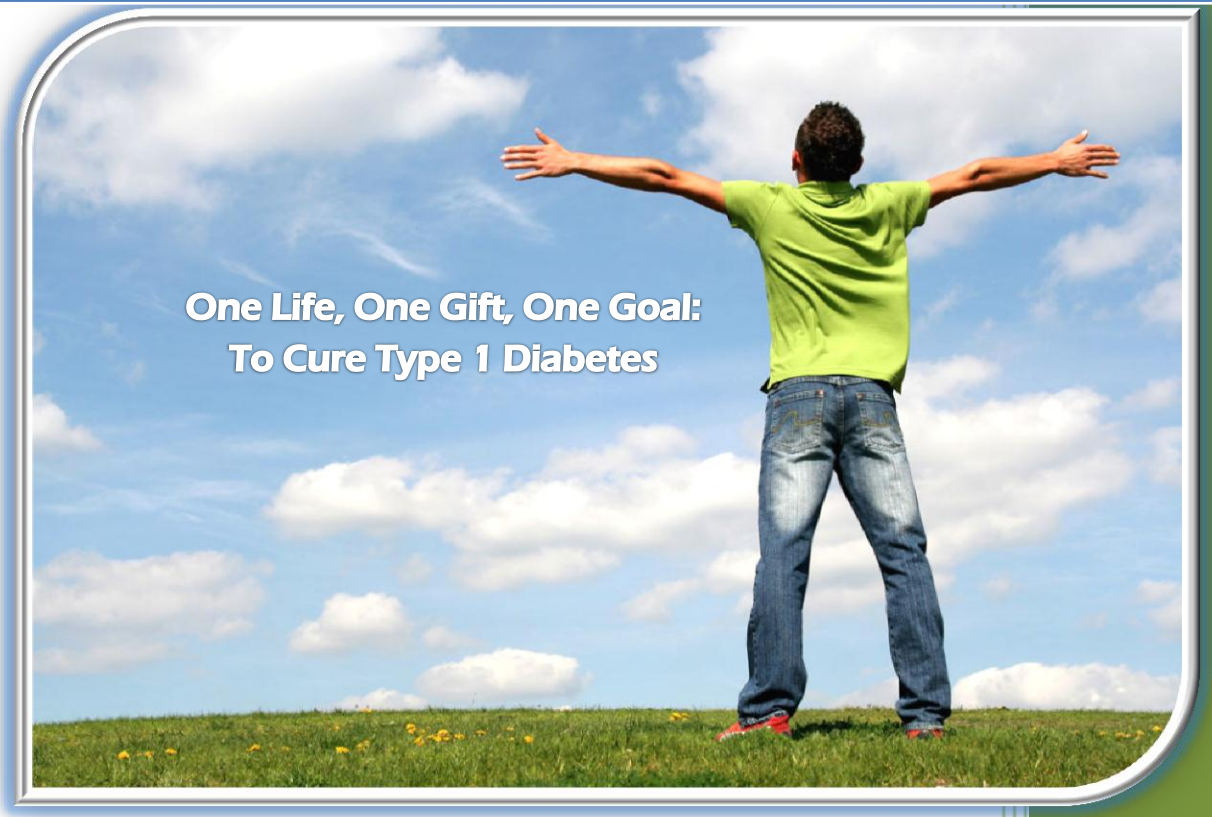
2013

nPOD 5th Annual Meeting



Meeting Syllabus

February 10-13, 2013

Atlantic Beach, Florida

nPOD 5th Annual Scientific Meeting

TABLE OF CONTENTS

February 10, 2013

Dear Participants,

Thank you for joining the 5th Annual nPOD Meeting. JDRF has been a proud supporter of nPOD since its inception nearly 6 years ago. At the end of last year, JDRF extended support to nPOD for an additional 5-year period, to continue to build on this critical community resource that has already provided novel insights into human T1D.

With the renewal of nPOD, JDRF looks forward to new opportunities to grow and strengthen the collaborative programs using the human tissue resources made available through nPOD. Specifically, JDRF sees great value in engaging industry and fostering robust academic-industry interactions – a topic that will be discussed during the meeting. We strongly support relentless efforts by nPod to identify the rare, yet most valuable, donor cases prior to, or at the time of, disease onset.

In addition, we applaud nPOD's leadership role in creating a culture of sharing and pooling of data and catalyzing collaborations and follow-on studies. The recent creation of nPOD-V exemplifies this community spirit. This meeting is a great example of how best to foster collaborative relationships to advance our understanding of the pathophysiology of T1D.

Again, thank you for your continued efforts and dedication to T1D. JDRF believes that the discoveries made through nPOD will help lead to transformative treatments that will improve the lives of those living with T1D and ultimately allow us to eliminate the disease entirely.

As the CEO of JDRF and the father of a child with type 1 diabetes, I would like to express my gratitude to everyone at nPOD for your dedication and ongoing efforts in support of this valuable initiative.

Have a great meeting.

Sincerely,

Jeffrey Brewer
President and CEO
Juvenile Diabetes Research Foundation

nPOD
Network for Pancreatic Organ Donors with Diabetes

February 10, 2013

Dear Colleague,

Welcome to Atlantic Beach and to the 5th Annual JDRF nPOD Scientific Meeting! We are thankful that you made it a priority to join us at this meeting and for what you have done to make the nPOD project a success.

The nPOD team has assembled a scientific program that is as inclusive as possible to allow all of us to share our collective progress and discoveries. In this setting, we would like to acknowledge the critical contributions of the nPOD Advisory Board, and all other committees. With their guidance and our collective input and participation, nPOD is bound to realize its full potential.

These were also the goals of one of the visionary founders of nPOD, Dr. George Eisenbarth. We at nPOD were extremely saddened when George passed away this last November, following more than a year-long battle with cancer. In honor of George and his contributions to nPOD, you will note a special program has been organized for Monday evening.

This year's program will have a number of new events and activities in order to help see the mission of nPOD expand. Amongst these, you will find computer "kiosks" that are designed to help introduce you to the new DATASHARE program. You will also note opportunities for smaller groups to meet in break-out sessions and then present recommendations for general discussion. Finally, we have included a session with leading representatives from the pharmaceutical industry with the hope of fostering academic-industry partnerships.

We would also like to thank the JDRF for their faith in the nPOD project and their continued support. During the past year, the JDRF has supported programmatic expansions and the formation of first formally recognized nPOD working group. Most critically, in late 2012, the JDRF renewed its funding commitment to nPOD for (up to) an additional five years.

At the end of this meeting, we ask that you complete a post-meeting survey found at the back of this booklet. Your feedback and candid opinions are critical to us for planning our meetings and activities. We look forward to an exciting meeting.

Mark Atkinson, Ph.D.
JDRF nPOD Executive Director

Alberto Pugliese, M.D.
JDRF nPOD Co-Executive Director

History and Mission of nPOD

The Network for Pancreatic Organ Donors with Diabetes (nPOD) is a collaborative type 1 diabetes research project funded by the Juvenile Diabetes Research Foundation (JDRF). nPOD supports scientific investigators by providing, without cost, rare and difficult to obtain tissues beneficial to their research. Approved investigators can participate in the nPOD program, even if they do not have outside funding to support their scientific research. Because of this precious and limited resource, data sharing and collaboration is strongly encouraged. nPOD began as a feasibility pilot project in 2007 and as of December 2011, supports over 70 type 1 diabetes-related scientific studies at institutions around the world.

The objective of the nPOD project is to recover and distribute, to qualified investigators, rare tissues obtained from deceased organ donors with type 1 diabetes and undiagnosed donors with anti-islet autoantibodies. nPOD also serves as an organizational hub for investigators who will utilize these tissues to answer fundamental questions about why type 1 diabetes occurs.

nPOD works closely with Organ Procurement Organizations (OPOs) to recover organs and tissues of interest. Inclusion and exclusion criteria are updated as necessary, based on feedback from the Scientific Advisory Board (SAB) and investigator needs. nPOD seeks donations from the following groups of organ donors:

1. Donors with recent onset of type 1 diabetes – These donors are potentially the key to helping our scientific investigators unlock the early disease process in type 1 diabetes and answer fundamental questions about the autoimmune process that leads to the destruction of the insulin producing beta cells in the pancreas.
2. Donors who have type 1 diabetes-related autoantibodies, but no clinical symptoms of the disease – These donors will help scientists study the autoimmune process at the very earliest stages of beta cell destruction.
3. Donors with pancreas transplant and history type 1 diabetes– These donors to improve our understanding of islet autoimmunity and its evolution, and how this may related to poorly understood mechanisms of pancreas regeneration/remodeling, and how both autoimmunity and regeneration may be affected by chronic immunosuppression
4. Donors without type 1 diabetes – These donors serve as normal control types when compared to other donor sets.

The nPOD website (www.jdrfnpod.org) provides additional information about the project and research efforts currently supported by nPOD, which are also listed in this booklet.

Acknowledgements

JDRF:

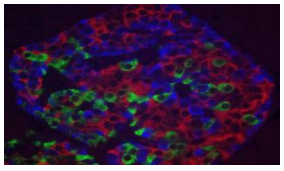
The nPOD team thanks JDRF for their constant support and dedication to "the little nPOD that could." JDRF has seen the project grow ten-fold within the four years it has been in operation. We are grateful that JDRF believes in nPOD and strives to see it succeed.

OPOs:

nPOD thanks the Organ Procurement Organizations (OPOs) that work tirelessly to refer and recover donor cases for nPOD. Without their support, nPOD would not exist.

Affiliates:

nPOD also thanks the scientific investigators and external committees that help guide and direct the project to success. Our investigators provide meaningful contributions to the field of type 1 diabetes research and encourage the nPOD team daily to continue to help them strive in their field.



General Information

Hotel Conference Room Map

Neighborhood Map

Atlantic Beach Area Attractions

Atlantic Beach is a relaxed beach village, with several eateries within walking distance. Below is a listing of a few eateries just outside of the hotel.

Beaches Town Center Map Legend—Restaurants & Bars

3. Al's Pizza—Winner of "Best Pizza" award by Folio Magazine 1995-2009 and voted "Beaches Favorite Pizza" 2000-2009! ● 303 Atlantic Blvd. ●alspizza.com ●904-249-0002

37. Jimmy John's—Founded in 1983, JJ's Sub Sandwich restaurant uses top quality meats, cheese, & award-winning French Bread baked fresh daily. ● 363 Atlantic Blvd.

16. Joseph's Pizza & Italian Restaurant
Jax's Oldest Pizzeria since 1956 features gourmet pizzas, pastas, subs, salads, calzones, zeppoles, gelato and a bakery. ● 30 Ocean Blvd. ● josephspizza.com ● 904-270-1122

11. Island Girl Cigar Bar—Upscale tropical atmosphere, outstanding cigar collection, 28 draft beers, and extensive wine list. Relax ...You're on island time. ●108 First Street ● islandgirljax.com ● 904-372-0943

22. M Shack—A modern day, road-side stand, serving up all natural burgers, dogs, shakes and more with culinary flare. ● 229 Atlantic Blvd ● mshackburgers.com ● 904-241-2599

12. Mezza Luna—Serving Italian classic and contemporary American ● 904-249-5573 ●110 First Street ● mezzalunaneptunebeach.com

23. North Beach Fish Camp—Southern fare, fresh seafood, and sought after bread pudding. ● 100 First Street ● northbeachfishcamp.com ● 904-249-3474

34. Ocean 60—Award winning restaurant offers Chef Created Continental Cuisine. Fine dining in a casual, elegant atmosphere. ●60 Ocean Blvd. ● ocean60.com ● 904-247-0060

13. Pete's Bar—Best prices at the beach, 25 cent pool. ●117 First Street ● 904-249-9158

8. Ragtime Tavern—Cuisines including Cajun and fresh seafood, chicken and pastas, and freshly brewed beers. ● 207 Atlantic Blvd ● ragtimetavern.com ● 904-241-7877

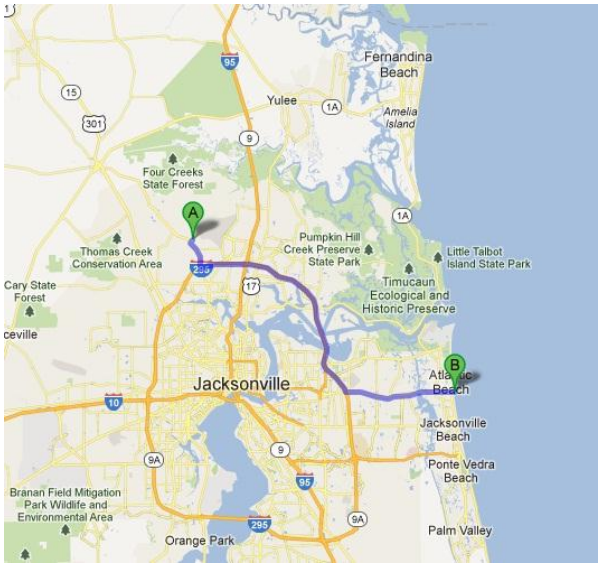
25. Sliders Seafood Grille—Floridian-style dinners and casual coastal atmosphere. Outside dining open year round! ● 218 First Street ● 904-246-0881

4. Poe's Tavern—Gourmet burgers, hand cut fries, fresh fish tacos and craft and import beers. ● 363 Atlantic Blvd. ● poestavern.com ● 904-241-7637

10. Tama's Sushi—Hakaru "Tama" Tamaki, sushi chef, formally trained in Japan, established in 1981. ● 106 First St. ● 904-241-0099

32. Lillie's Coffee Bar—Open at 7 a.m. Coffee, breakfast, lunch specials and desserts. Evening appetizers with beer & wine pairings. ● 200 First Street lilliescoffeebar.com ● 904-249-2922

Airport Map



A = Jacksonville International Airport B = One Ocean Resort in Atlantic Beach

Emergency Information

Please call Jayne Moraski at 352-359-2741 for any emergencies you might have.

Transportation Information

Transportation from the Jacksonville International Airport to the One Ocean Resort is estimated at approximately thirty minutes. Prices below are for a one way trip.

East Coast Transportation
904-525-8600
$79 airport shuttle each way

GO Airport Shuttle—Shared Shuttle Service
904-353-8880
$30 first person, $8 each additional person

Checker Cab
904-345-3333
$55 per person from hotel to airport

Floridian Transportation—Shared Shuttle Service
904-353-8880
$45 per person

nPOD
Network for Pancreatic Organ Donors with Diabetes

nPOD Annual Meeting Agenda
February 10-13, 2013 ♦ One Ocean Resort ♦ Atlantic Beach, FL

MEETING GOALS

While previous Annual nPOD Meetings have received much in the way of positive feedback, this year's meeting has been organized to not only continue in that tradition, but also to maximize discussion and see the potential for collaborative research efforts move to new levels. We hope participants will find this new format both intellectually stimulating and rewarding.

The goals of the 5th Annual JDRF nPOD meeting are:

1. In *cutting edge seminars and oral abstract presentations*, we will review and discuss progress and new findings from nPOD supported projects, including (but not limited to):
    a. Data pertaining to the immunopathogenesis of human type 1 diabetes
    b. New abnormalities/possible pathogenic mechanisms/therapeutic targets.
2. In *working group sessions*, we seek to present the evolution of the nPOD research model, based on super-structured working groups focused on key topics in T1D research, including the concept of real time data sharing within the new nPOD DataShare program. This concept will be illustrated by:
    a. Providing a progress report from the nPOD-V group.
    b. Presentation of bioinformatic infrastructure (DataShare), alongside investigator discussion and feedback.
    c. Noting our desire for nPOD investigators to develop their own concepts and ideas, for analysis, as a collective action.
3. In *general sessions*, we will apply the nPOD approach to key research priority areas, by:
    a. Reviewing status of knowledge via talks and discussions in each main area
    b. Tasking small groups of investigators (*break-out sessions*) to draft key questions in each main area.
    c. Following presentation, provide review and discussion of small group .recommendations, and formulation of integrated list of key questions for each area.
    d. Ascertaining the provisional formation of new, dedicated working groups.
4. Guiding questions throughout the conference include:
    a. How can DataShare make investigation easier?
    b. What are the new questions that can be asked?
    c. How do the results diverge from conventional wisdom?
    d. Is there another sample set that could be used to inform/further develop these results?
    e. What other experiments inside or outside of nPOD should be performed to extend results/analysis?

Time	AGENDA Function	Location
Sunday, February 10, 2013		
5:00-7:00 pm	Registration opens	Palmara Room
Monday, February 11, 2013		
7:00 am	Registration opens	Palmara Room
7:00–7:45 am	Breakfast	Pristina Room
7:45–8:30 am	**Welcome and Introductions** Co-Chairs: Mark Atkinson and Teo Staeva • The State of nPOD: This session will include discussion of, "What do we want to see accomplished in the meeting?" • Desmond Schatz will present the new T1D Classification Scheme	Atlantica Ballroom
8:30–9:00 am	**Session 1: nPOD-Virus (nPOD-V) Working Group Update** Chair: Alberto Pugliese Introduction– (5 min) <u>Presentations from Task Group Leaders - (5 min each)</u> Task 1. Sarah Richardson Task 2. Joseph Petrosino Task 3. Noel Morgan Task 4. Dirk Homann Task 5. Rick Lloyd	Atlantica Ballroom
9:00–9:30 am	**Session 2: DataShare Presentation** Chairs: John Kaddis, Les Jebson, and Suzanne Ball This session will illustrate the nPOD-V working group concept by example from Task 6 leader and demonstrate use of DataShare.	Atlantica Ballroom
9:30–9:45 am	Break	Pristina Room
9:45–11:15 am	**Session 3: Oral Abstracts–Viral Etiology** Chair: Mark Atkinson (10 minutes + 5 minutes Q&A for each) 1. Isabella Spagnuolo–Beta cell expression of enteroviral receptor CAR 2. Ivan Gerling–Gene expression profiles from laser captured nPOD donor islets 3. Vincent Plagnol–Link between viral infections and T1D 4. Maarit Oikarinen–Correlation between enterovirus positivity in different tissues	Atlantica Ballroom
11:15 am–12:15 pm	**Session 4: Review of nPOD Case 6195** This session hopes to examine one case from different points of view, and discuss summary of findings. Discussants: David Leslie, Alberto Pugliese, Martha Campbell-Thompson	Atlantica Ballroom

Monday, February 11, 2013 (continued)		
Time	**Function**	**Location**
12:15–1:15 pm	Lunch	Pristina Room
1:15–2:30 pm	**Session 5: Cutting Edge Seminars I** Chair: Dale Greiner (2 talks, 20 min each) 1. Decio Eizirik–Mechanisms of beta cell death 2. Matthias Hebrok– Beta cell trans-differentiation or regeneration	Atlantica Ballroom
2:30–3:00 pm	**Session 6:** **General Discussion on Cutting Edge Seminars I** Chairs: Dale Greiner and Clayton Mathews Following previous seminar presentations, this session will formulate a list of key questions for the creation of new, dedicated working groups.	Atlantica Ballroom
3:00–3:30 pm	Break	Pristina
3:30–5:00 pm	**Session 7: Oral Abstracts–Islet Biology** Chair: Teo Staeva (10 minutes +5 minutes Q&A for each) 1. Ben Giepmans–Anatomy of human islets 2. Roberto Gianani–Diabetic pathology syndromes distinguished by beta cell mass and islet phenotype 3. Marika Bogdani–Hyaluronan accumulates in islets and lymphoid tissues of T1D patients 4. Maggie Morris–Expression patterns in lipoxygenases 5. Linda Yip–Diminished A1 receptor function in pancreatic alpha cells 6. Giacomo Lanzoni–Stem and progenitor cell signatures	Atlantica Ballroom
5:00–6:30 pm	End of Afternoon (Break) We encourage you to explore the DataShare program in the *Serenoa* and *Caretta* Rooms at this time (space is limited)	On your own
6:30–7:30 pm	Reception	Verandina patio
7:30 pm	Dinner–With program in honor of George Eisenbarth	Solaria Rooom

Tuesday, February 12, 2013 AGENDA		
Time	**Function**	**Location**
7:00–7:45 am	Registration opens	Palmara Room
7:00–7:45 am	Breakfast	Pristina Room
7:00–7:45 am	Executive Committee Meeting (closed session)	Board Room
7:45–8:30 am	**Session 8:** **nPOD-Transplantation (nPOD-T) Update and Discussion** Chair: Alberto Pugliese 1. George Burke–nPOD-T keynote talk (20 min) 2. Alberto Pugliese–nPOD updates from (10 min) 3. Tim Tree–JDRF Immune Memory Consortium updates (10 min)	Atlantica Ballroom
8:30–9:00 am	**Session 9: Oral Abstracts–Transplantation** Chair: Luca Inverardi (10 minutes +5 minutes Q&A for each) 1. Clayton Mathews–Reduced B cell expression of glucokinase and ATP synthase linked to impaired insulin secretion 2. Francesco Vendrame–Memory T cells in the transplanted pancreas with recurrent diabetes	Atlantica Ballroom
9:00–10:00 am	**Session 10: nPOD-Europe (nPOD-E) Discussion and Updates** Co-chairs: Olle Korsgren and Noel Morgan Francesco Dotta–nPOD-E Keynote talk (20 min) Updates (10 minutes each): 1. Heikki Hyöty–Finland 2. Gun Frisk–Sweden 3. Eduard Montanya–Spain	Atlantica Ballroom
10:00–10:30 am	Break	Pristina Room
10:30–12:00 pm	**Session 11: Breakout Brainstorming Sessions** Small groups will assemble, discuss ideas, goals and key questions to collaboratively address in the following key research areas: 1. Insulitis–Chairs: Peter In't veld and Martha Campbell Thompson 2. Beta Cell replication–Chair: Jake Kushner 3. Biomarkers–Chairs: Bart Roep and Jerry Nepom	Breakout rooms
12:00–1:00 pm	Lunch	Pristina Room
1:00–2:30 pm	**Session 12: Reports from Breakout Session Chairs**	Atlantica Ballroom

Tuesday, February 12, 2013 (continued)		
Time	**Function**	**Location**
2:30–3:00 pm	Break	Pristina Room
3:00–4:30 pm	**Session 13: Cutting Edge Seminars II** Chair: Ron Gill (3 talks, 20 min each) 1. Alexandra Butler–The pancreas in T1D, a pathologist's perspective 2. Andrew Stewart–Beta cell replication 3. Olle Korsgren–Revisiting the notion of Type 1 Diabetes being a T cell-mediated autoimmune disease	Atlantica Ballroom
4:30–6:00 pm	End of Afternoon (Break) We encourage you to explore the DataShare program in the *Serenoa* and *Caretta* Rooms at this time (space is limited)	On your own
6:00–7:00 pm	**Session 14: Poster Session** Wine & Cheese Reception	Atlantica Ballroom
	Dinner on your own	On your own

Wednesday, February 13, 2013 (Half day)		
Time	**Function**	**Location**
7:00 am	Registration opens	Palmara Room
7:00–7:45 am	Breakfast	Pristina Room
7:45–10:00 am	**Session 15: Oral Abstracts–Immunology** Chair: Todd Brusko (10 minutes +5 minutes Q&A) 1. Darius Schneider–Cytokine signatures of cells 2. Kathleen Gillespie–Maternal microchimerism in healthy and T1D pancreas 3. Howard Seay–Global CpG methylation signature of pancreatic draining lymph node CD8+ T cells in type 1 diabetes 4. Sally Kent–Splenic autoreactive B cells from T1D subjects 5. Helena Reijonen– Islet autoreactive CD4 T cells restricted by T1D protective HLA molecules in a T2D donor and Islet autoantigen specific CD4 T cells with distinct HLA restrictions, epitope specificities and chemokine profiles in the blood and spleen of a T1D patient 6. Mercè Marti–Redundancy of CDR3 sequences in the TCR VB& family of intrapancreatic T cell infiltrates 7. Melanie Stumpf–Identification of potential disease associated T cell receptor (TCR) sequences in pancreatic tissue from patients with recent-onset type 1 diabetes	Atlantica Ballroom
10:00–10:30 am	Break	Pristina Room
10:30 am–11:30 am	**Session 16:** **Moving Discovery Towards Clinical Application** *NEW!* Chair: Dick Insel <u>Roundtable discussion of potential partnership opportunities</u> 1. Phil Ambery and Soumitra Ghosh–GlaxoSmithKline 2. Matthias von Herrath–Novo 3. Jim Lenhard–Johnson & Johnson 4. Michele Youd–Genzyme	Atlantica Ballroom

Wednesday, February 13, 2013 (continued)		
Time	**Function**	**Time**
11:30 am–12:30 pm	**Session 17: Wrap Up–General Discussion** Chairs: Mark Atkinson and Alberto Pugliese • What general principles have we learned from nPOD to date that has changed our understanding of T1D and may affect therapeutic strategies? • Is the nPOD culture and resource providing a new paradigm for conducting research? • What could nPOD be exploring that it is currently not doing? • What do we not know about T1D that nPOD could address?	Atlantica Ballroom
12:30 pm	Boxed lunch provided for all attendees	Atlantica Ballroom
12:30 pm	**End of Meeting**	
12:30–3:00 pm	nPOD SAB meeting (closed session)	Solaria Room

Invited Speaker Biographical Information

Note: Sessions 1, 2, 4, 5, 8, 10, and 13 have invited speakers. Biographical information below is provided by each speaker. The other sessions have discussion chairs as indicated, or are included in the Scientific Abstract section that follows this section.

Session 1: nPOD-Virus Group Invited Speakers

Chair: **Alberto Pugliese** obtained his MD degree from the University of Palermo, Italy. He then trained with George Eisenbarth at the Joslin Diabetes Center and at the Barbara Davis Center. He then established his own laboratory at the Diabetes Research Institute, University of Miami, in 1994. His research interests span across various disciplines, but are focused on type 1 diabetes, and in particular on the immunology and genetics of the disease. Since 2000, he has been involved in clinical trials as a steering committee member of the Type 1 Diabetes TrialNet and co-PI of the TrialNet Center at the University of Miami. He is also Executive Co-director of the JDRF nPOD. Amongst his past contributions is the observation that autoantibody-positive relatives maintain an extremely low risk if they carry the HLA-DQB1*0602 allele, which is relevant to the design of clinical trials. The discovery that the insulin gene is transcribed in the human thymus, where transcription levels are affected by two mechanisms, allelic variation and parent-of-origin effects, provided a mechanistic explanation for insulin gene-mediated susceptibility. During the past seven years, he has also worked with Dr. G.W. Burke, Director of Pancreas-Kidney Transplantation at UM, in leading a study on the recurrence of type 1 diabetes in pancreas-kidney transplant recipients.

nPOD-V Task 1 Leader: Sarah Richardson's career in type 1 diabetes research began six years ago when she joined Professor Noel Morgan's group at the University of Exeter Medical School. Prior to this her postdoctoral work was in the field of apoptosis based at both the University of Sheffield and the Walter and Eliza Hall Institute in Melbourne. She utilises two unique cohorts of type 1 diabetes patient pancreas samples and her research is centered around developing a clearer understanding of the disease processes by which beta cells are targeted and destroyed. She has a particular interest in the role that enteroviruses may play in the disease pathogenesis. Her current research projects are aimed at confirming whether enterovirus can be detected in the pancreas of patients, and enhancing knowledge of the pathogenic signaling mechanisms involved in the recruitment and activation of immune cells. The success of this work enabled her to gain a Diabetes Research Wellness Foundation (DRWF) Non-Clinical Research Fellowship and an nPOD Junior Investigator Award and she is an Innovators in Diabetes participant with Diabetes UK. Sarah is an enthusiastic and pro-active member of both the nPOD-V and EU FP7 PEVNET networks.

nPOD-V Task 2 Leader: Joseph F. Petrosino, Ph.D., is an Assistant Professor of Molecular Virology and Microbiology at Baylor College of Medicine where he is also the director of the Alkek Center for Metagenomics and Microbiome Research. He holds joint appointments in the Human Genome Sequencing Center, and Department of Ophthalmology. Dr. Petrosino has authored more than 40 original papers. Among 14 published in 2012 are the June HMP flagship manuscripts in Nature, collaborative studies examining microbiome associations with cystic fibrosis, pregnancy, nutritional intervention in colitis, rotavirus infection, and the shaping of the microbiome from birth in murine systems. In recognition of these efforts and others, Dr. Petrosino has been named an American Society for Microbiology Distinguished Lecturer for 2012-2014.

nPOD-V Task 3 Leader: Noel Morgan began his research career studying islet biology at University of Leicester and undertook postdoctoral work on the hormonal control of liver glycogenolysis at Vanderbilt University (Nashville, Tn). He subsequently developed an independent research programme in islet biology while on the faculty of Keele University (UK). He was recipient of the inaugural Albert E. Renold Fellowship of EASD in 1990 to work in the laboratory of Robert J Lefkowitz (2012 Nobel laureate) at Duke University, Durham, NC studying adrenoceptor signalling in β-cells. Noel was appointed to a Personal Chair at Keele University in 1993 and moved to University of Exeter, Devon, UK in 2002, as Professor of Endocrine Pharmacology. He is currently Director of the Institute of Biomedical & Clinical research within the University of Exeter Medical School. His group is delighted to participate in both the EU FP7 PEVNET and nPOD-V consortia.

nPOD-V Task 4 Leader: Dirk Homann, **MD, PhD**, Assistant Professor of Pediatrics, and Immunology. Dr. Homann's research interests lie in the areas of autoimmunity, immunological memory, and persistent viral infections. The common theme among these topics is a focus on T cell immunity under conditions where specific T cells may cause pathology (autoimmunity), provide protection upon reencounter with a pathogen (immunological memory), or are impaired in their capacity to control an infection (persistent virus infections).

nPOD-V Task 5 Leader: Rick Lloyd, PhD, is a Professor, Department of Molecular Virology and Microbiology at the Baylor College of Medicine. Projects in Dr. Lloyd's lab center on mechanisms of translation control in mammalian cells, mechanisms of RNA granule (Stress granule and P-body) gene regulation and interference in these regulatory processes by enteroviruses such as poliovirus and coxsackievirus. He is also working on the Virome and Microbiome of the TEDDY study together with Joseph Petrosino, also at Baylor College of Medicine.

Session 2: DataShare Presentation

nPOD-V Task 6 Leader: John S. Kaddis, **Ph.D**. Staff Scientist at City of Hope National Medical Center, Department of Information Sciences.

Session 3: Please see Scientific Abstract Section of the program

Session 4: Review of nPOD Case 6195

4.1 **David Leslie** is Professor of Diabetes and Autoimmunity at the Blizard Institute, University of London and a consultant physician at St Bartholomews Hospital. He is current President of the Association of Physicians of Great Britain and Ireland. Dr Leslie is Principle Investigator of the UK Diabetic Twin Study, the European Action LADA study and Work Package leader in BLUEPRINT, a major EU study of epigenomics.

4.2 **Alberto Pugliese** is listed above in Session 1.

Session 4: Review of nPOD Case 6195 (continued)

4.3 Martha Campbell-Thompson, D.V.M., Ph.D, serves as the Pathology Core Principal Investigator at the University of Florida, where she earned doctorates in veterinary medicine and veterinary physiology. A board certified large animal veterinary surgeon, Dr. Campbell-Thompson moved into basic research after receiving her Ph.D. She has over 20 years' experience in animal models of human disease with an emphasis on type 1 diabetes. She brings managerial experience to her role as primary administrator of the Pathology Core, providing histological and immunolocalization services to over 100 investigators at the University of Florida, in the United States, and overseas.

Session 5: Cutting Edge Seminars I

5.1 Decio L. Eizirik—Mechanisms of Beta cell death. Dr. Eizirik is an MD and PhD, trained in Brazil (Universities of Rio Grande do Sul and Sao Paulo) and Sweden (Uppsala University). Since 2002 has been Professor at the Medical Faculty, Universite Libre de Bruxelles (ULB), Belgium, where he directs the Laboratory of Experimental Medicine and leads a group focusing on pancreatic beta cell dysfunction and death in diabetes. He has investigated the molecular pathways involved in immune-induced beta cell impairment and apoptosis in type 1 diabetes (T1D), leading to fundamental concepts such as the dialogue between the immune system and beta cells that trigger and amplify insulitis and beta cell damage. By using functional genomics and bioinformatics tools he and his group have clarified the cytokineregulated gene networks that define beta cell outcome, including the recent discoveries of the role of endoplasmic reticulum stress and alternative splicing in this process, and the identification of the mitochondrial pathways that ultimately trigger beta cell apoptosis. His scientific contributions have been recognized by several awards, including a Career Development Award from the Juvenile Diabetes Research Foundation International (JDRFI), the Research Prize Pharmacia & Upjohn (Belgium), the JDRFI Diabetes Care Research Award, the Trondheim Honorary Lecture and the EASD Albert Renold Award Lecture.

5.2 Matthias Hebrok, Ph.D.—Beta cell trans-dfferentiation or regeneration. Dr. Hebrok is the *Hurlbut-Johnson Distinguished Professor in Diabetes Research* and Director of the UCSF Diabetes Center. He is the recipient of several honors and awards, including the JDRF Scholar Award. He received his Diploma degree in cellular biology from Albert-Ludwigs University in Freiburg, Germany, and performed his PhD thesis at the Max-Planck-Institute for Immunobiology. His postdoctoral research was performed at HHMI at Harvard University. His laboratory uses Cell, Molecular, and Developmental Biology tools to decipher the mechanisms that underlie mammalian pancreas organogenesis and pancreatic diseases, including diabetes and pancreatic cancer. The goals of these studies are *a)* to generate functional ß-cells from human stem cell populations for cell therapy purposes and *b)* to prevent the formation and growth of pancreatic tumors. Dr. Hebrok has served on numerous Grant Review Committees for the JDRF and NIH and is currently a full member of the NIH Cellular Aspects of Diabetes and Obesity (CADO) study section. He was a Member of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Diabetes Mellitus Interagency Coordinating Committee (DMICC) and continues to participate as a Member of the Scientific Advisory Board for the Speman Graduate School at the Albert-Ludwigs University in Freiburg, Germany.

Session 7: Please see Scientific Abstract Section of the program

Session 8: nPOD-Transplantation

8.1 George W. Burke, III, M.D., F.A.C.S., American Board of Surgery, Director, Division of Kidney and Pancreas Transplantation Professor of Surgery. His research interests include recurrence of autoimmunity after pancreas transplantation, prevention of proteinuria in children with nephrotic syndrome after kidney transplantation, use of machine preservation for kidney transplantation clinical benefits, bariatric surgery for patients with ESRD and BMI greater than 35 randomized to omentectomy or not, pilot study to evaluate portal vein adipocytokine and approach to tolerance induction in kidney and kidney/pancreas transplant.

8.2 Alberto Pugliese (please see biographical information in Session 1 Chair above)

8.3 Timothy Tree is a Senior Lecturer of Immunology, Infection and Inflammatory Disease (DIIID) at King's College London. His research interests include the role of autoreactive and regulatory T cells in human health, disease and transplantation. Developing statergies to strengthen immune regulation.

Session 9: Please see Scientific Abstract Section of the program

Session 10: nPOD-Europe

Keynote speaker: Francesco Dotta graduated in Medicine and Specialized in Endocrinology and Metabolism at University of Rome "La Sapienza". Post-doctoral research fellow at the Joslin Diabetes Center - Harvard medical School, Boston, USA, under the supervision of Prof. George Eisenbarth. Investigator and then Associate Professor of Endocrinology, University of Rome "La Sapienza", Italy. Since 2003, Director Diabetes Unit, University of Siena, Italy and Scientific Director of The Umberto Di Mario Research Foundation. His research focuses on te characterization of diabetes-associated islet autoimmune response and of islet pathology in human pancreas and the identification of pathways involved in islet cell damage and regeneration represents the major research focus in Dotta's laboratory. Dr. Dotta is author of more than 140 publications in peer reviewed international journals

10.1 Heikki Hyöty—Finland. Dr. Hyöty is a Professor of Biomedicine and Virology, University of Tampere, Finland. Selected among the top ten Opinion Leader Scientists in health science sector by a Finnish medical journal MediUutiset (16.12.2011), Dr. Hyoty is also coordinator of three EU-funded international multicenter studies.

10.2 Gun Frisk—Sweden. Dr. Frisk is an Associate Professor, Department of Immunology, Genetics and Pathology, Uppsala University, Sweden. Her research interests include: aetiology of type 1 diabetes, human enterovirus infection and innate immunity in primary human cells; and tropism of Human enterovirus in various human tissues.

10.3 Eduard Montanya—Spain. Dr. Montanya is Senior Professor of Medicine at the University of Barcelona, Head of the Diabetes Section of the Endocrinology Department of Bellvitge University Hospital, and Head of the Diabetes and Metabolism Research Group at Bellvitge Biomedical Research Institute (IDIBELL). He has been Chair of the Islet Study Group of the Spanish Diabetes Association, and is the current President of the Diabetes Advisory Board of the Catalan Government, and the President of the Catalan Diabetes Association.

Session 13: Cutting Edge Seminars II

13.1 Alexandra Butler—The pancreas in T1D, a pathologist's perspective. Alexandra Butler MD attended medical school at the University of Newcastle upon Tyne in northeast England. Upon moving to the United States, she did a Pathology residency at Mayo Clinic. Since moving to Los Angeles, she has been involved in research into the pathology and underlying mechanisms in type 1 and type 2 diabetes, with a particular emphasis on human disease.

13.2 Andrew Stewart–Beta cell replication. Dr. Andrew Stewart has led a career devoted to patient care and basic and clinical research in endocrinology and diabetes research for over 30 years. Dr. Stewart is a leading authority on human pancreatic beta cell replication and regeneration. His research focuses on understanding and developing novel means for inducing beta cell regeneration. His group was the first to demonstrate that growth factors could drive beta cell replication in vivo in mammals, and also improve glucose control in living animals. Dr. Stewart has received numerous honors, including Councilor of both the Endocrine Society as well as the American Society for Bone and Mineral Research (ASBMR); Secretary-Treasurer of the Endocrine Society, and Chair of the Program Committee for the ADA. He has published more than 230 scientific papers, with many in journals of the highest quality, including the *Proceedings of the National Academy of Sciences*, the *New England Journal of Medicine*, and *Science*.

13.3 Olle Korsgren–Revisiting the notion of Type 1 Diabetes being a T cell-mediated autoimmune disease. Dr. Korsgren started his medical studies at the University of Uppsala, Sweden. He then received a Research Trainee Award from the Swedish Medical Research Council. In 2002 he was appointed Professor of Transplantation Immunology at Uppsala University. Since 2006, he's held the position as Professor of Cell Transplantation at the same University. Dr. Korsgren's research activity has been focused on making islet transplantation a possible treatment for patients with type I diabetes. He is the Principal Investigator of the Nordic Network for clinical islet transplantation.

Scientific Abstracts For Oral Presentations

Scientific abstracts from Sessions 3, 7, 9, and 15 are included in this section. **All are printed as submitted by the authors. No edits to content, spelling, or grammar have been made.** Abstracts are listed in the order which they are presented throughout the meeting.

Session 3: Oral Abstracts–Viral Etiology

3:1 Beta cell expression of enteroviral receptor CAR and islet inflammation in human type 1 diabetes

Isabella Spagnuolo, Guido Sebastiani, Aurora Patti, Fabio Arturo Grieco, Francesca Mancarella, Francesco Dotta

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Purpose: Type 1 diabetes mellitus (T1D) is a chronic autoimmune disease in which pancreatic beta cells are selectively destroyed by an autoimmune process. The onset is caused by the interaction of genetic, immunological and environmental factors. Among the environmental factors potentially involved in the T1D etiology, viral infections play a key role. Previous studies have demonstrated signs of enteroviral infection in pancreatic islets, and most intriguingly in beta cells, from recent-onset T1D patients. Moreover it has been suggested that Coxsackie B4 virus (CVB4) directly trigger beta-cell destruction, suggesting a link between enteroviral infections and T1D. The susceptibility to viral infections is caused by viral variants and by the nature of infection, while the specific tropism of viruses is modulated by the local expression of cellular receptors, such as hCAR (coxsackievirus and adenovirus receptor). In addition, intracellular viral RNA sensors, such as RNA helicases RIG-I and MDA5 can trigger the antiviral response such as the production of IFNs by activation of two transcription factors, NF-kB and IRF-3. We here aimed at characterizing hCAR and RIG-I islet expression as well as inflammatory phenomena in human type 1 diabetic and control pancreas.

Materials and methods: We studied pancreatic specimens obtained from 4 recent onset T1D, from 2 long standing (disease duration: 7 and 14 years) T1D and from 10 non-diabetic organ donors. Formalin–fixed and paraffin embedded pancreatic sections were used in immunohistochemical experiments for the expression of proinflammatory molecules (CXCL10, CCL2, Fas) as well as of viral receptor hCAR and dsRNA sensor RIG-I. In addition, double immunofluorescence with confocal microscopy analysis was utilized for the identification islet cell subset(s) expressing the molecule of interest (i.e. alpha-, beta- and delta cells identified by anti-glucagon, anti-insulin and anti-somatostatin antibodies respectively).

Summary of results: In T1D pancreata, insulitis was present in 44% of islets and was characterized by CD3 expressing T-cells. Ongoing islet inflammation was detected in all T1D cases, but not in control donors, with in situ detection of CXCL10, Fas and of CCL2; interestingly, this latter chemokine showed a remarkable beta-cell specific expression. Viral receptor hCAR was expressed both in T1D and in control donors mainly in islet cells and, of note, almost exclusively at beta cell level, suggesting the existence of a differential distribution of enterovirus

receptors among islet cell subsets, possibly influencing the susceptibility of these cells to enteroviral infection. Intriguingly, the only rare alpha cells expressing hCAR were single cells located outside islet structures and sparse throughout the exocrine tissue. We also detected the presence of viral sensor RIG-I in a subclass of human islet cell both in T1D that in the healthy control; of note, such cells resulted to be delta cells by confocal microscopy.

Conclusion: In conclusion, we have shown that islet inflammation can be detected at disease onset, persists years after T1D diagnosis and is characterized proinflammatory molecules and by a beta-cell specific expression chemokine CCL2. In addition, we uncovered the beta-cell specific expression of enteroviral receptor hCAR, which may represent a mechanism responsible for the beta-cell tropism observed in the case of some enteroviruses.

3:2 Gene Expresssion Profiles from Laser Captured nPOD Donor Islets

Nataliya I. Lenchik, Shannon G. Matta, Ivan C. Gerling

Department of Medicine, University of Tennessee Health Science Center

Purpose: The purpose of our study was to use laser-capture microscopy to collect islets from non-diabetic auto-antibody positive (ab+) and matched autoantibody negative (ab-) control nPOD donors, and obtain islet mRNA expression profiles.

Methods: Cryo-sections (8µm thick) were shipped from the nPOD laboratory in Gainesville. Slides were fixed, dried, and 60-80 islets captured from each donor. RNA was immediately extracted from the tissue and stored at -80C. The quantity and integrity (RIN number) of total RNA was determined. RNA was amplified using kits appropriate for each downstream transcriptome platform. Affymetrix expression arrays and RNA sequencing technologies (SoLID) were used to obtain expression profiles.

Summary of Results: We found great variability in the quality of RNA obtained from different donors (RIN: 2.2 – 7.3, n=33). Although the main source of this variability seemed to be donor differences, we did find that using a different block from the same donor could produce as much as a 2.5 difference in RIN. In our first round of islet collection from 12 donors, we found that by isolating RNA from two different blocks (from the same donor), islet RNA with RIN $\geq$3.9 could be obtained from all but one donor. Pools of islet RNA with variable RIN were created, to test the ability of samples with low RIN to produce expression profiles. RNAseq on the SoLID platform required a large amount of RNA and did not produce good results with low RIN samples. In contrast, the Affymetrix platform could be used with much less RNA and was able to produce expression profiles even from a sample with RIN 3.2. Next, we obtained tissue from 8 ab+ and 8 age and sex matched ab- controls. In this experiment we were able to find islets in all but one (an ab+) donor pancreas. All RNA isolated from the 15 islet samples had RIN $\geq$3.2. We conducted expression profiling on these 15 samples using Affymetrix arrays. Different analysis and data-mining approaches were used, and showed that the genes separating islets gene expression in ab+ from ab- donors, are associated with mitochondrial dysfunction. Among the genes differentiating the two groups were HLA class I, oxidative stress related genes, proteasome subunits and multiple genes regulated by HNF4A.

Conclusions: We have demonstrated the feasibility of obtaining comprehensive islet gene expression signatures from >90% of the nPOD donor pancreata using laser-capture. A comparison of expression from islets of non-diabetic autoantibody positive and negative organ donors suggest mitochondrial dysfunction and tissue stress in islets from autoantibody positive donors.

3:3 Different Species of Enteroviruses (EV) in Peripheral Blood Leukocytes (PBL) of Children at the Clinical Onset of Type 1 Diabetes

Sofia Morfopoulou, Vincent Plagnol

University College London Genetics Institute, London

Purpose: A growing body of genetic and epidemiological evidence suggests a link between viral infections and type 1 diabetes (T1D). Viruses may act as a trigger for T1D onset and the identification of viral agents that are present in young onset cases may provide new clues into T1D pathogenesis. Transcriptome sequencing, or RNA-Seq, is a powerful technology to detect active viral infections in human tissue. To identify viruses relevant to T1D etiology, we undertook as part of the network of pancreatic donors virus group (nPOD-V) initiative a RNA-Seq experiment for a set of post-mortem pancreatic samples from young onset T1D cases and controls.

Methods: We sequenced total RNA from n = 9 pancreatic samples in two batches using deep Illumina short sequencing reads. We used a metagenomic analysis to detect pathogens present in these samples. To maximize the power of this study, we developed a novel Bayesian statistical approach to detect organisms expressed at very low levels in metagenomic mixtures.

Results: Our results highlight the difficulties and opportunities provided by deep short read sequencing to generate insights into the contribution of viral infections to T1D. At least 10% of the reads cannot be reliably assigned to a known organism. We identified a wide arrays of organisms present in these samples, including two potential bacterial (E coli) and viral (murine leukemia virus) contaminations but also multiple bacteriophages. We have not detected enteroviruses which are prime candidate for a role in the etiology of type 1 diabetes. However this analysis is still ongoing and methodological refinements may uncover organisms present at very low levels.

Conclusions: The interpretation of deep sequencing metagenomics dataset is a challenging process. Based on our ongoing analysis, we can rule out based on our analysis the presence of acute enterovirus infections. The large fraction of unassigned sequencing reads suggests that much can still be discovered as analytical methods improve.

3:4 Correlation between enterovirus positivity in different tissues of cadaver organ donors with type 1 diabetes

Maarit Oikarinen, Sisko Tauriainen, Sami Oikarinen, Heikki Hyöty

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Purpose: An association between enterovirus (EV) infections and type 1 diabetes (T1D) has been shown by epidemiological studies. Potential causal relationship has got support from studies showing EV in the pancreatic islets and intestinal mucosa of T1D patients. The aim of the present study was to analyze the correlation between EV positivity in the pancreas, spleen, duodenum and pancreatic lymph nodes (PLN) in T1D patients, islet-autoantibody-positive (auto-ab+) individuals and healthy controls.

Methods: Tissues were collected from cadaver organ donors in the Network for Pancreatic Organ donors with Diabetes (nPOD) study. Formalin-fixed paraffin-embedded tissue samples were analyzed using EV-specific in situ hybridization (ISH) and immunohistochemical (IHC, clone 5-D8/1, DakoCytomation) assays. Study series included 35 donors (15 T1D patients, 3 auto-ab+ individuals and 17 controls) from whom, in addition to pancreatic sample, at least one other tissue sample was available.

Summary of results: The presence of EV (either viral genome in ISH or protein in IHC) in the pancreas correlated with that in the other tissues. 80 % (8/10) of those donors who were EV-positive in the pancreas were also EV-positive in the spleen compared to 32 % (8/25) of the donors who were EV-negative in the pancreas (p=0,01). Similarly, all three donors who were EV-positive in the pancreas were also positive in the pancreatic lymph nodes (p=0,04). 67 % (4/6) of the donors who were EV-positive in the pancreas were also positive in the duodenum compared to only one (13 %) of the eight donors who were EV-negative in the pancreas (p=0,04). All the donors with enterovirus positivity in multiple organs were T1D patients or auto-ab+ individuals.

Conclusions: Simultaneous detection of EV in multiple organs in type 1 diabetic patients and islet-autoantibody-positive individuals supports the presence of the virus. These findings fit with a scenario where the infection in T1D patients starts from the duodenal mucosa from which it spreads to the pancreas and other organs. Possible role of EV in the pathogenesis of type 1 diabetes merits further studies.

Session 7: Oral Abstracts—Islet Biology

7:1 Nanotomy of human Islets of Langerhans during Type I diabetes

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Purpose: High resolution imaging; data-sharing; and analysis (nano-anatomy; or "nanotomy") of Islets of Langerhans of nPOD donors. Data will be analyzed for hallmarks that might play a role in diabetes development (i.e. presence of viruses; ER- stress; insulitus; insulin content; etc.). All hallmarks are in this single dataset.

Methods: We image complete cross-sections of human Islets during TypeI diabetes or therapy with nanometer resolution [1,2] at an electron microscope (EM) for large-scale imaging.We implement Nanotomy to sample Islets of different donors (nPOD). Pancreas will be processed for EM. Data will be acquired, and initial analysis will be performed, focused on insulitis and small virus(-like) particles. Importantly, we obtain unbiased information from all cells sampled in the cross-section of Islets.

Summary of Results: This project is part of nPODv and started September 1st 2012. Currently, the first large scale maps of donors have been imaged, and are being analyzed. Technical challenges have been solved in a prior study, focusing on a T1D rat model [2]. In this animal dataset we found particles resemble structures such as viruses or glycogen. During analysis of the nPOD datasets, special attention will be on the possible presence of these particles in human Islets from diabetic donors versus controls. Given our unbiased large-scale approach, we can not directly determine all (rare) events. Therefore, the data should become available of as much donors as possible to allow a complete accessible analysis by nPOD investigators.

Conclusions: Nanotomy is a new technique that helps to unravel the hallmarks of beta cell destruction and Islet condition in macromolecular detail.

References: 1. Faas, F. G. *et al*. Virtual nanoscopy: Generation of ultra-large high resolution electron microscopy maps. *J. Cell Biol.* 198, 457-469 (2012).
2. Raimond, Kalicharan, Avramut, Sjollema, Pronk, Dijk, Koster, Visser, Faas, Giepmans. Destruction of Tissue, Cells and Organelles in Type I Diabetes Presented at Macromolecular Resolution– *submitted*.

The first two authors contributed equally.

7:2 Diabetic syndromes with differential pathology and beta cell phenotype

Roberto Gianani

University of Colorado Denver

Purpose: Diabetes mellitus is a syndrome caused by a variety of different etiologies. At the pathological levels, however, two fundamental patterns of beta cell loss can be recognized on the basis of the presence (pattern A) or absence (pattern B) of pseudo-atrophic islets (i.e. islets completely devoid of beta cells). There are, however no, data on the quantification of beta and alpha cell mass in these two patterns. In addition, it is also unknown whether the beta cells in pattern A display a fundamentally different phenotype than beta cells in normal controls and patients with pattern B pathology.
To answers these questions, we have expanded our initial characterization of nPOD (network of pancreatic organ donors with diabetes) organ donor pancreata from normal controls and patients with diabetes mellitus.

Methods: Fifty eight donors with DM and twenty six controls were studied. For each subjects, measurements of islet auto antibodies to the islet autoantigens GAD, IA-2 and Znt8 as well as DR HLA typing and C-peptide levels were obtained.

Summary of results: Forty eight subjects with pattern A and 10 subjects with pattern B were identified. Morphometric analysis of beta and alpha cell reveal that both were reduced in patients with pattern A and pattern B diabetes mellitus in relation to normal controls. The beta cells mass was significantly lower in donors with pattern A than in donors with pattern B while the alpha cell mass was not significantly different among the two groups.
There was a strong association between autoimmune diabetes (defined by the presence of at least one positive islet autoantibodies, and/or HLA DR3/DR4 status and insulitis) and pattern A (as compared to pattern B) ($P < 0.001$).
The residual beta cells in pattern A had “lobular” distributions with clusters of islets containing beta cells interspersed among pseudo-atrophic islets. The beta cell in subjects with pattern A often expressed a peculiar phenotype characterized by positivity for survivin similarly to fetal beta cells. In control and pattern B donors, the majority of islets did not contain survivin positive beta cells but, in some instances, clusters of islets with survivin positive beta cells were also seen.

Conclusion: These data show that pattern A and pattern B are differentiated by their relation with autoimmune diabetes, the amount of residual beta cell mass as well as a specific fetal like phenotype of the beta cells.

7:3 Hyaluronan accumulates in islets and lymphoid tissues of type 1 diabetic patients and associates with inflammatory cells in insulitis

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Purpose. The process of β-cell destruction in type 1 diabetes (T1D) relies on migration of inflammatory cells from the blood stream into pancreatic islets via interaction with the extracellular matrix (ECM). In this study, we focus on hyaluronan (HA), an ECM glycosaminoglycan and examine the presence and distribution of HA in normal pancreatic islets and lymphoid tissues, and determine the changes that occur in the amount and distribution of HA in T1D pancreatic islets and lymphoid tissues.

Methods. Pancreas and lymphoid tissue sections from 16 T1D patients with T1D 1-10 years or more than 30 years, and 17 age-matched controls were provided by nPOD (under grant # 25-2010-648). HA presence and location were examined by affinity histochemistry and HA accumulation in both tissues determined quantitatively.

Summary of Results. In the non-diabetic control pancreas, HA staining was present at the islet periphery and within the islets. HA was observed as scanty accumulations forming a loose fine fiber-like or bead-like structures occupying 2.4 ± 0.4 % of the islet area. HA was enriched in the T1D islets occupying 8.9 ± 2.6 % of the islet area. Some differences were noted in the percentage of intra-islet HA staining depending on the duration of T1D. Non-diabetic and T1D islets were examined for the presence of inflammatory cells by double immunohistochemistry. T1D tissues contained more islets with CD45 cells than the non-diabetics, and a larger number of CD45 cells per islet mostly observed in a row or clustered inside the islets or at islet periphery in close contact with islet endocrine cells. All the T1D tissues characterized by the presence of inflammatory cells in the islets also showed elevated HA staining. Further, the inflammatory cells were often embedded in the HA-enriched ECM. A significant increase in the total area stained by HA was observed in the pancreatic lymph nodes in T1D as compared to the non-diabetic tissues (8% and 0.2%, respectively), both in B-cell follicular germinal centers and T-cell areas. A similar pattern was observed in the T1D spleen B-cell follicles, in which HA occupied 8.6% of the follicular germinal centers, which was higher than that observed in the non-diabetics (0.7%). Intense HA staining was observed within these areas forming thick fibers along the T-cell cords and occupied 12% of the T-cell area compared to 2.7% of the area observed in the non-diabetic controls.

Conclusions. Our observations show that major ECM changes occur in islets and lymphoid tissues of T1D patients. In diabetic islets, HA accumulates in islet ECM and occurs in close physical association with infiltrating leukocytes in insulitis, both in human and rodent T1D tissues. In T1D lymphoid tissues, HA amasses in pancreatic lymph nodes and spleens, in the T-cell areas and enlarged B-cell follicular germinal center. Our results point to the involvement of HA in the inflammatory processes in T1D. The importance of this ECM component in T-cell events associated with the development of T1D awaits further investigations.

7:4 Expression patterns of lipoxygenases in human islets

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Purpose*:* The key pathways leading to autoimmune inflammatory damage to beta cells in Type 1 diabetes (T1D) remain unclear. Identifying new targets for therapy remains as a high priority given the failure of current treatments to prevent or reverse beta cell damage in T1D. 12-Lipoxygenase (12-LO) is an enzyme that generates a lipid leading to human beta cell dysfunction and death. 12-LO is expressed in human islets, and expression increases after the addition of pro-inflammtory cytokines (Ma, 2010). 12-lipoxygenase is integral in the development of inflammatory responses in diabetes. Our group has shown that 12/15-Lipoxygenases are important in the development of Type 1 Diabetes in the NOD mouse model (*McDuffie, 2008)*. We have not previously localized this expression within the islets. The goal of our studies was to delineate the pattern of expression of main lipoxygenase (ALOX12 and ALOX15) genes products (as potentially of importance in the pathophysiology of diabetes) in human islets in situ.

Methods*:* Indirect immuno-detection system (immunofluorescence and immunohistochemistry) was employed using a panel of commercially available antibodies specific for ALOX12-*S* and ALOX15-1 proteins. Normal (i.e., non diabetic), autoantibody positive, and diabetic tissues (paraffin-embedded, 7 μm thick sections) obtained from the nPOD consortium were used.

Summary of Results: By immunostaining, ALOX-12*S* was not detectable in normal islets, nor in islets of patients with long-term type 1 or type 2 diabetes. In contrast, we found ALOX-12S signal in islets from several of the autoantibody positive patients (2 out of 5), as well as in early type 1 diabetes and short-duration type 2 diabetes specimens (when beta cells are present in both types). Interestingly, ALOX-12 appears to be predominantly expressed by cells that are also positive for pancreatic polypeptide (PP). In contrast to ALOX12-*S*, ALOX15-1 protein was absent in pancreatic islets, regardless of the diabetic status of the patient.

Conclusion: Based on the finding of this study, ALOX-12*S* appears to be expressed in islets during very early stages of diabetes, supporting its role as a potential therapeutic target.

7:5 Diminished A1 receptor function in pancreatic alpha cells may contribute to T1D

Linda Yip, C. Garrison Fathman

Department of Medicine, Division of Immunology and Rheumatology, Stanford University

Recent studies have shown that loss of glucagon signaling can protect against streptozotocin-induced diabetes in mice. Glucagon secretion is controlled by various factors, including adenosine. Adenosine acts on adenosine A1 receptors (ADORA1) to inhibit glucagon secretion. We have previously shown that *Adora1* gene expression is significantly reduced in the pancreatic lymph node of Non-obese diabetic mice (NOD) compared to NOD.B10 controls during the onset of disease.

Purpose: Here, we studied whether the gene and protein expression of ADORA1 is altered in the pancreas of NOD vs. NOD.B10 mice, and non-diabetic vs. auto-AB positive (AA^+) and T1D patients.

Methods: Double staining immunohistochemistry and confocal microscopy was performed to localize ADORA1, insulin, and glucagon in mouse and human pancreas sections. RT-PCR, QPCR, cloning and sequencing were used to detect, quantify, sequence, and synthesize fusion proteins of the wild-type (WT) and the spliced isoform of *Adora1 (*A1-VAR*)*. cAMP ELISA assays were used to assess ADORA1 and A1-VAR receptor function in transfected cell lines.

Summary of results: In NOD.B10 mice of all ages (4, 8, 12, and 20 wks), ADORA1 was expressed on all alpha cells, and on few beta cells. The expression pattern was similar in NOD mice at 4 and 8 wks of age, prior to disease onset. However, as islet infiltration increased at 12 wks of age, ADORA1 expression was significantly reduced. By 20 wks of age, ADORA1 expression was barely detectable even though glucagon expression remained abundant. Similar findings were observed in patient samples. In control patients, the majority of ADORA1 expression was observed on alpha cells. In AA+ and T1D patients, ADORA1 expression was significantly lower and did not co-localize with glucagon. RT-PCR experiments demonstrate that *Adora1* is alternatively spliced to form a novel isoform (A1-VAR) in the pancreas of NOD mice. At 12 wks of age, the expression of this isoform is significantly higher in the pancreas of NOD vs. NOD.B10 mice. PCR primers were designed to determine if an equivalent isoform is expressed in human pancreas. Using these primers, we identified an AA^+ patient who expressed the A1-VAR isoform. The protein sequence of the human and mouse A1-VAR show 93% homology. ADORA1 and A1-VAR function was assessed in transfected HEK 293 cells. Surprisingly, the A1-VAR isoform was found to act as a dominant negative receptor, abolishing the ability of the ADORA1 WT isoform to inhibit forskolin-induced accumulation of cAMP.

Conclusion: These data demonstrate that A1 receptor expression and function is diminished in alpha cells during the progression of T1D. This may lead to increased glucagon secretion and signaling and contribute to the pathogenesis of T1D.

7:6 Stem and progenitor cell signatures in niches of the adult biliary tree and pancreas

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Purpose: The existence and the phenotypic traits of stem/progenitor cells in pediatric and adult pancreas are actively debated. This has hindered investigations aimed at determining whether such populations might be involved in pathological processes in type 1 and type 2 diabetes. We investigated the presence of stem and progenitor cells in peribiliary glands (PBGs) of the biliary tree and in pancreatic duct glands (PDGs) of pancreas derived from donors of all ages.

Methods: We obtained tissues from non-diabetic donors. We performed in situ immunohistochemistry, immunofluorescence and cell isolation under cell culture conditions designed for stem cells. We then assessed the differentiation potential of the isolated cells *in vitro* and *in vivo*.

Summary of Results: Stem cell populations were found in PBGs and committed progenitors in PDGs, as assessed by expression of markers of pluripotency (NANOG, OCT4, SOX2, SALL4), proliferation (Ki67), early hepato-pancreatic commitment (SOX9, SOX17, PDX1, LGR5), pancreatic endocrine commitment and maturation (NGN3, INSULIN). Cultures of stem cells from biliary tree and committed progenitors from pancreas were isolated using serum-free Kubota's Medium (KM) and culture plastic. They were differentiated to a mature fate in a serum-free hormonally defined medium (HDM-P) and extracellular matrix components tailored for an islet fate. The cells matured into glucose-regulatable, insulin-producing cells both in culture and after transplantation in vivo. The net findings were that bile ducts and their PBGs, pancreatic ducts and their associated PDGs comprise a continuous network of cells organized in maturational lineages from stem cell populations transitioning to committed progenitors and thence to mature cells. Proximal-to-distal lineages start in peribiliary glands (PBGs) near the duodenum with cells expressing markers of pluripotency, proliferation and early hepato-pancreatic commitment. The lineages progress to committed progenitors in PDGs with loss of pluripotency, hepatic and proliferation markers and increased expression of pancreatic endocrine maturational markers. Radial axis lineages start within bile duct walls progressing from stem cells in PBGs near to the fibromuscular layer and transitioning to mature cells at the bile duct lumens.

Conclusions: Biliary tree stem cells are precursors for committed progenitors within the pancreas and thence to islet cells. Our findings with respect to the maturational lineages in healthy subjects constitute the basis for analyses of stem and progenitor cell pools as affected by or responsive to the pathology in type 1 and type 2 diabetes.

Session 9 Oral Abstracts—Transplantation

9:1 Reduced B cell expression of glucokinase and ATP synthase linked to impaired insulin secretion but only modest B cell loss in the transplanted pancreas of patients with recent onset, recurrent T1D

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Purpose: We describe results from 2 T1D patients with simultaneous pancreas-kidney transplants (SPKTx) and developed recurrent T1D following the reappearance of multiple autoantibodies, beginning 2 and 4 yr after SPKTx. Hyperglycemia developed at 3.5 and 9 yrs with HbA1c levels of 12% and 10.7%, respectively.

Methods: Patients were tested for autoreactive T cells, ex-vivo, using tetramers. Patients underwent biopsy of the pancreas transplant tail and metabolic testing. The biopsy was subjected to immunofluorescent histochemistry [IF] analysis for insulin, insulitis, glucokinase [GCK], and ATP Synthase [ATPase]. For the IF experiments, we included sections from an insulin-independent SPKTx control, as well as sections from T1D-free and T1D donors with insulin positive islets from nPOD.

Results: Onset of insulin-dependence in both patients was associated with autoreactive T cells, in the circulation and the pancreas transplant associated lymph node tissue. Patient 1 had a mixed meal tolerance test (MMTT) 5 d prior to biopsy. Baseline blood glucose and c-peptide levels were 173 mg/dl and 1.3 ng/ml, respectively; c-peptide response was flat with a peak c-peptide level of 1.65 ng/ml at 2 hr. In Patient 2, blood glucose and c-peptide levels at MMTT baseline were 192 mg/dl and 1.28 ng/ml; c-peptide response was impaired (peak level 2.99 ng/ml at 1 hr; 2.33 ng/ml at 2 hr). Post-biopsy [2 mo], Patient 2 underwent an oral glucose tolerance test (OGTT), when HbA1c had decreased to 8.9%: baseline blood glucose and c-peptide levels were 198 mg/dl and 1.05 ng/ml, respectively; the c-peptide response was flat: peak c-peptide level of 1.27 ng/ml at 2 hr. Immunostaining of the pancreas transplant biopsies showed strong insulin staining that did not segregate the 3 SPKTx donors or nPOD cases from controls. Patient 1: 105/118 (89%) islets, 13/118 (11%) islets only stained for glucagon, and 16/118 (14%) islets had insulitis (mostly in insulin^{+} islets). patients with recurrent T1D in the transplanted pancreas. Biopsy tissue from Patient 2, obtained 11 d post MMTT, showed no evidence of acute rejection but rather insulitis, consisting predominantly of CD8 T cells, in 13/45 (29%) islets; there was strong insulin staining in 34/45 (76%) islets, with 11/45 (24%) islets only staining for glucagon. Significant decreases in both GCK and ATPase were observed in the B cells of the 2 SPKTx patients with recurrent T1D when compared to the insulin-independent SPKTx control; moreover, the reduction in GCK and ATPase levels in these 2 transplant biopsies was similar to that observed in nine nPOD cases with T1D and insulin positive islets.

Conclusions: There are no studies that correlate fasting and stimulated B cell function with assessment of B cell mass at the onset of T1D. Our 2 SPKTx patients provide such an assessment in the context of recent onset, recurrent T1D. Impairment of stimulated insulin secretion in the context of frank T1D with fasting hyperglycemia [300-400 mg/dl] range, and recurrent islet autoimmunity demonstrated by circulating autoantibodies and autoreactive T cells, significant insulitis, yet limited B cell loss was observed. Thus, in SPKTx patients with recently diagnosed recurrent T1D, hyperglycemia and impaired stimulated insulin secretion may be explained by severe functional impairment of a largely preserved B cell mass. These data suggest the existence of mechanisms of B cell dysfunction, besides immune mediated destruction, both in nPOD cases with spontaneous disease in the native pancreas and patients with recurrent T1D in the transplanted pancreas.

9:2 Memory T Cells in the transplanted pancreas with recurrent diabetes.

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PURPOSE: Type 1 diabetes recurrence (T1DR) may develop in 5% of recipients with simultaneous pancreas-kidney (SPK) transplants, despite immunosuppression that prevents rejection, typically after several years of normal transplant function. Earlier studies in patients with recent onset T1D and in islet cell transplant recipients implicate memory autoreactive T cells in islet autoimmunity. Here, we investigated whether memory autoreactive T cells are associated with T1DR.

METHODS: We recently identified two SPK recipients who developed T1DR. The first patient (SPK#1) was a White Hispanic who developed T1D at age 14, and received the SPK transplant when 39 years old. The second patient (SPK#2) was a White Caucasian who developed T1D at age 12, and received the SPK transplant when 35 years old. Both SPK patients developed multiple autoantibodies, at 4 and 2 years after transplantation. Hyperglycemia became evident at about 8 and 4 years, respectively. Biopsies of the pancreas transplant tail were performed about a year after HbA1c levels had begun to rise and hyperglycemia required insulin therapy to control. Autoreactive T cells were analyzed with class I and/or class II tetramers based on the HLA types, and included CD45RO/RA assessment to distinguish naïve and memory T cells, in peripheral blood and pancreas transplant lymph nodes. For SPK#1, cells were also studied from the pancreas transplant. Transplant biopsies were assessed for the presence of endocrine cells and for insulitis, by staining for CD3, CD4, CD8, CD20 and CD45RO (memory marker).

SUMMARY OF RESULTS: In SPK patient #1, ex-vivo assessment of autoreactive T cells using HLA-A2 pentamers demonstrated circulating GAD65-reactive CD8 T cells (and earlier time points), in the pancreas transplant biopsy and the associated lymph node tissue. In SPK patient #2, pooled GAD and insulin HLA-DR3 tetramers demonstrated autoreactive T cells in the circulation and in the pancreas transplant associated lymph node tissue. In both patients, the autoreactive T cells included memory cells in the circulation, pancreas transplant and associated lymph node tissue. Roughly 40-60% of the autoreactive T cells were CD45RO+ memory cells. The biopsies revealed insulitis and ß-cell loss with features that are similar to those reported for patients with spontaneous disease. In SPK patient #1, there was no evidence of acute rejection but rather insulitis, consisting predominantly of CD8 T cells, in about 30% of the islets examined; there was strong insulin staining in ~70% of the islets, while ~30% only stained for glucagon. In SPK patient #2, ~90% of the islets expressed insulin, while ~10% only stained for glucagon; insulitis affected about 14% of the islets, which were mostly insulin-positive. Also in this patient CD8 T cells were more abundant than CD4 T cells and B cells. Importantly, we demonstrated the presence of memory cells CD45RO+ T cells in the infiltrates of both patients. The majority of infiltrating T cells expressed CD45RO.

CONCLUSIONS: The localization of memory T cells in islet lymphocytic infiltrates and the presence of memory autoreactive T cells, as assessed with tetramers, in the circulation and pancreas transplant lymph node, links memory autoreactive memory T cells with T1DR. Studying their functional/phenotypic features could identify new therapeutic targets. Importantly, biopsies from these two patients are now part the JDRF nPOD repository and are available to nPOD investigators for further studies.

Session 15 Oral Abstracts—Immunology

15:1 Cytokine signature of cells in the pancreatic islet in patients with type 1 diabetes

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Rationale: The role of cytokines as immunopathological factors in autoimmune disease has been demonstrated, as well as the possible use as therapeutic target. However, our knowledge about the cytokine milieu of the pancreatic islet in T1D pathogenesis has been dampened by the scarcity of donor organs and the intricate histology techniques needed for cytokine detection. Nevertheless, directly identifying these cytokines as pivotal factors in T1D pathogenesis opens new therapeutic avenues, since cytokine blockade could be integrated in a combination therapy aimed at long term tolerance induction, ideally complementing immunization regimens that induce regulatory T cells. Here, we developed and evaluated a new immunohistochemical method to study the expression profile of selected cytokines (IL-6, IL-10, IL-12B, IFN-γ, IL-1, IL-17, TNF, IFN-alpha) in samples from diabetic donors and relate this data to known immunopathological factors like MHC-I upregulation.

Methods: Cryopreserved sections of pancreata and spleens from pancreata obtained through the nPOD concortium were incubated with selected cytokine specific antibodies after fixation with PFA and using saponin to permabilise cell membranes.
Results: The immunohistochemical method yielded reproducible and distinct staining patterns, in which cytokines accumulated mainly in the Golgi apparatus of producer cells, thus indicating local production rather than cytokine uptake. Thus far, we found preliminary evidence of interferon-alpha expression in pancreatic islets, other cytokines from the above mentioned panel will follow shortly.

Conclusion: We have established an improved method for high accuracy sampling of nPOD samples for cytokine production that will help us determine the local production of a wide array of cytokines within the pancreatic islet.

15:2 Maternal microchimerism in healthy and type 1 Diabetes pancreas

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Purpose*:* Maternal cells acquired from placental cell transfer exist in multiple parenchymal tissues. Encounter of maternal antigens in fetal development results in anti-maternal tolerance in healthy subjects. Nevertheless, increased levels of MMc (maternal microchimeric cells) have been observed in several autoimmune diseases including type 1 diabetes, but their role is unknown. Several different hypotheses have been put forward to explain these observations: 1) that MMc are effector cells of the immune response, 2) that MMc are targets of the autoimmune response or, 3) that MMc play a role in tissue repair. The aim of this study was to define the cellular phenotype and genotype of MMc in healthy and type 1 diabetic human pancreas.

Methods*:* Archival male human pancreas from 9 healthy (aged from late gestational to pediatric), 2 recent-onset pediatric, and 1 long-standing adult type 1 diabetes adult were analyzed as well as male fresh human islets and cultured islet derived cells. Female cells in male tissues were identified using X/Y chromosome-based fluorescence *in-situ* hybridization and phenotypes were determined by concomitant immunofluorescence for a range of phenotypic markers. Single cell laser capture with DNA profiling was explored to determine whether individual MMc in pancreatic tissue could be genotyped.

Summary of Results: In healthy pancreas, MMc positive for endocrine and exocrine markers, were identified. Rare MMc also exist in the $CD34^+$ endothelial fraction. MMc positive for Ki67 were identified, suggesting replication. In healthy individuals the frequencies of $insulin^+$ MMc beta cells varied from 0.05% to 0.8% and increased in frequency after the age of two ($p=0.0003$). MMc were identified in fresh human islets as well as islet derived cells and *in vitro* some expressed nestin, an indicator of dedifferentiation. In both recent onset and long-standing type 1 diabetes pancreases, no MMc were observed in $CD45^+$ infiltrates. Single cell DNA profiling to confirm the maternal origin of female cells in pancreas was shown to be feasible.

Conclusion: These data provide support for the hypothesis that MMc in human pancreas are derived from multipotent maternal progenitors. Increased levels of MMc beta cells in type 1 diabetes pancreas could potentially be a trigger of autoimmunity or may contribute to attempted tissue repair, but they are not effectors of the autoimmune response in type 1 diabetes.

15:3 Global CpG methylation signature of pancreatic draining lymph node CD8+ T cells in type 1 diabetes

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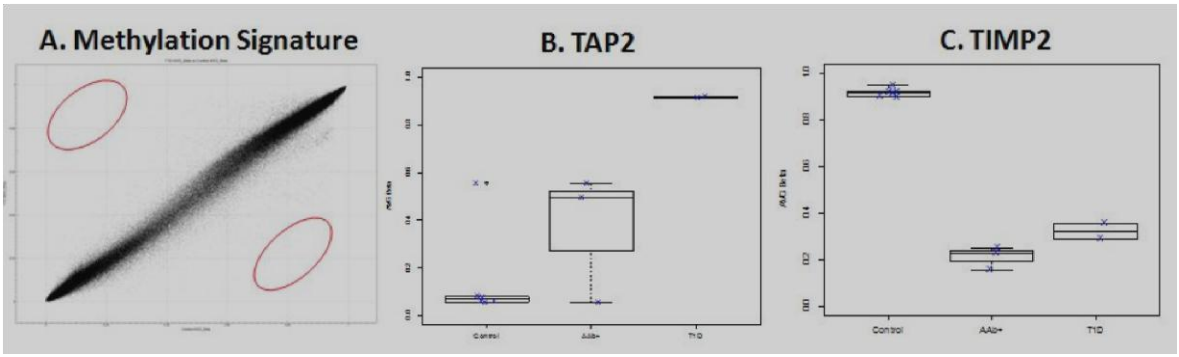
Purpose: HLA class I overexpression within islets and the expansion of autoreactive CD8+ T cells are characteristic events during the pathogenesis of type 1 diabetes (T1D). We propose that CD8+ T cells display epigenetic markers, including CpG methylation, that are initiated during T cell activation and differentiation and persist in established disease. Further, we predict these marks foretell the transcriptional profile and effector phenotypes of these autoreactive effector populations. To address this hypothesis, we assessed the global methylation signature of CD8+ T cells isolated from tissues derived from individuals with T1D, multiple autoantibody positive at-risk subjects, and normal healthy controls.

Methods: CD8+ T lymphocytes were isolated from peripheral blood mononuclear cells (PBMCs), pancreatic draining lymph nodes (PLN), or irrelevant draining lymph nodes by Fluorescence-Activated Cell Sorting (FACS). Total genomic DNA was extracted and bisulfite converted prior to CpG-methylation analysis on an Illumina Infinium HumanMethylation450 BeadChip comprising 485,000 CpG sites. Data were exported to R statistical program and vialized in Genome Browser.

Summary of Results: Preliminary analyses demonstrated variable DNA methylation patterns at a number of unique loci between patients with T1D and controls (e.g., TAP2, TIMP2, IDI2, RPH3AL, FAM20C) (Fig 1, below).

Fig. 1 - *Differential CpG methylation pattern of isolated CD8+ T cells in T1D*. *A*) A representative plot depicting the average methylation of a subject with T1D and control subject, with differentially methylated loci identified by their non-linear position on the graph. Two representative genes of 22 highly differentially methylated at CpG residues are depicted in *B*) for TAP2, and *C)* for TIMP2. Graphs shown compare methylation at each designated loci for T1D, AAb+ subjects, and controls (1=methylated CpG residue, 0=demethylated CpG)



Conclusion: These data demonstrate our ability to isolate CD8+ T cells from the PDLN of nPOD subjects for epigenetic studies. Preliminary analyses suggest several distinct loci display differential methylation patterns in T1D, at-risk, and controls subjects. Ongoing studies are underway to validate targets differentially methylated in additional nPOD and PBMC samples, and subsequently assess their functional impact on CD8+ T cells in T1D.

15:4 Splenic autoreactive B cells from Type 1 diabetes subjects

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Purpose: The purpose of this study is to determine the *ex vivo* frequency and function after polyclonal activation of autoreactive B cells from the spleen from subjects without Type 1 diabetes (T1D), with autoantibody, but without disease and from recent and long-term T1D subjects, in order to understand autoreactive B cell function and to develop methods to tract autoreactive B cells in in the course of T1D and in therapies.

Methods: Autoantibodies from single splenic B-cells were detected by microengraving and ELISpot. Both of these methods were tested with the use of the human anti-GAD B cell clone, b96.11. Splenocytes were polyclonally stimulated with BCR cross-linking, soluble CD40L and pokeweed mitogen for 18 hours (short stimulation) and then dispersed into the wells (100,000/slide). Glass slides are coated with antigen (rh-GAD65 or -proinsulin) and blocked. The supernatant of the nanowells is exposed to the antigen-coated surface of the glass slide for 2 hours and antibody from the supernatant bound to the antigen is detected with anti-human Ig isotype antibodies labeled with different flurochromes. After imaging, percentages of CD20+ B cells secreting autoreactive antibodies of different isotypes and subisotypes were calculated. In order to detect B-cells secreting cytokines upon polyclonal stimulation, we have developed a two-color ELISpot for detection of splenic CD20+ B cells secreting anti-GAD autoantibody and either IL-4, IL-6, IL-10 or LT-α after 48 hours of polyclonal stimulation.

Summary of Results: The range of autoreactive B cells was from undetectable to 0.006-0.48% of polyclonally stimulated CD20+ B cells at 18 hours. From 4 spleen samples from subjects without diabetes, no GAD65- or proinsulin-reactive B cells secreting isotyped switched immunoglobulin were detected, though B cells from 2 non-diabetic samples showed IgM reactive with GAD or proinsulin. In cases of subjects ('pre-diabetic') positive for autoantibody in the circulation at the time of demise, but without a history of T1D, splenic B cells from two subjects secreted IgM reactive with GAD and one subject's B cells secreted IgM reactive with proinsulin. From 7 subjects with recent onset of T1D (<7 years of diagnosis), the percentage of splenicCD20+ B cells secreting antibody reactive with proinsulin or GAD65 ranged from undetectable to 0.006-0.48%. There were statistically significantly more B cells secreting IgM or isotype switched antibody reactive with GAD or proinsulin from the recent onset group as compared to the non-diabetic, pre-clinical or long-term diabetic subjects. From the spleen from 5 long term (>10 yrs from diagnosis) T1D subjects, frequencies of secreted GAD reactive antibody in the range from undetectable to 0.006%-0.06% were seen; IgM and isotype-switched antibodies reactive with proinsulin were seen from the long term diabetes subjects, possibly due to long-term exogenous insulin administration. Polyclonally stimulated CD20+ B-cells from PLN examined for cytokine expression by quantitative PCR and ICS, expression of IL-6, IL-10, LTα LTα, and TNFα at was only seen Day 2-3 post stimulation. We developed a two-color ELISpot to detect single B cells secreting both an autoantibody and a cytokine in order to determine to functional phenotype of autoreactive B cells.

Conclusions: Splenic CD20+ B-cell secreting antibodies reactive with GAD65 and proinsulin were detected. An increased frequency of B cells secreting IgM and isotype-switched antibodies reactive with GAD65 was seen from the spleen from recent onset T1D subjects as compared to the frequencies seen from controls, long-term T1D subjects and those with serum autoantibodies, but without disease. B cells secreting autoantibody reactive with GAD or proinsulin were seen in from the spleen of pre-clinical subjects. Proinflammatory cytokine expression was detected from CD20+ PLN B cells only after 48 hrs of polyclonal stimulation and a two-color ELISpot was developed to detect B cells secreting both an autoantibody and a cytokine. These studies will aid in defining the frequency/function of autoreactive B cells in during the course of human T1D and in during therapies in which there are responder and non-responder patient groups in therapies in which B cells or other immune cells are manipulated.

15:5:1 Islet Autoreactive CD4 T cells restricted by T1D Protective HLA molecules in a T2D donor

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Purpose: HLA class II molecules associated with T1D risk (DRB1*0401-DQB1*0302) or protection (DRB1*1501-DRB5*0101-DQB1*0602) can present the same immunodominant islet antigen epitopes from to CD4 T cells. We investigated such responses in two nPOD donors with type 2 diabetes (T2D) with protective HLA.

Methods: We studied PBMC, spleen and peri-pancreatic lymph nodes (PLN) from nPOD case #6114 and PBMC and PLN from nPOD donor #6124. Case #6114 was 42.5 years old at his passing, 2 years after the diagnosis of T2D; multiple family members also had diabetes. The donor was autoantibody negative and carried the HLA haplotypes DRB1*0701-DRB4*0101-DQB1*0303 and DRB1*1501-DRB5*0101-DQB1*0602. Histological examination of the pancreas showed reduced insulin staining and severe amyloidosis. Donor #6124 was 62.3 years old at his passing (3 years with T2D), beta cells were still present in the pancreas, and his HLA haplotypes were DRB1*0401-DRB4*0101 and DRB1*1501-DRB5*0101-DQB1*0602. Tetramers used were DR0401, DRB4-0101 (predisposing), DR1501, DRB5-0101 and DQ0602 (protective); all were loaded with the same peptides. An aliquot of the cells was further expanded with HLA-matched APCs and autoantigen peptides, and tetramer stained. Strongly tetramer stained CD4 T cells were single cells sorted, expanded and evaluated for antigen/epitope specificity.

Summary of results: Case #6114: tetramer positive CD4 T cells were detected in PBMC, PLN and spleen samples when using DQ0602, DRB5-0101 and DR1501 tetramers containing GAD65 and PPI/insulin peptides. The expression of chemokine receptors CXCR3 and CCR4 on tetramer positive cells was distinct: the DQ0602 tetramer positive cells identified in PBMC, spleen and PLN were almost exclusively CCR4+/CXCR3-, indicating a predominant TH2 phenotype. The DR1501 and DRB5-0101 restricted T cells expressed both CCR4 and CXCR3. Tetramer staining was confirmed with *in vitro* antigen stimulated cells. Furthermore, DRB5-0101 and DR1501 restricted GAD65 552-572 specific T cell clones were isolated from PLN and spleen but not PBMC. Staining with T1D risk associated DRB4-0101 tetramers was negative in both *ex vivo* and *in vitro* analyses. Case #6124: We did not identify autoreactive T cells in PBMC and PLN.

Conclusions: The study of T2D nPOD case #6114 demonstrates autoreactive CD4 T cells in blood, PLN and spleen, which recognized immunodominant epitopes from islet autoantigens presented by three different protective HLA class II molecules; in contrast, we could not identify similar responses restricted by the other predisposing HLA molecules carried by this donor. We suggest that HLA protective alleles may favor the generation of CD4 T cells with a Th2 phenotype that may either compete or regulate effector cells restricted by the other haplotype.

15:5:2 Islet autoantigen specific CD4 T cells with distinct HLA restrictions, epitope specificities and chemokine profiles in the blood and spleen of a T1D patient

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Purpose: T cell studies of human T1D have mostly been done using peripheral blood samples. However, it is unclear how well the peripheral blood represents the autoreactive T cell repertoire relevant to disease pathogenesis. Access to the pancreas, spleen and peri-pancreatic lymph nodes (PLN) before or around the time of disease onset through nPOD allowed us to study T cells that are present at or near the target organ.

Methods: We received cryopreserved specimen of PBMC, spleen and PLN from a T1D case (nPOD # 6119). The donor was 27.8 years old at the time of his passing (14 years with T1D), carried the high-risk HLA haplotypes DRB1*0404-DRB4*0101-DQB1*0302 and DRB1*0301-DQB1*0201 and was positive for GAD65ab+, mIAA+. Histological examination by nPOD investigators showed lymhocytic infiltration of CD3 T cells in the islets. DR0404 and DR0301 tetramers containing peptides from GAD65, proinsulin and Zn-T8 were used in the analysis of CD4 T cells from the PBMC and spleen. Recovery of the cells from PLN was too low for our tetramer analysis. We also studied PBMC, PLN and spleen specimen from two healthy autoantibody negative donors with similar T1D risk associated HLA genotypes, nPOD #6102 (HLA-DRB1*0401/*0301), #6122 (DRB1*0404/*1401), and PBMC and spleen from an autoantibody+ T2D case (#6127, DRB1*0404-DRB4*0101/DRB1*0301).

Results: CD4 T cells from both PBMC and spleen stained with separate pools of DR0404 and DR0301 GAD65/proinsulin tetramers. In addition, DR0404-ZnT8 tetramer pool binding cells were identified in PBMC but not in spleen. DR0404 restricted T cells were predominantly CXCR3+ and CCR4-, suggesting a Th1 cytokine profile. In contrast, DR0301 restricted GAD65 specific T cells expressed both receptors suggesting a Th0 phenotype. The expression pattern of the chemokine receptors and association with the HLA restriction and/or epitope specificity was similar in both the spleen and the PBMC. An aliquot of the cells was further expanded and analyzed for epitope specificity by using single tetramers: DR0404-ZnT8 staining was observed only in the PBMC but not in the spleen, which confirmed the *ex vivo* findings. We detected binding to DR0404-GAD65 555-567 and DR0301-GAD247-262 tetramers and weaker staining with DR0404-PPI 73-90 tetramers in both PBMC and spleen. Binding of peptide expanded cells to the DR0301-GAD65 247-262 tetramer but not to the other DR0301 tetramers suggests that GAD247-262 is the dominant DR0301 restricted epitope in this subject. The T cell clones generated from the spleen and PBMC displayed high affinity tetramer staining and proliferation. Tetramer positive T cells were not detected in the two healthy controls and the T2D patient.

Conclusions: Distinct chemokine patterns between islet reactive T cells with different HLA restrictions and epitope specificities may suggest dominance and persistence of DR0404 restricted T cell responses in the disease pathogenesis. The presence of islet autoantigen specific T cells with Th1 cytokine profile and homing receptors associated with islet directed trafficking in both the blood and spleen, over 10 years after the clinical diagnosis of T1D, correlates with the presence of CD3 T cell islet infiltrates in the pancreas of this donor.

15:6 Redundancy of CDR3 sequences in the TCR Vβ7 family of intrapancreatic T cell infiltrates.

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TCR repertoires are more redundant than previously proposed, i.e. there are TCRs that occur more frequently than expected, the so-called public TCRs. Public TCRs have been demonstrated in NOD by Quinn et al (Internat Immnol 2006) from isolated islet infiltrating T cells immunized with the GAD65 peptide P530-543. The same clone was detected in purified islets of prediabetic NOD animals, suggesting their presence in the target organ prior to the disease establishment. Li et al (J.Immunol. 2009) analyzed the repertoire of BDC2.5 mimotope-specific islet infiltrating T cells, showing the predominance of certain CDR3 motifs. In a recent report, Liu et al (Diabetes 2012) showed that the allele TCRB-V13S1A1 is an element of susceptibility to autoimmunity in rats, suggesting the TCR as a putative biomarker for T1D. Our group has described the intrapancreatic infiltrate from a recent onset-diabetic donor. Four TRBV families contained monoclonal expansions, each of which was associated with a dominant CDR3 sequence (Codina-Busqueta et al. J. Immunol 2011). The Vβ7-expanded CDR3 (CASSQVAGAGTGELFF) had been previously found by Conrad et al. (Nature 1994) expressed in the purified islets from a diabetic donor that shared HLA-A2, HLA-Cw3 and HLA-DR4 with our donor. An increase of Vβ7 in periphery has also been reported a by Luppi et al (Diabetologia 2000). These evidences prompted us to study the Vβ7 (TRBV4) family and to hypothesize that autoreactive public TCR could also be involved in the development of the autoimmune process. M&M. TRBV repertoire was analyzed by multiplex PCR. Vβ7 amplimers from diabetic (n=4; Case 1 to Case 4)) and healthy (n=3; C1 to C3) donors' pancreas samples were cloned and sequenced.
Results. Both diabetic and control pancreas expressed a diverse repertoire although Vβ7 and Vβ22 were dominant families in all diabetic samples. All four diabetic pancreas had monoclonal expansions of some Vβ families. Interestingly control pancreas also showed some monoclonal expansions, suggesting that TCR expansion may also take place also in homeostasis. Moreover, Vβ7-CDR3 length was analyzed by spectratyping, and Vβ7 amplimers were cloned and sequenced. Three CDR3 sequences were identified that were common to two or three samples (Table 1). Common sequences were found in samples that shared at least one HLA allele, independent of disease. Moreover, peripheral TRBV repertoire showed that some families, including Vβ7 and Vβ22, were increased in patients compared to controls.
Conclusions. The presence of TCR expansions shared between diabetic pancreas suggest the presence of public TCRs in the infiltrates that could be potential biomarkers of diabetes.

Table 1. Identical CDR3 sequences between samples

		HLA class I			HLA class II		CDR3	TCR (aa)		
Sample		HLA-A	HLA-B	HLA-Cw	HLA-DR	HLA-DQ	size	TRBV	NDN	TRBJ
T1D	Case 2	02/24	39/	07/	07/08	*0202/*0402	10	CASSQ	EGR	EQFF
T1D	Case 3	01/29	07/08	07/	03/	*0201/	10	CASSQ	EGR	EQFF
T1D	Case 2	02/24	39/	07/	07/08	*0202/*0402	13	CASSQ	EPGL	TGELFF
C	C1	03/32	18/44	04/07	07/08	*0202/*0613	13	CASSQ	EPGL	TGELFF
T1D	Case 2	02/24	39/	07/	07/08	*0202/*0402	11	CASSQ	GG	QETQYF
T1D	Case 3	01/29	07/08	07/	03/	*0201/	11	CASSQ	GG	QETQYF
C	C1	03/32	18/44	04/07	07/08	*0202/*0613	11	CASSQ	GG	QETQYF

15:7 Identification of potential disease associated T cell receptor (TCR) sequences in pancreatic tissue from patients with recent-onset type 1 diabetes

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Purpose: Tri-molecular complexes targeting pancreatic beta cells play a central role in the development of type 1 diabetes (T1D). The goal of this study is to directly identify TCR alpha and beta chain sequences, as well as their target antigens, by analysis of T cells infiltrating the pancreatic islets in patients with T1D.

Methods: We utilized 454 high-throughput sequencing and established a protocol to specifically detect human TCR sequences from limited sample quantities. RNA for 454 sequencing was isolated using histological sections from pancreas as well as spleen and pancreatic lymph node (PLN) tissue from JDRF nPOD T1D cases (#6113: HLA DR1/DR3, 1 week after diagnosis; #6195: DR2/DR4, 5 weeks after diagnosis). Having developed and validated a responsive system for analysis of human TCR, we also tested T cell lines that express multiple combinations of TCR alpha and beta chains frequently detected in the pancreas for their reactivity to pancreatic islets as well as several beta cell antigens, including insulin peptides.

Summary of results: We analyzed the TCR repertoire in different portions of the pancreas from both donors (Pancreatic head and body, case #6113; body and tail, case #6195) and identified numerous unique TCR alpha and beta chain sequences (69 alpha and 65 beta chains, case #6113; 132 alpha and 134 beta chains, case #6195). While there were no unique TCR sequences shared by the two cases, within each nPOD case, several highly frequent alpha and beta chains were found in both pancreatic regions analyzed. Six out of seven sequences shared by the different regions of pancreas were also detected in the PLN (case #6195). In both patients, a significant number of TCR sequences that were found in the pancreas were detected in peripheral tissues (spleen, case #6113; PLN, case #6195). Additionally, we discovered a distinct signature of V-alpha and V-beta chain genes specifically used by T cells infiltrating the pancreas compared to peripheral lymphoid organs. We are currently in the process of initial screening of multiple TCR chain combinations that were detected in pancreas of case #6113 for response to beta cell antigens. Strikingly, these early efforts identified a particular TCR alpha-beta chain pair responding to the insulin peptide B:1-15.

Conclusion: T cells with identical TCR sequences infiltrate different regions of the pancreas as well as PLN. The strategy determining TCR pairs successfully identified one reactive to the insulin peptide. Further screening should enable us to identify additional TCR pairs responding to islet antigens. Such information will greatly enhance our understanding of the molecular immunological events that underlie the formation of T1D as well as uncover novel targets for interventions seeking to prevent disease development.

Poster Abstracts

Abstracts accepted for poster presentation during Session 14 are listed in alphabetical order by the primary author.

Poster: Baj

Novel Molecular Methods for Enterovirus Detection

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Purpose: Persistent enterovirus (EV) infections are frequent in the early stages of type 1 diabetes (T1D). The current taxonomy of human EV includes four species (A, B, C, D) comprising 111 virus types (not considering human rhinoviruses). EV undergo remarkable genetic variation and evolution that are linked to the high mutation rate proper of RNA viruses, recombination among different EV types, deletions, and "codon deoptimization" that may slow down virus replication. Thus, the genetic structure of currently active EV is often different from that of prototype strains. Public databases contain over 1,000 complete EV sequences. Very low levels of EV genome are usually present in the course of persistent infections. Under these conditions, commercial RT-PCR assays for EV detection, rarely give positive results. Thus, we developed sensitive RT-PCR methods based on minimally degenerated primer pairs aimed at different EV genome regions. These methods are characterized by extreme sensitivity (≤50 copies/ml) and capable of detecting virtually all reported EV types. When RT-PCR assays were applied to studying T1D cases, results were taken as those producing when amplicons were obtained from two or more genomic regions. Methods: Bioinformatic tools were used for designing primers to the following EV regions: 5'UTR (untranslated region), 5'UTR-VP2 (capsid), 2C (membrane- and RNA-binding, helicase), and 3D (RNA polymerase). Twenty primer pairs were tested against selected EV reference strains and clinical/environmental isolates belonging to the A, B, C, and D species. Positive results were confirmed by direct sequencing. This allowed identifying the virus type of control strains. In studying diabetes specimens, the sensitivity of EV detection was enhanced by combining biological amplification (i.e., culture in susceptible cell lines) followed by molecular amplification of cell culture supernatants (0.6 to 1.0 ml for RNA extraction). Retrotranscription was performed at 46°C using thermostable enzymes. Templates were amplified using prolonged annealing and extension times for 40 cycles. Positivity of selected cultured samples was confirmed by direct amplification of original specimens. Summary of

Results: Amplification of serial dilutions of EV reference strains and clinical/environmental isolates demonstrated a sensitivity of ≤50 copies/ml. Amplification of the 5'UTR region gave no clue to EV type identification. Amplification of the 5'UTR-VP2 region (5 primer sets) and the 3D region (7 primer sets) allowed identifying the virus species. When amplicons were obtained in sufficient amounts from clinical specimens, they were purified from gel bands and sequenced directly. Using these methods, strains of coxsackie A20 and echovirus 9 were detected in two children at the clinical onset of T1D. In the majority of EV-positive diabetes cases, only the

species of the infecting virus could be identified. The A, B, C, and D species were found in different cases, with the B species being the most prevalent.

Conclusions: The reported primer sets are being used for EV detection in our own T1D patient cohort and nPOD tissue specimens. Cell separation techniques, coupled to sensitive molecular methods, are expected to identify the lymphoid cell types carrying EV in lymph node and spleen samples from the nPOD collection.

Poster: Chapman

Persistence of enteroviruses in the pancreas post-acute infection

Nora M. Chapman, Steven Tracy

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Purpose: To determine whether the persistence of enteroviruses in the pancreas post-acute infection is due to the selection of 5' terminally deleted genomes. Coxsackievirus B3 with these genomes have been shown to have reduced positive strand RNA replication, leading to low level persistent infections in the heart.

Methods: Mice susceptible to enterovirus-induced myocarditis and pancreatitis were inoculated with coxsackievirus B3. Pancreatic tissue (day 3 and day 28 post-inoculation) was analyzed for the presence of virus using cytopathic assay and viral RNA (using RT-PCR, with viral specific primers). RT-PCR amplified viral cDNA was sequenced to determine the alterations in selection in the pancreatic tissue.

Summary of Results: Viral RNA was detected in pancreas of mice inoculated with CVB3 at a stage at which detection of the enterovirus with cytopathic assays in cell culture was no longer possible.

Conclusions: In cases of human enterovirus-associated myocarditis and in the murine model of coxsackievirus- induced myocarditis, persistence of enterovirus is associated with the selection of viruses with deletions of a 5' terminal portion of the genome. This region is important for the initiation of transcription of the enterovirus (positive strand) genome and the deletions have been shown to decrease positive strand RNA replication relative to the negative strand antigenome. As the positive strand viral RNA serves as the template for all enterovirus proteins, this decrease results in a slower production of viral proteins and reduced replication of enterovirus. Persistence may be correlated with prolonged intracellular replication as enterovirus clearance correlates with antibody response to the virus, occurring once virus is released from cells. Persistence in the pancreas of mice demonstrates a mechanism for enterovirus persistence after acute enterovirus infection of the pancreas, an infection which may exacerbate the process leading to type 1 diabetes onset.

Poster: Chen

Co-expression of IL-15 and IL-15Rα on pancreatic β islet cells induced insulin dependent type 1 diabetes

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Viral infections are thought to play a major role in the pathogenesis of Type 1 diabetes (T1D). Interleukin-15 (IL-15) is a pro-inflammatory cytokine that is often induced by viral infection and interferon. Increased serum levels of IL-15 are reported in type 1 diabetes. Here we showed increased serum levels of soluble IL-15Rα in T1D. To investigate the role of IL-15/IL15Rα in the pathogenesis of T1D, we generated double transgenic mice with β islet cell expression of IL-15 and IL-15Rα. These mice developed hyperglycemia, marked mononuclear cell infiltration, β cell destruction and insulin autoantibodies that mimic early human T1D. Inhibiting IL-15 signaling with a monoclonal antibody TMβ1 (anti-CD122) that blocks IL-15 trans-presentation or a Jak2/3 inhibitor Tofacitinib completely reversed the diabetes. Increased expression of pro-inflammatory cytokines/chemokines and increased surface expression of MHC class I/II and ICAM-1 were found in the purified islets from the double transgenic mice. Depletion of CD4 cell at the onset of diabetes reversed hyperglycemia and CD4 cells isolated from the double transgenic mice were able to recognize both wild type and transgenic islets in vitro. Moreover, in non-obese diabetic (NOD) mice, IL15/IL15Rα expression was increased in islet cells at the pre-diabetic stage and inhibiting IL-15 signaling with TMβ1 delayed the diabetes development. Taken together, our data suggest that disordered IL-15 and IL-15Rα may be involved in the pathogenesis of T1D and the IL-15/IL15Rα pathway may be a rational therapeutic target for T1D.

To follow the observations in mice and investigate whether IL-15 and IL-15Rα were involved in the pathogenesis of type 1 diabetes in humans, we applied to nPOD, JDRF and got approval to obtain pancreatic tissue slides from patients with type 1 diabetes, autoantibody positive donors and normal donors. We plan to examine the protein expression of IL-15/IL-15Rα by immunochemistry and the mRNA message by micro-dissecting islets and then analyzing IL-15, IL-15Rα expression by Nanostring Technology. Initial data from islets micro-dissected from normal donors suggested no expression of IL-15/IL-15 Rα in normal donors. Immunohistochemistry of pancreas slides showed high levels of IL-15 expression in patients with T1D. The data obtained from patients with T1D will be important to determine whether IL-15/IL-15Rα play roles in the pathogenesis of T1D in humans.

Poster: Cheung

Variations in Islet B7-H1 Expression in Type 1 Diabetes Pancreas Segments

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Purpose: Recent studies have highlighted the central role of the negative costimulatory molecule B7-H1, also called PD-1 pathway ligand PD-L1, in suppressing the proliferation of autoreactive T-cells and consequently halting the progression of autoimmune diabetes in NOD mouse model. Our earlier study shows that weak B7-H1 expression is found in the islet β cells and strong B-H1 expression in the islet periphery in normal human pancreas and reduction in islet B7-H1 expression in Type 1 Diabetes (T1D) pancreas. We report here islet B7-H1 expression from two cases of T1D pancreas donor which show variations in different segments of the pancreas gland.

Methods: Multifluorescence and bright-field immunohistochemistry (IHC) with 3, 3-diaminobenzidine as chromogen were used to study B7-H1 expression on formalin-fixed paraffin-processed tissue sections from the head, body and tail segments of two T1D patients. IHC on B7-H4, insulin and glucagon markers expression were included in sequential tissue sections in the study. Sample #1 was from a T1D donor while sample #2 was an explant pancreas from a T1D patient with failed pancreas allotransplant. For comparison and reference, T1D pancreas sections were provided by the Network for Pancreatic Organ Donors with Diabetes (nPOD) initiatives and normal pancreas tissues were provided by Ike Barber Human Islet Transplantation Laboratory and British Columbia Transplant Society.

Summary of results: In sample #1, islet B7-H1 expression was lost in the pancreas head but was retained in the body and tail segment. In sample #2, loss of B7-H1 expression was observed in the head, body and tail segments. Islet insulin and B7-H4 expression was lost or reduced in the head, body and tail pancreas segments from both samples. In addition, insulitis was either minimal or absent in all pancreas segments of these two samples.

Conclusions: These results suggest that B7-H1 might play a role in islet pathophysiology. This new information from the two clinical samples lends support to observation in murine model that B7-H1 might play a role in T1D development.

Poster: Hodick

The extracellular domain of the Coxsackie-Adenovirus-Receptor (CAR) is more often expressed in islets from type 1 diabetic and islet autoantibody positive organ donors than in controls.

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Background: Type 1 diabetes (T1D) is believed to be an autoimmune disease triggered by some environmental factor(s). One such factor is a virus infection, and the prime candidate is the Enteroviruses, especially the Coxsackie B viruses. These viruses use a tight junction protein, CAR, as their receptor. There are six known iso-forms of CAR, and many cells express the gene encoding the CAR protein, but not all cells express the protein that has the extracellular domain.
Purpose: The aim was to study the expression of this domain of CAR in human islets of Langerhans in four groups of organ donors. One additional aim was to study the expression of the gene encoding CAR in human islets infected *in vitro* with different Enteroviruses.
Methods: Isolated human islets were infected *in vitro* with three strains of Coxsackie B virus-1 and one strain of Echovirus 6, total RNA was extracted day three post infection. Mock infected islets were treated the likewise. After cDNA synthesis, real time-PCR was performed with primers against the genes encoding CAR and 18S.
Sections from pancreatic biopsies from the head of the pancreas from three T1D related groups of donors and from control organ donors were stained with the RmBc antibody that detect the extracellular domain of CAR. Number of positively stained cells in the islets was counted in section from all donors. Groups of donors studied was T1D close to onset n=9, T1D with longer duration n=4, auto-antibody positive n=14 and controls n=24.
Results: The gene encoding CAR was significantly less expressed ($p<0.001$) in islets infected with any of the CBV-1 strains. In islets infected with Echovirus 6 no effect on the expression level of the gene encoding CAR was seen compared to mock infected controls. Detection of the extracellular domain of CAR in islets from T1D donors with resent onset were 44%, in the T1D with longstanding disease 50%, in the islet autoantibody positive donors 57% and in the non-T1D control donors 22%. There were a significant difference between the difference between auto-antibody positive donors ($p<0.039$) between the T1D donors ($p<0.033$) and the controls. There were no differences between any of the diabetes related groups. The odds ration was 3.877, meaning that the risk was increased three times to be CAR positive if you had T1D.
Discussion/Conclusion: The down regulation of the gene encoding the viral receptor CAR might be due to the loss of beta cells since the gene encoding insulin also is down regulated while the gene encoding glucagon is not affected in infected islets. However, Echovirus 6 also replicate in isolated islets but no effect on the expression level of CAR was seen, suggesting that the down regulation is virus induced and receptor usage specific. The higher expression levels of the extracellular domain of CAR in all groups related to T1D might explain why some individuals get T1D while the majority don't after an infection with Coxsackie B virus. However, CAR is an inducible protein and the expression of CAR could be caused by an increased degree of inflammation. What causes the higher expression in the T1D pancreatic islets is not known, but the mere presence of the viral receptor in the islets will facilitate binding of virus to these cells.

Poster: Laiho

Development of *in situ* hybridization assay for the detection of coxsackievirus B1 in tissue samples

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Purpose Several studies have shown an association between enterovirus (EV) infections and type 1 diabetes (T1D). Particularly Coxsackie B viruses (CBV) have been associated with the onset of the disease and recent studies have suggested CBV1 to be the T1D risk serotype. The aim of the present study was to develop an *in situ* hybridization (ISH) assay which detects CBV1 viruses with high sensitivity in formalin-fixed paraffin-embedded (FFPE) tissue samples.

Methods Conserved CBV1- specific sequences were searched by bioinformatics, gathering the CBV1 sequences from the GenBank®, aligning them with several other EV strains (using ClustalX and GeneDoc), followed by a Basic Local Alignment Tool (BLAST) search to avoid any sequence similarities with host proteins. Probes were designed for the chosen sequences and tested individually and as a combination for the detection of CBV1 in FFPE tissue samples from CBV1-infected mice in *in situ* hybridization assay.

Summary of results Three CBV1 specific genome regions; 39, 60 and 126 bases in length, were identified to be optimal for the design of hybridization probes. To cover the variation within the CBV1 strains several probes were designed for each of the three regions. Subsequently, the probes within a region were unified to create a region-specific probe and optimized for their binding to virus-infected cells. Clear positive staining was obtained from the pancreas tissue of CBV1-infected mice by each of these three probes while tissues from non-infected mice were negative. Additionally, the sensitivity and specificity of the detection was further improved by using the three individual CBV1 probes in a cocktail.

Conclusions The CBV1 probes were able to detect the virus in FFPE tissue sections of CBV1-infected mice in ISH. These probes will be further tested for their specificity against other enterovirus serotypes.

Poster: Leslie

The epigenetics of risk of autoimmune diseases and self-antigen expression

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PURPOSE: We aim to identify changes in DNA methylation associated with type 1 diabetes (T1DM) using disease-relevant tissue provided by nPOD. In addition we aim to study DNA methylation in selected tissue including thymus cells and to provide normal thymus to nPOD as a resource.

METHODS: We studied monozygotic twins discordant for T1DM and blood spots from Guthrie cards taken at birth. Peripheral blood monocytes were purified using magnetic beads. Whole genome scale DNA methylation was performed using Illumina arrays (initially 27k and now 450K) plus Bis-Seq. Thymuses (n=24 to date) were obtained from the Institute of Child Health, London, and isolation procedures for selected cells are being established.

SUMMARY OF RESULTS: We identified T1DM-associated methylation variable positions (T1D-MVPs) in blood monocytes and then showed that the changes antedated the disease (Plos Genetics 2011). We now seek these same T1D-MVPs in pancreatic lymph nodes from T1DM patients using nPOD samples. We validated two methodologies to generating comprehensive DNA methylomes from Guthrie cards. Using integrated epigenomic/genomic analysis of these cards and follow-up blood samples, we identified inter-individual DNA methylation variation (iiDMRs) both at birth and three years later (Genome Research 2012). Finally, we demonstrated that non-genetically determined iiDMRs can be temporally stable for at least two years with concomitant changes in chromatin state estimated by levels of the histone variant H2A.Z. Interestingly, promoter iiDMRs did not correlate with gene expression implicating a major non-genetic effect on non-coding DNA regions (submitted).

CONCLUSION: In summary, despite remarkable twin concordance for DNA methylation, MVPs can be found in small tissue samples and were shown to be stable with time and potentially T1DM-associated. Non-genetically determined DNA methylation variation often involves non-coding DNA.

Poster: Richardson

Hyperexpression of class I MHC on pancreatic islet cells

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Purpose: The hyperexpression of class I MHC on pancreatic islet cells is a unique characteristic of type 1 diabetes but the underlying mechanisms are unknown. In a UK cohort of type 1 diabetes patients, islet MHC I hyperexpression has been correlated with the presence of interferon-alpha, suggesting that viral infection might be involved in driving MHC I expression. Since the enteroviral capsid protein, VP1, has also been detected immunologically in the islet cells of certain type 1 diabetes patients, we have now assessed the relationship between class I MHC and VP1 expression in the islets of such patients.

Methods: Formalin-fixed paraffin embedded pancreatic sections from 13 nPOD type 1 diabetes cases (with varying durations of disease) and a similar number of cases from a UK cohort (all with recent-onset diabetes (<18mth duration)) were examined by immunohistochemistry for the presence of the enteroviral capsid protein vp1 (Dako antibody; 5D8/1), insulin and class I MHC. Frozen sections from a selection of the nPOD cases were also examined for the presence of class I MHC, independently.

Summary of Results: Of the 13 nPOD cases examined, 8 retained insulin-containing islets (ICIs) and, among these, 5 had evidence of class I MHC hyperexpression. The cases which hyperexpressed class I MHC within their ICIs (6046, 6052, 6070, 6051 and 6038) had islets which were also immunopositive for VP1; albeit in small numbers of individual cells. However, in all cases, islets were also seen which hyperexpressed MHC I but were immunonegative for VP1 within the plane of the section. In a separate UK cohort of recent-onset type 1 diabetes patients (n=13) all ICIs that were VP1 positive again hyperexpressed MHC I but some islets were observed which hyperexpressed class I MHC and had no evidence of vp1 expression within the plane of the section. A further three nPOD cases contained ICI (6061, 6065, 6069) in which none of the cells expressed VP1 or hyperexpressed MHC I. Neither MHC I hyperexpression nor VP1 immunoreactivity was detected in any of the 5 nPOD cases whose islets lacked residual beta-cells.

Conclusions: All islets in which enteroviral VP1 immunopositivity could be detected within beta cells also hyperexpressed class I MHC. Thus, if VP1 immunopositivity is indicative of enteroviral infection, then such infections could be a trigger for the hyperexpression of class I MHC. However, since some islets were immunonegative for VP1 but still hyperexpressed MHC I, it cannot be excluded that other stimuli may also induce class I MHC expression. Alternatively, it is also possible that, in these cases, MHC I expression might be driven by a persistent infection in which VP1 production is minimal.

Poster: Rodriguez-Calvo

Spontaneous immune activation and pancreatic T cell infiltration in diabetic patients

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Purpose: Type 1 diabetes (T1D) results from a complex interplay between varying degrees of genetic susceptibility and environmental factors which have been implicated in the pathogenesis of T1D both as triggers and potentiators of β-cell destruction. Chronic exposure to low doses of antigen can lead to activated autoantigen-specific T cells that are significantly skewed toward a proinflammatory phenotype. Here we report that T1D individuals have a general and spontaneous immune T cell activation and that T-cell infiltration may not be an islet-restricted phenomenon, rather a consequence of spontaneous immune activation, affecting both exocrine and endocrine pancreas.

Methods: For the ELISpot assay DR4 restricted epitopes of IA-2 and proinsulin as well as a DR3 restricted GAD 65 epitope were used to stimulate PBMCs. Lymphocytes were incubated with the above epitopes for 48 hrs. Then, PBMC were harvested and plated in triplicates in ELISA plates pre-coated with capture antibody. Twenty-four hours later, cells were lysed, the contents were incubated with a biotinylated detection antibody and spots were developed and counted.
For immunofluorescence, frozen pancreases from T1D, T2D, autoantibody positive donors and healthy controls from the Network for Pancreatic Organ Donors (nPOD) were stained for insulin and CD8 T cells. T cell infiltration was quantified in both endocrine and exocrine pancreas.

Results: Analysis of antigen specific cytokine production by ELISPOT revealed that T1D subjects showed a strong trend toward increased IFN-γ production when compared to controls. In contrast, subjects with T2D showed a statistically significant increase in IFN-γ spot production compared to controls. There was no statistically significant difference between subjects with T1D or T2D. Interestingly, a more detailed analysis of cytokine production revealed significant spontaneous production of both IFN-γ and IL-10 by T-cells from T1D donors, even without any antigenic stimulation (spots in media only).
Further analysis of pancreatic samples from T1D nPOD donors showed a general infiltration of CD8 T cells in pancreas, both in exocrine and endocrine tissue. T1D donors presented 1.3 times more infiltrating cells in pancreas than autoantibody positive donors and 3.75 times more than healthy controls.

Poster: Salvatoni

INTRAFAMILIAL SPREAD OF DIFFERENT ENTEROVIRUS SPECIES IS COMMON AT THE CLINICAL ONSET OF TYPE 1 DIABETES

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Purpose: At the clinical onset of type 1 diabetes (T1D), enteroviruses (EV) of different species can be detected in blood of over two thirds of probands. Family spread of EV infections and the temporal/geographic clustering of cases have been documented in the past (Kogon et al., 1969). At the time of T1D diagnosis, we checked whether EV were present in blood of diabetic probands as well as their consenting parents and siblings.

Methods: Blood was drawn from 19 newly-diagnosed diabetic children, their consenting siblings (15) and parents (26). Sixty-nine non-diabetic individuals matched by age, time, and geographic site were used as controls. EV-susceptible cell lines were co-cultured with PBL of all individuals. RT-PCR assays detecting virtually all EV serotypes (5'UTR, 5'UTR-VP2, and 3D genome regions) were run both on plasma samples and medium of cell lines that were co-cultured with PBL for one month. Detected EV were typed at the species level based on RT-PCR results. Clinical data of virus-positive children were then compared to those of a series of 26 diabetic children that were EV-negative at the time of clinical onset (patients from the Varese and Pisa cohorts). Time from the early symptoms and clinical onset, blood glucose, basal and stimulated C-peptide, HLA-II haplotypes, and diabetes-related auto-Abs (GAD65, IA2, ZnT88) were evaluated at the time of diagnosis. HbA1c and insulin requirement were measured both at diagnosis and one year post-diagnosis.

Summary of Results: EV infectivity and genome were found in blood of 15/19 (79%) diabetic children, 9/15 (60%) non-diabetic asymptomatic siblings, 15/26 (58%) asymptomatic parents, 3/69 (4%) non-diabetic controls. EV of the A, B, C, and D species were detected. The B species was the most prevalent. Two children and their family members were shown to be infected by coxsackievirus A20 and echovirus-9 (both enteroviruses of the B species). In all cases, virus-positive members of each family shared the same EV species. During follow-up, 4/15 (25%) siblings of diabetic probands developed T1D with a latency of 2-8 months. All of them were EV-positive at the time of T1D onset in their index case. Comparison of EV-positive vs. matched EV-negative T1D children showed that at the time of clinical onset, EV-positive patients had enhanced HbA1c levels as compared to EV-negative patients and significantly lower glucagon-stimulated C-peptide ($P<0.05$). This indicates a particularly severe reduction of beta cell mass. However, 1 month and 1 year post-diagnosis, insulin requirements were not significantly **different between the two groups.**

Conclusions: The high prevalence of systemic EV infections at clinical onset, the familial spread of infection, the development of diabetes in a high percentage of EV-infected siblings indicate that EV in blood may represent a biomarker of T1D. The data show that, in early diabetes, EV positivity is associated with a severe reduction of insulin production.

Reference: Kogon et al. 1969. Am J Epidemiol 89:51-61.

Poster: Sebastiani

MicroRNA profiling of unfitted $CD4^+$ T regulatory cells residing in the pancreatic draining lymph nodes of patients with type 1 diabetes

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PURPOSE: We collect pancreatic draining lymph-nodes (PLN) and peripheral blood (PB) of patients with type 1 diabetes (T1D) undergoing pancreas transplantation and of non-diabetic individuals. PLN of patients with T1D have $CD4^+CD25^{bright}$ T cells epigenetically imprinted to be regulatory but that, for still unknown reasons, do not function as such in vitro. Importantly, the defect is present only within the PLN and not in PB (Ferraro et al. Diabetes 2011). Recent pioneering studies clearly indicate that not only protein-coding genes but also non-protein-coding small RNAs control Treg-cell development and function. MicroRNAs (miRNAs) are an abundant class of evolutionarily conserved small non-coding RNAs that regulate gene expression post-transcriptionally by affecting the degradation and translation of target mRNAs. When poorly regulated, miRNAs are critically involved in a range of human diseases. We hypothesize that there might be post-transcriptional regulations in Treg cells and these regulations may affect Treg-cell function only within the PLN but not in the periphery of subjects with T1D.

METHODS: $CD4^+$ Treg and T conventional cells were sorted from PLN and PB of patients with T1D and non-diabetic donors. The miRNA expression profiles were performed by RT-PCR using Taqman low-density miRNAs array cards.

RESULTS: Specific miRNAs differentially expressed between Treg cells residing in the PLN of T1D patients and those in their PB or in PLN of non-diabetic donors were identified.

CONCLUSIONS: Studies are ongoing to confirm such a profile in a bigger cohort of donors from nPOD and to assess the miRNAs' role in the unfitness of Treg cells residing in the PLN of patients with T1D.

Poster: Udin

FasL: Unorthodox but potential therapeutic target in type 1 diabetes

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Type1 diabetes (T1D) results from autoimmune destruction of pancreatic insulin-producing ß-cells. Autoreactive T cells are activated by islet autoantigens in pancreatic lymph nodes (PLN) from where they infiltrate pancreatic islets of Langerhans to cause insulitis and ß-cell destruction. Although present within the normal repertoire of healthy individuals, autoreactive T cells are held in check by suppressive cytokines and specialized subsets of regulatory cells. In disease-susceptible individuals and the widely used "non-obese diabetic (NOD) mouse model," these immunoregulatory mechanisms fail, thereby permitting diabetogenic T cells to infiltrate islets, cause insulitis, and destroy ß-cells. What causes these mechanisms to fail and how the failures can be avoided/rectified are current questions of paramount scientific and clinical significance.

Our group is interested in understanding mechanisms that potently control ß-cell-specific autoreactive T-cells when Fas ligand (FasL), an apoptosis-inducing member of the tumor necrosis factor (TNF) family, is genetically or pharmacologically inactivated. Disruption of the Fas pathway causes massive T cell lymphoproliferation but instead of causing or aggravating organ specific autoimmunity, as is the case with CTLA-4 or Foxp3 deficiency, it restores organ-specific T cell tolerance in several autoimmune models. This paradox is best illustrated in NOD mice carrying homozygous gld or lpr mutations. Introduction of homozygous gld mutations into NOD mice confers complete protection from diabetes even though these mice develop massive lymphadenitis and splenomegaly. This experiment-in-nature indicates a key but still undefined role for the Fas/FasL system in the pathogenesis of autoimmune diabetes. Initially it was thought that the protection is due to abrogation of Fas-mediated death of beta cells. However, subsequent studies show a disposable role for the Fas/FasL system in the death of beta cells. Our previous studies show that disease resistance at least in NOD-gld/+ mice is not due to deletion of diabetogenic T cells, suggesting that FasL inactivation protects from T1D by an immunoregulatory mechanism(s) that keeps diabetogenic T cells in check.

Our mouse data support the hypothesis that defects in IL-10 utilization underlie autoimmune diabetes and that IL-10-producing B cells induced in pancreata by inactivating FasL are capable of controlling diabetogenic T cells. The goal of this study is to test the relevance of this hypothesis to the human disease using PBMC from newly diagnosed patients and from nine nPOD cases. Our results showed significantly higher production of IL-10 by PBMCs in general and B cells in particular from not only new cases but long term cases than in controls. We conclude that there is no systemic defect but rather enhanced production of IL-10 by the immune cells of newly diagnosed and established patients perhaps in unsuccessful but sustained effort to quell the autoimmune process. We will discuss the role of FasL in compromising and negating the role of IL-10 in antagonizing the diabetogenic process especially at the effector site, the pancreas.

Poster: Usero

iNKT cells modulate effector T cells suppression by Treg cells in T1D

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T regulatory (Tregs) and invariant natural killer T cells (iNKT) are both involved in the control of pathogenic autoreactivity in Type 1 Diabetes (T1D). Previous results from our group showed that although their predominant anatomical location in the pancreas changed as the disease developed, both cell types were present at the diabetic pancreas. These results suggest a scenario where, an active cross-talk between Tregs and iNKTs at the target organ could result on a better regulation of autoagressive pancreas-specific T cells. Thus, our aim was to study their interaction and the functional consequences of this interaction in T1D patients.
iNKT (TCRVα24Jα18^{+}) and Treg (CD4^{+}CD25^{++}) cells from healthy donors and T1D patients PBMCs were expanded *in vitro* with αGalactosylCeramide pulsed CD1d^{+} APCs or αCD3, respectively. We analyzed the capacity of the expanded iNKTs cells to modify the suppressor effect of Tregs in the proliferation of T effector (Teff) cells. We determined if the effect of iNKT cells required cell-cell contact using a transwell system with different combinations of iNKT:Treg:Teff together with a cytometric bead array to analyze cytokines secretion.

The results showed that Treg cells from healthy donors and T1D patients suppressed autologous Teff cells proliferation at the same Treg:Teff ratio. This was accompanied by the abolishment of IL2 secretion in a cell-cell contact-dependent manner only in healthy donors. In T1D patients the production of IL2 by Teff cells was not reduced in the presence of Treg cells. Interestingly, the suppressor effect of Treg cells correlated with their capacity to secrete IL-13. iNKT cells, both in healthy donors and T1D patients, showed an adjuvant effect on the suppressor function of Tregs as their addition to the culture further decreased Teff cells proliferation. When added to the culture, iNKT cells did maintain the minimal IL-2 production depending upon cell contact with Treg cells. Moreover, the presence of iNKT cells induced the secretion of large amounts of effector cytokines both inflammatory and regulatory. Indeed, the presence of iNKTs in the cocultures resulted in increased concentrations of regulatory soluble factors (IL4, IL10, IL13, TNFα and GM-CSF) that could be responsible for the adjuvant suppression effect.
These results show that iNKT cells have an adjuvant effect on Treg cells function in T1D patients as they increase their capacity to inhibit Teff cell proliferation *in vitro*. This effect is mediated by the secretion of effector and regulatory soluble factors by iNKTs contributing to the suppressor effect and even enhancing the suppression of T1D Teff cells proliferation by Tregs.

Current nPOD Scientific Projects

Beta Cell Regeneration

Investigator	Institution	Project Title
Mark Atkinson & Patrick Rowe	University of Florida	The ductal pancreatic progenitor microenvironment in type 1 diabetes
Juan Dominguez-Bendala, Giacoma Lanzoni & Luca Inverardi	University of Miami	Progenitors of the beta cell lineage in the pancreas and biliary tree of diabetic patients
Dieter Egli, Rudy Leibel & Scott Noggle	Columbia University	Generation of pluripotent stem cells from diabetics
Leonard Harrison	The Walter & Eliza Hall Institute of Medical Research	Adult islet progenitor cell markers
Matthias Hebrok	University of California, San Francisco	Investigating the de-differentiated state of the ß-cell in human diabetic patient tissues
Jake Kushner	Baylor	Do de-granulated ß-cells persist in type 1 diabetes?
Fred Levine	Sanford-Burnham Medical Research Institute	Beta-cell regeneration by transdifferentiation
Douglas Melton	Harvard University	Generation of tools to distinguish human pancreatic cell populations
Christopher Rhodes	University of Chicago	"Empty beta cells" in type 1 diabetes
Srinath Sanda	University of California, San Francisco	Analysis of beta cell senescence in type 1 diabetes subjects
Andrew Stewart & Amy Cox	University of Pittsburgh Medical Center	Multidisciplinary approaches to driving human beta cell replication

Immunology

Investigator	Institution	Project Title
Mark Stuart Anderson	University of California, San Francisco	Identification of novel tissue-specific antigens expressed by human extrathymic Aire-expressing cells and determination of their potential contribution to the prevention of type 1 diabetes
Manuela Battaglia & Ezio Bonifacio	San Raffaele Scientific Institute	Dissecting the effector/regulatory compartments in the target tissues of T1D
Todd Brusko	University of Florida	Molecular signature of autoimmune T cells in type 1 diabetes
Raphael Clynes	Columbia University	TCR signal transduction in diabetogenic T cells
Howard Davidson	University of Colorado Health Science Center	Characterization of autoantigen-specific T cell receptors from PLNs
Garry Fathman	Stanford University	Microarray analysis of PLN from autoantibody positive donors

Investigator	Institution	Project Title
Dale Greiner & Leonard Shultz	University of Massachusetts	Diabetogenic function of autoimmune donor splenocytes in humanized mice
Abdel Hamad & Thomas Donner	Johns Hopkins University	Fas ligand: Unorthodox target for prevention of type 1 diabetes
Dolores Jaraquemada, Mercè Martí & Carme Roura-Mir	Universitat Autonoma de Barcelona	Tracing effector and regulatory T cell populations in type 1 diabetes
Sally Kent	University of Massachusetts Medical Center	Investigation of B cells in human islets and PLN in T1D
Richard David Leslie	University of London	Risk of autoimmune disease and human self-antigen expression
Maki Nakayama & Joe Larkin	University of Colorado	Direct replication of T cell receptors specific for type 1 diabetes
Jerry Nepom	Benaroya Research Institute at Virginia Mason	TCR repertoire analysis in type 1 diabetes
Massimo Pietropaolo & Michael Morran	The Brehm Center for Diabetes Research, University of Michigan	Identification of T1D-specific Fab fragments of IA-2 dominant conformational epitope
Alberto Pugliese	Diabetes Research Institute, University of Miami	Beta cells and autoimmunity correlations
Helena Reijonen	Benaroya Research Institute at Virginia Mason	Correlation of islet autoantigen specific T cells repertoire in the pancreatic lymph nodes and peripheral blood in T1D autoimmunity
Bart Roep	Leiden University	Detection of islet autoreactive CD8 T-cells in insulitis versus periphery
Suparna Sarkar & Dirk Homann	University of Colorado, Denver	Pancreatic expression of chemokines in human type 1 diabetes
Brad Stone, Bill Kwok & Matthias von Herrath	Benaroya Research Institute	Systematic assessment of autoreactivity in human type 1 diabetes
Matthias von Herrath	University of California, San Diego	In situ detection of pro-inflammatory cytokines within pancreatic islets from diabetic subjects
Thomas Waldmann & Jing Chen	NCI/NIH	The role of IL-15 and IL-15Ra in the pathogenesis of type 1 diabetes
Clive Wasserfall, Mark Atkinson & Pat Rowe	University of Florida	Humoral immunity in type 1 diabetes

Metabolic and Developmental

Investigator	Institution	Project Title
David Carpenter, Miguel Porta & Sarah Howard	SUNY Albany	Persistent organic pollutants in the pancreatic tissue of people with and without diabetes
Yuval Dor & Benjamin Glaser	Hebrew University of Jerusalem	Markers of stress in diabetic islets
Decio Eizirik & Ihsane Marhfour	Univeristè Libre de Bruxelles	Characterization of beta cell ER stress markers in type 1 diabetes
Ivan Gerling	University of Tennessee Health Science Center	Sequencing the islet transcriptome
Maria Grant	University of Florida	Bone marrow progenitor cell (BMPCs) dysfunction in diabetes is mediated by reduced bioavailability of NO
Paul Harris, Masanori Ichise & Matthew Freeby	Columbia University	Determination of specific and non-specific binding of 18F-FP-DTBZ in whole pancreas homogenates obtained from controls and patients with longstanding type 1 diabetes
Kevan Herold & Alfred Bothwell	Yale University	Reconstitution of HHLS mice with bone marrow from patients with T1DM
Gokhan Hotamisligil & Feyza Engin	Harvard University	Examination of ER stress markers in type 1 diabetes samples in humans
Rudy Leibel & Nao Wakae	Columbia University	E-cadherin mediates developmental effects on the proliferation and the function of ß-cells in the islet of Langerhans
Rudy Leibel & Streamson Chua	Columbia University	Identification of a gene regulating pancreatic beta cell replication
Clayton Mathews	University of Florida	Islet resistance to T1D
Sara Michie & Baohui Xu	Stanford University	Tissue-selective adhesion molecules in human type 1 diabetes
Anna Moore & Amol Kavishwar	Massachusetts General Hospital	In vivo imaging of islets
Jerry Nadler, Margaret Morris & Kaiwen Ma	Eastern Virginia Medical School	12/15 Lipoxygenase expression in type 1 diabetes
Jerry Nadler & Julius Nyalwidhe	Eastern Virginia Medical School	Protein based biomarkers for type 1 diabetes
Al Powers	Vanderbilt University	Pancreatic islet biology and vascularization
Shiva Reddy	The University of Auckland, New Zealand	Immunohistochemical identification of molecular markers of oxidative and nitrosative stress in ß-cells during the early stages of human type 1 diabetes mellitus
Stephen Rich	University of Virginia	ImmunoChip data on nPOD samples

Investigator	Institution	Project Title
Salvatore Sechi & Akos Vertes	George Washington University	Molecular profile of the pancreatic tissue of long-standing type 1 diabetes patients
Charmaine Simeonovic, Christopher Parish, J. Dennis Wilson & Andrew Ziolowski	Australian National University	Heparan sulfate levels mark the health status of human islet ß-cells
Gerald Taborsky, Jr.	University of Washington	Glucagon secretion and islet neuropathy
Shannon Wallet	University of Florida	Role of mucosal epithelium in autoreactivity

Pathology

Investigator	Institution	Project Title
Vitaly Ablamunits, Kevan Herold & Jasmin Lebastchi	Yale University	Comparison of total pancreatic insulin content and peripheral markers of beta cell function and destruction
Domenico Accili & Chutima Talchai	Columbia University	Beta cell dedifferentiation in type 2 diabetes
Jeffrey Bluestone & Armando Villalta	University of California, San Francisco	Vascular endothelial growth factor receptor signaling regulates the pathogenesis of type 1 diabetes
Peter Butler, Alexandra Butler & Mark Atkinson	University of California, Los Angeles	Pancreatic duct glands in type 1 diabetes
Martha Campbell-Thompson, Alberto Pugliese, Clive Wasserfall & Mark Atkinson	University of Florida	Pancreatic immunologic and metabolic parameters
Gun Frisk & Olle Korsgren	Uppsala University	Different expression of extracellular CAR in islets in pancreatic sections and isolated islets
Roberto Gianani & Mark Atkinson	University of Colorado, Denver	Characterization of type 1 diabetes subsets
Ben Giepmans & Martha Campbell-Thompson	University Medical Center Groningen	Nanotomy of human Islets of Langerhans in type 1 diabetes
Kathleen Gillespie	University of Bristol	Maternal microchimerism in T1D pancreas
Akihisa Imagawa & Toshiaki Hanafusa	Osaka University	Histological differences between Japanese and Western type 1 diabetes
George King & Susan Bonner-Weir	Harvard University	Joslin Medalist Study
Paolo Maddedu & Costanza Emanueli	University of Bristol	Microangiopathy in diabetic bone marrow
Lydia Sorokin & Eva Korpos	University of Muenster	Peri-capsular basement membrane degradation during leukocyte penetration into the pancreatic islet during development of human type 1 diabetes
Scott Swenson	Yale University	Polyploidy in the exocrine pancreas

Investigator	Institution	Project Title
Ranjeny Thomas & Katharine Irvine	University of Queensland	Pathological changes associated with chronic RelB activation in T1D
Thomas Wight & Susan Perigo	Benaroya Research Institute at Virginia Mason	Extracellular matrix involvement in type 1 diabetes pancreatic islet destruction

Type 1 Diabetes Etiology

Investigator	Institution	Project Title
Heikki Hyöty	University of Tampere	Detection of enteroviruses in the pancreas and other organs
Richard Lloyd & Joseph Petrosino	Baylor College of Medicine	Virome and microbiome in T1D onset
Noel Morgan & Sarah Richardson	Peninsula Medical School	Enteroviral infection as a causative factor in type 1 diabetes
Åke Sjöholm	Karolinska Institute	Ljungan virus in type 1 diabetes patients
John Todd, Vincent Plagnol & Herbert Virgin, IV	University of Cambridge	Characterization of the nPOD samples virome by high throughput DNA sequencing
Antonio Toniolo, Andreina Baj &Roberto Accolla	University of Insubria	Detection of enteroviruses in lymphoid tissues of donors with T1D of short duration and attempts to identify the infected cell type(s)
Steven Tracy	University of Nebraska	Enterovirus involvement in the etiology of T1D
Eric Triplett, Clive Wasserfall & Mark Atkinson	University of Florida	Gut microbiota in early type 1 diabetes

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Post-Meeting Survey

The nPOD team is so pleased that you were able to join us at the 5th Annual nPOD Meeting. Each day, our goal is to ensure that we provide you with the best service and resources to conduct your research. We always welcome your feedback and seek to continually improve to meet your needs. Please use this survey to let us know how we are doing. You may tear out this page and leave with an nPOD staff member at the end of the meeting. Thank you!

Please rate your satisfaction on the following points:

	Very Satisfied	Somewhat Satisfied	Neutral	Somewhat Dissatisfied	Very Dissatisfied
Facilities and Timing					
Space					
Location					
Time of Year					
Travel Ease					
Event Length					
Food/Accommodations					
Research Content					
Speakers/Presentations					
Exhibit/Session Topics					
Time Allotted					
Comprehensive Overview					
Collaborations					
Communication					
Save-the-Date Announcements					
Abstract Requests					
Registration Requests					
Email Reminders					

Other

- Overall, how would you rate the meeting?

  ☐ Excellent ☐ Very Good ☐ Good ☐ Fair ☐ Poor

- What were your greatest take-aways from the meeting?

- What can we do to improve?

- May we contact you after the meeting to discuss any concerns you might have?

  ☐ Yes Name: Email:

Notes