Correction of Type 1 Diabetes Treg Activation Defect with In Vitro TNFR2 Agonism

Yoshiaki Okubo, Heather Torrey, John Butterworth, Hui Zheng, Denise Faustman
Massachusetts General Hospital/Harvard Medical, Boston, USA

Tumor necrosis factor receptor 2 (TNFR2) is obligatory for induction, maintenance and expansion of activated regulatory T cells (aTregs), which are known to prevent or halt various forms of autoimmunity in animal models and humans. In this study, we show that although type 1 diabetics (T1D) have normal numbers of total Tregs, they have an increase in resting Tregs (rTregs) and a decrease in aTregs compared to controls (n= 55 T1D, n=45 controls, p=0.01), defined by CD45 protein. A large cross-sectional study of children and adults with T1D reveals that this Treg activation defect is lifelong (n=100 T1D, p<0.01). Lower numbers of aTregs were associated with having less residual C-peptide secretion from the pancreas (p=0.08) and poorer HbA1C control (p=0.03). Using two separate in vitro Treg expansion protocols, TNFR2 antibody agonism corrected the T1D activation defect by triggering conversion of rTregs into aTregs (n=54 T1D, p<0.001). TNFR2 antibody agonism was superior to standard protocols of Treg expansion and superior to tumor necrosis factor (TNF) in expanding the most potent subsets of Tregs. TNFR2 antibody expansion protocols exclusively expanded Treg cells but not CD4 T cells, thus creating homogenous populations of potent human Tregs in culture. In T1D, TNFR2 agonist-expanded Tregs were functionally potent by virtue of suppressing autologous cytotoxic T cells in a dose-dependent manner compared to controls. Targeting the TNFR2 receptor for Treg expansion in vitro and perhaps in vivo may be a means to correct the Treg activation defect in T1D children and adults.
Update on Clinical Program Using BCG for Reversal of Longstanding Type 1 Diabetes

Denise Faustman
Massachusetts General Hospital/Harvard Medical, Boston, MA, USA

The bacillus Calmette-Guerin (BCG) vaccine, first used nearly 100 years ago for tuberculosis prevention, represents the most continuously used and safest vaccine in world history. Currently, 10 human clinical trials globally are testing repeat BCG vaccination in diverse forms of autoimmunity and allergies for both prevention and treatment (including patients with new onset and longstanding conditions). Phase I study of the BCG vaccine in longstanding type 1 diabetes (T1D) reveals potential disease modulating effects after repeated BCG vaccination, including death of autoreactive cells, transient and modest restoration of insulin secretion and induction of beneficial regulatory T cells (Tregs). A Phase II clinical trial using multi-dose BCG in longstanding T1D was initiated in June 2015. This double-blinded, placebo controlled immuno-interventional trial protocol was approved by the FDA and is unique in testing the efficacy of the BCG vaccine in long-term diabetic subjects (average disease duration: 15-20 years) with small but detectable levels of C-peptide secretion from the pancreas. Based on published Phase II clinical trial data of BCG in multiple sclerosis subjects, the therapeutic effects of this vaccine appear to improve over the passage of time; therefore, potential clinical benefits in the diabetes trial will be followed for 5 years. The primary endpoint is decrease in HbA1c in treated vs placebo subjects. The selection of BCG as an immuno-intervention in T1D is based on the protective host TNF response, including induction of Tregs, and potential long-term modulation of the immuno-inflammatory profile of vaccinated subjects.
**Insulin-degrading enzyme deficiency protects from type 1 diabetes by regulating autoantigenicity and proliferation of pancreatic beta cells**

Marie-Andrée Bessard¹, Moser Anna¹, Waeckel-Énée Emmanuelle¹, Chhuon Cerina², Lipecka Joanna², Kim Jessica¹, Guenette Suzanne³, Santamaria Pere⁴, Wong F. Susan⁵, Diana Julien¹, Guerrera Chiara², Unanue Emil⁶, van Endert Peter¹

¹INSERM U1151, Paris, France, ²INSERM US24, Paris, France, ³Mass. Gen. Inst. for Neurodegenerative Diseases, Charlestown, USA, ⁴University of Calgary, Calgary, Canada, ⁵University of Cardiff, Cardiff, UK, ⁶Washington University School of Medicine, Saint Louis, USA

Type 1 diabetes is the result of the destruction of pancreatic beta cells by autoreactive T cells. Proinsulin as an autoantigen with beta cell-restricted expression triggers and sustains the autoimmune CD4⁺ and CD8⁺ T cell response and islet inflammation. We hypothesized that insulin-degrading enzyme (IDE), a protease genetically associated with type 2 diabetes possessing very high insulin affinity, might be involved in proinsulin processing and presentation. We find high expression of IDE and an increased number of autoantigenic insulin B chain fragments in IDE-deficient beta cells of non-obese diabetic (NOD) mice, and normal to increased stimulation of insulin-specific CD8 and CD4 T cells by IDE-deficient islets. This suggests that IDE physiologically degrades (pro)insulin in beta cells. However, surprisingly, IDE-deficient NOD mice are more resistant to diabetes transfer by T cells specific for insulin but not for another key autoantigen, harbor fewer diabetogenic splenocytes and display strongly reduced diabetes incidence. Moreover, IDE-deficient islet grafts are more resistant to autoimmune rejection. Seeking to explain the apparent paradox between normal to increased insulin presentation and resistance to the diabetogenic action of insulin-specific T cells, we find that IDE deficiency results in upregulated beta cell regeneration in response to autoimmune inflammation. Diabetes protection in IDE-deficient mice most likely result from moderately increased beta cell stress recently shown to induce beta cell proliferation. Thus IDE acts both in processing of the key autoantigen in murine type 1 diabetes and as a regulator of beta cell stress, ultimately enhancing autoimmune pathology and diabetes.
Hyperglycemia in patients with type 1 diabetes reduces the ability of tolerogenic dendritic cells to induce stable antigen-specific T cell hyporesponsiveness and to generate functional regulatory T cells

Klara Danova1,2, Anna Grohova1,2, Pavla Strnadova1, David Funda3, Zdenek Sumnik4, Jan Lebl4, Ondrej Cinek4, Stepanka PruhoVA4, Stanislava Kolouskova4, Barbora Obermannova4, Lenka Petruzelkova4, Anna Sediva2, Petra Fundova3, Karsten Buschard5, Radek Spisek1,2, Lenka Palova-Jelinkova1,2

1SOTIO a.s., Prague, Czech Republic, 2Department of Immunology, Charles University in Prague, Second Faculty of Medicine and University Hospital Motol, Prague, Czech Republic, 3Department of Immunology and Gnotobiology, Institute of Microbiology of the CAS, v. v. i., Prague, Czech Republic, 4Department of Pediatrics, Charles University in Prague, Second Faculty of Medicine and University Hospital Motol, Prague, Czech Republic, 5The Bartholin Institute, Rigshospitalet, Copenhagen, Denmark

Tolerogenic dendritic cells (tolDCs) may offer an intervention strategy to re-establish antigen-specific tolerance in autoimmune diseases, including type 1 diabetes (T1D). T1D results from destruction of β cells leading to hyperglycemia that in turn markedly affects the patient's immune system. We prepared monocyte-derived tolDCs using dexamethasone and vitamin D2 from 31 T1D patients with optimal and 60 T1D patients with suboptimal glycemic control and assessed their tolerogenic properties. tolDCs differentiated from both groups of patients acquired regulatory phenotype; however, tolDCs from well-controlled patients expressed significantly higher levels of inhibitory molecules IL-T3 and PD-L1. Additionally, GAD65-loaded tolDCs from well-controlled patients decreased significantly primary Th1/Th17 responses, induced stable GAD65-specific T cell hyporesponsiveness and suppressed markedly control DC-induced GAD65-specific T cell activation compared to poorly controlled patients. We confirmed that the ability of tolDCs to induce GAD65-specific T cell hyporesponsiveness depends on the glycemic control, since tolDCs from poorly controlled patients restored their ability to induce stable GAD65-specific T cell hyporesponsiveness once they improved the glycemic control. In both groups of patients, tolDCs were able to induce T regulatory cells (Tregs) from autologous naïve CD4+ T cells. However, Tregs from well-controlled patients had better suppressive abilities and produced more IL-10 than Tregs from poorly controlled patients. These results suggest that metabolic control of T1D affects the functional characteristics of tolDCs and subsequent effector T cell responses. This may be relevant for refining inclusion criteria of future clinical trials. This project was supported by the Charles University in Prague, GAUK 132215.
Maternal 1,25-dihydroxy-Vitamin D during Pregnancy and Risk of Childhood onset Type 1 Diabetes

Lars C Stene1, Ingvild M Sørensen2, Sandra R Dahl3, Anne Eskild4,5, Pål A Jenum6,5, Geir Joner2,5
1Norwegian Institute of Public Health, Oslo, Norway, 2Oslo University Hospital, Department of Pediatric Medicine, Oslo, Norway, 3Oslo University Hospital, Hormone Laboratory, Oslo, Norway, 4Akershus University Hospital, Department of Obstetrics and Gynaecology, Lørenskog, Norway, 5University of Oslo, Department of Clinical Medicine, Oslo, Norway, 6Vestre Viken Hospital Trust, Department of Laboratory Medicine, Drammen, Norway

Maternal vitamin D status during pregnancy has been inconsistently associated with type 1 diabetes (T1D) in children. 25-hydroxyvitamin D is the clinically relevant marker of vitamin D status, but 1,25-dihydroxyvitamin D is the active form, increases during pregnancy, and has never been studied in relation to offspring T1D. We aimed to investigate whether serum calcitriol during pregnancy was lower in women whose offspring developed T1D. We designed a case-control study nested within a cohort of pregnant women in Norway, with up to three biobanked serum samples from the first, second and third trimester. Cases whose child developed T1D before age 15 years were identified by record linkage to the Norwegian Childhood Diabetes Registry, and controls were randomly selected from the cohort. We measured 1,25-dihydroxyvitamin D using immunoextraction and enzyme immunoassay (IDS Nordic) in 217 serum samples from cases and in 419 samples from controls (25-hydroxyvitamin D had previously been measured and published from this material). As expected, 1,25-dihydroxyvitamin D concentration increased from the first to the second trimester, and was significantly correlated with 25-hydroxyvitamin D (r=0.46). Overall mean 1,25-dihydroxyvitamin D was 219.4 pM (SD: 74) in cases, and 225.9 pM (SD: 79.4) in controls (p=0.5 in linear mixed model with random intercept for each woman). We conclude from this first study of its kind, that there was no significant difference in maternal serum 1,25-dihydroxyvitamin D during pregnancy in women whose offspring later developed childhood onset type 1 diabetes, and controls.
We aimed to identify subtypes of type 1 diabetes with differences in ethiopathogenesis by analyzing associations between pattern of islet autoantibodies at the diagnosis and specificity of the first autoantibody initiating the autoimmunity. The follow-up cohort of the Finnish Type 1 Diabetes Prediction and Preventon (DIPP) study was analyzed for the first detectable autoantibody and autoantibodies present at diagnosis. Autoantibodies present at diagnosis in children from the Finnish Pediatric Diabetes Register were also compared in relation to HLA-DR/DQ genotypes and SNP markers in the INS and IKZF4 genes. Identification of a single first autoantibody at seroconversion and autoantibody data at diagnosis were available from 128 children participating the DIPP study. The most common primary autoantibodies were specific for insulin (IAA; N=68) followed by those for glutamic acid decarboxylase (GADA; N=38), insulinoma-associated antigen-2 (IA-2A; N=13) and zinc transporter8 (ZnT8A; N=9), whereas at diagnosis, IA-2A were most frequent (N=103) followed by IAA (N=78), ZnT8A (N=73), and GADA (N=71). Accordingly the presence of a specific autoantibody at diagnosis correlated poorly with the primary autoantibody. However, autoantibody combinations at diagnosis could be assembled into groups associated with either IAA or GADA at the seroconversion, which also were associated with either HLA-DR4-DQ8 or HLA-DR3-DQ2 homozygosity. These groups showed stronger associations with type 1 diabetes associated genetic markers in the INS and IKZF4 genes than the presence of single autoantibodies at diagnosis. Patterns of specific autoantibodies at diagnosis can thus be used to identify heterogenic subtypes of type 1 diabetes.
Comparative analysis of antigen-presenting cell subsets from the pancreatic lymph nodes of control and T1D donors

Jorge Postigo-Fernandez, Remi Creusot
Columbia University, New York, USA

Various subsets of antigen-presenting cells (APCs) in lymph nodes contribute to the maintenance of peripheral tolerance. Recent studies have revealed the existence of a local imbalance in the pancreatic lymph nodes (PLNs) of T1D patients, with exacerbated effector responses and defective regulatory processes, as well as a number of gene dysregulations. We hypothesized that these changes may partly result from possible defects affecting the ability of particular APCs to engage self-reactive T cells (e.g., expression and presentation of relevant autoantigens) and/or to properly educate them (expression of tolerogenic products). We analyzed the phenotype and expression of tolerance-related genes and beta-cell antigens in sorted APC subsets from the PLNs of T1D patients and healthy donors, using flow cytometry and BioMark qPCR assays, to address their possible contribution in the local disease processes. We observed that dendritic and stromal cell subsets from the PLNs of T1D patients display an altered phenotype and gene expression profile compared with the same subsets isolated from non-diabetic PLNs. Changes in population frequency and increase in HLA-DR expression were observed in specific stromal cell subsets between T1D and control PLNs. Gene expression also differed between APC subsets, suggesting distinct tolerogenic potential. Our studies will contribute to a better understanding of the genes and pathways that are altered in different APC subsets of T1D PLNs and benefit the development of therapeutic approaches that address these deficiencies to help restore immune tolerance.
Improving efficacy of tolerogenic DNA vaccines for Type 1 diabetes

Jorge Postigo-Fernandez, Remi Creusot
Columbia University, New York, USA

Type 1 diabetes (T1D) is an autoimmune disease where pancreatic beta-cells are destroyed by CD4 and CD8 T cells. Antigen-specific therapies (ASTs) aim to restore T cell tolerance and block the response of diabetogenic T cells. Various attempts at applying ASTs to treat T1D in humans have failed. One approach, using tolerogenic DNA vaccines, has completed safety clinical studies showing deletion of CD8 T cells specific for the encoded autoantigen. Few antigens have been used as DNA vaccines, and their poor efficacy might be due in part to the use of single antigens, with epitopes primarily presented to CD8 T cells. We have developed a DNA construct encoding major epitopes and mimotopes for several beta-cell antigens that allows simultaneous engagement of CD4 and CD8 T cells, and with different targeting signals for antigen presentation of all epitopes confined to transfected cells or for secretion and acquisition by other antigen-presenting cells. We tested different routes of injection, and the most pronounced antigen-specific response, assessed with adoptively transferred T cells and tetramer-identified endogenous T cells, was observed after intradermal injection of the secreted construct. This response included the induction of antigen-specific regulatory CD4 T cells in the draining lymph-nodes after a single treatment, suggestive of a tolerogenic outcome. We are also validating that these constructs remain efficient at deleting CD8 T cells and evaluating new formulations for DNA delivery. Our studies so far suggest that DNA constructs can be improved to more efficiently target diabetogenic T cells and enhance protection against T1D.
Reactive oxygen species contribute to the development of autoimmune diseases, including Type 1 diabetes (T1D). Our lab recently demonstrated that superoxide-deficient CD4 T cells exhibited increased effector responses and were highly diabetogenic. Others have shown that enhanced susceptibility for autoimmune arthritis is attributed to increased reduced thiols on arthritogenic CD4 T cells. These results demonstrate that one mechanism of redox regulation of autoreactive CD4 T cells is an increase in cell surface thiols, but how T cell surface thiols are regulated and contribute to T1D is unclear. We hypothesized that during T1D, activated CD4 T cells will display an increase in cell surface reduced thiols and a concomitant enhancement in diabetogenic effector responses. To test this, fluorescein-5-maleimide (F5M)-labeling of cell surface reduced thiols on diabetogenic mouse and human CD4 T cells was performed. We observed an increase in the percentage and geometric mean fluorescence intensity (gMFI) of F5M from peripheral NOD CD4 T cells during progression to overt diabetes. Cognate autoantigen stimulation elicited a 2- and 20-fold increase in CD4+ F5M+ T cell percentage and gMFI, respectively. More importantly, analysis of cell surface reduced thiols from human CD4 T cells with T1D displayed a distinct high, medium, and low F5M+ expression profile, unlike healthy controls, providing evidence that F5M-labeling could be used as a novel marker of autoreactive T cells. Our studies point to the exciting potential that oxidation of cell surface thiols on diabetogenic CD4 T cells could potentially abrogate effector responses and serve as an autoreactive signature.
T cell receptor affinity for self instructs regulatory T cell function during autoimmunity

Maria Bettini, Maran Sprouse, Ivan Shevchenko, Thomas Lee, Samuel Blum
Department of Pediatrics, Section of Diabetes and Endocrinology, McNair Medical Institute, Baylor College of Medicine, Texas Children’s Hospital, Houston, Texas, USA

Multiple studies suggest that the strength of the T cell receptor (TCR) signal leading to Foxp3+ regulatory T cell (Treg) selection, expansion, and survival is unique and distinct from T effector cells (Teffs). However, it is unknown whether the TCR affinities of pancreas-infiltrating Tregs are important for Treg accumulation and function in autoimmune diabetes. In the current study, we addressed this question by using retroviral-mediated gene expression of TCRs specific to the insulin B9-23 self-peptide. We generated mixed bone marrow (BM) chimeras with BM from NOD.scid (‘wt’) or NOD.scid.scurfy mice, with two pairs of low and high affinity TCRs. For each TCR pair, we generated four groups of animals with intact, deficient, or completely absent Treg compartment (wt+wt, wt+scurfy, curfy+wt, or curfy+scurfy). This strategy allowed for comparing the function of Tregs expressing different TCRs in the context of identical pairs of Teff populations. Complete deletion of Tregs led to accelerated diabetes development; however, deletion of low or high affinity Tregs resulted in similar disease course. To assess the role of TCR affinity in a polyclonal NOD Treg population we sorted and transferred islet-infiltrating Tregs expressing either high or low levels of CD5 (a marker of self-reactivity). CD5hi Tregs were more protective against diabetes, expressed higher levels of GITR and CTLA4, and exhibited more complete demethylation of key Treg genes. These results indicate that TCR signals early in Treg development shape their functional potential; however, high affinity for tissue antigen is not critical for Treg function in periphery.
Determination of Proinsulin T cell Epitopes Restricted by Type 1 Diabetes-associated HLA Class II Molecules

Emmi-Leena Ihantola1, Henna Ilmonen1, Anssi Kailaanmäki1, Riitta Veijola2, Jorma Toppari3, Mikael Knip4, Jorma Ilonen3, Tuure Kinnunen1
1University of Eastern Finland, Kuopio, Finland, 2University of Oulu, Oulu, Finland, 3University of Turku, Turku, Finland, 4University of Helsinki, Helsinki, Finland

Type 1 diabetes (T1D) is caused by a T-cell mediated destruction of pancreatic beta cells. Several antigens have been implicated as targets of the autoimmune CD4+ T-cell response in T1D but recent studies suggest that (pro)insulin may be the most important autoantigen.

The HLA class II region has the strongest impact on the genetic risk of T1D and the DR3-DQ2 and DR4-DQ8 are the main risk haplotypes for the disease. Hence, the identification of proinsulin epitopes restricted by HLA class II molecules encoded by the DR3-DQ2 and DR4-DQ8 haplotypes is important for the understanding of the disease pathogenesis.

In this study, we have used a well-established T-cell cloning method based on CFSE-dilution to generate proinsulin-specific T-cell clones from healthy individuals who carry only the high-risk DR3-DQ2 and/or DR4-DQ8 haplotypes. Epitope specificity of the T-cell clones was determined by stimulating them with individual peptides spanning the proinsulin sequence, and HLA-restriction was determined by using anti-DR, -DQ and -DP antibodies and transfected cell lines expressing single HLA class II molecules.

We have generated 28 unique T-cell clones that recognize at least nine distinct epitopes within proinsulin. With our approach we have both confirmed the existence of previously reported DR4-restricted epitopes and also identified DR3- and DQ2-restricted epitopes that have not been reported before.

The identification of novel autoantigen epitopes recognized by human CD4+ T cells may be utilized in the development of both antigen-specific therapies as well as better T-cell assays for immune monitoring in clinical trials of T1D.
Autoantigen (GAD-alum) given into lymph-nodes together with oral Vitamin D to preserve beta cell function in Type 1 diabetes. The DIAGNODE pilot trial.

Johnny Ludvigsson, Beatriz Tavira, Hugo Barcenilla, Jeanette Wahlberg, Rosaura Casas
Linköping university, Linköping, Sweden

In allergy allergen administration into lymph-nodes seems much more effective than sc administration For the first time intra-lymphatic route is tried in T1D. Vitamin D might help to gain additional efficacy.

Objectives: To evaluate the safety as well as clinical and immunological response

Patients and methods: DIAGNODE-1 is a single-center open-label pilot Phase I trial designed to enroll approximately 9 subjects between 12-30 years of age, T1D duration < 6 months, positive for GAD65- antibodies (GADA) and a fasting C-peptide ≥0.12 nmol/L. They get Vitamin D 2000 U/d Day 0-120 and 4 µg GAD-alum into an inguinal lymph-node Day 30, 60 and 90. So far 7 patients have been recruited. Four have been followed for 6 months (soon 15 months). GADA, has been measured and the effect of GAD65 stimulation on cytokines in cell supernatants, cell proliferation and T cell phenotypes. Beta cell function has been evaluated by MMTT

Results: The treatment has been feasible, well tolerated, without any concerns regarding safety. From baseline to 6 months the C-peptide AUC (nmol/l) decreased 2% and 29% in two patients, and increased 32% and 6% respectively in two patients. A strong immune response is found suggesting a pronounced Th2-deviation of the immune system.

Conclusion: A low dose GAD-alum given into lymph-node in recent onset T1D is feasible, tolerable, seems to be safe, and gives a strong Th2-deviation of the immune response which together with Vitamin D might preserve beta cell function.
Modulation of the gastrointestinal microbiota normalizes systemic inflammation and islet immunocyte recruitment potential associated with autoimmune diabetes susceptibility

Angela Henschel¹, Susanne Cabrera¹, Mary Kaldunski¹, Shaung Jia¹, Rhonda Geoffrey¹, Mark Roethle¹, Vy Lam¹, Dr. Yi-Guang Chen¹, Xujing Wang², Nita Salzman¹, Martin Hessner¹
¹The Medical College of Wisconsin, Milwaukee, WI, USA, ²Systems Biology Center, National Heart, Lung, and Blood Institute, the National Institutes of Health, Bethesda, MD, USA

The incidence of Type 1 diabetes (T1D), a T-cell mediated autoimmune disease that targets the pancreatic β-cells, has risen significantly over the past half century, suggesting an increase in environmental pressure. Our earlier studies of T1D families and the BioBreeding (BB) rat model identified a peripheral innate inflammatory state associated with diabetes susceptibility, consistent with pattern recognition receptor (PRR) ligation, yet independent of disease progression. Here, compared to control strains, islets of spontaneously diabetic BB DRlyp/lyp and nondiabetic BB DR+/+ weanlings provided a standard cereal diet were found to temporally express a proinflammatory transcriptional program consistent with microbial antigen exposure that included numerous cytokines/chemokines. The dependence of this proinflammatory phenotype on diet and gastrointestinal microbiota was investigated by transitioning DR+/+ weanlings to a hydrolyzed casein diet (HCD) or treating them with antibiotics to alter or reduce PRR ligand exposure. Sequencing of the bacterial 16S rRNA gene revealed that these treatments significantly altered the ileal and cecal microbiota, resulting in an increased Firmicutes:Bacteriodes ratio, and greater relative abundances of lactobacilli and butyrate producing taxa. Both treatments partially normalized the peripheral inflammatory state, reducing plasma cytokine, chemokine and TLR-4 activity levels. Further, both treatments normalized the islet proinflammatory transcriptome and reduced β-cell specific chemokine expression. Neither HCD nor antibiotic treatment normalized BB rat PBMC hyper-responsiveness to ex vivo mitogen stimulation. Combined, these studies link islet-level T1D susceptibility, as measured by β-cell chemokine expression, to a genetically controlled innate inflammatory state that is modulated by diet and the intestinal microbiota.
The Type 1 Diabetes-Protective rs5979785 SNP is Associated with an Altered Innate Immune Response to TLR7 and TLR8 Ligation

MacKenzie Williams, Clive Wasserfall, Mark Atkinson
University of Florida, Gainesville, Florida, USA

Type 1 diabetes (T1D) is thought to occur in genetically susceptible individuals who encounter a triggering environmental insult, resulting in an autoimmune attack on pancreatic beta cells. There is a need to understand the relationship between aberrant innate immune responses and adaptive autoimmunity. Genome-wide association studies found the rs5979785 (T→C) single nucleotide polymorphism (SNP) to be protective against T1D. This SNP, located on the X chromosome, is in close proximity to its candidate genes encoding Toll-like receptors (TLRs) 7 and 8. TLR7 and TLR8 recognize single-stranded viral RNA, and viral infections have long been hypothesized to initiate or promote T1D pathogenesis, though direct evidence supporting a causative role for viruses remains elusive. Our objective was to determine the mechanism by which the TLR7/TLR8 SNP protects against T1D development. We hypothesized that the T1D-protective allele at the TLR7/TLR8 SNP, through the modulation of TLR7 and/or TLR8 signaling, confers a tolerogenic environment for enhanced regulation of adaptive immune responses. Peripheral blood was collected from consented T1D (n=41) and control (n=56) donors. We stimulated whole blood and peripheral blood mononuclear cells (PBMC) using TLR7 and TLR8 ligands and measured IL-6 secretion using ELISA. We observed reduced IL-6 production from R848-stimulated whole blood (p=0.0321), but not PBMCs, obtained from individuals carrying the T1D-protective TLR7/TLR8 SNP allele (CC protective allele n=20, TT susceptibility allele n=56, CT heterozygous n=25) regardless of disease state. These data suggest that the T1D-protective allele of the TLR7/TLR8 SNP alters host response to viral infection, conferring resistance to T1D development.
Inducible IL-7 hyperexpression in a novel immunosuppressive mouse model influences lymphocyte development and function leading to enhanced allograft rejection

Maria Schreiber1,2, Marc Weigelt1,2, Anne Karasinsky1, Annett Lindner1,2, Konstantinos Anastassiadis3, Ezio Bonifacio1,2, Karsten Kretschmer1,2, Angela Hommel1,2

1DFG-Center for Regenerative Therapies TU Dresden (CRTD), Dresden, Germany, 2Paul Langerhans Institute Dresden, German Center for Diabetes Research (DZD), Dresden, Germany, 3BIOTEC, Dresden, Germany

Cell replacement therapy in Type-1-Diabetes must consider strategies to control immune-mediated loss of graft tissue. The IL-7/IL-7R pathway has been suggested to play an important role in T cell-mediated loss of insulin-producing beta-cells following islet transplantation in Type-1-Diabetes.

Here, we report the establishment of an experimental in vivo immunosuppressive mouse model and findings from these transgenic mice (C57BL/6 background) carrying a tetracycline inducible IL-7 expression cassette, which allows the temporally controlled induction of IL-7 hyperexpression by Dexamethasone and Doxycycline treatment. Upon induction of IL-7, both percentages and numbers of the CD19+B220+c-Kit+ Pro/Pre-B-I compartment in the bone marrow increased correlating with enhanced IL-7 serum levels as compared to control mice. Moreover, cytotoxic memory T cells were preferentially expanded in secondary lymphoid organs. To study the effect of IL-7 on islet graft survival in a mismatched allograft model, Balb/c mice were rendered diabetic by streptozotocin und transplanted with C57BL/6 IL-7-inducible or control islets. As expected, Dexamethasone and Doxycycline treatment did significantly prolong graft median survival compared to the untreated control group representing the immunosuppressive situation in this transplantation mouse model. Upon induction of local IL-7 hyperexpression in the islets, graft survival was decreased as assessed by determination of hyperglycemia correlating with increased CD4+ and CD8+ T cell infiltration in the islets.

Altogether, this novel model provides the possibility to elucidate the role of IL-7/IL-7R signalling during graft rejection and to develop strategies to impede IL-7 promoted graft rejection and study the effects of homeostatic immune cell proliferation during cell replacement therapy.
The *Idd7*-associated gene *Nfkbid* modulates thymic negative selection in NOD mice

Maximiliano Presa, Deana Lamont, Harold Chapman, Jennifer Allocco, David Serreze

*The Jackson Laboratory, Bar Harbor, ME, USA*

More than 50 genetic loci (*ldd*) are associated with susceptibility or resistance to T1D in both humans and NOD mice, with particular major histocompatibility complex (MHC) haplotypes providing a primary risk factor. However, when expressed in the context of other *ldd* genes, certain relatively common MHC class I variants in both human and NOD mice aberrantly contribute to T1D by enabling the development and functional activation of autoreactive CD8 T-cell responses. Using NOD.AI4 TCR transgenic mice, we previously demonstrated genes mapping to the *Idd7* locus on Chromosome 7 contribute to less efficient thymic negative selection of diabetogenic CD8 T-cells in NOD.AI4 compared to B6.*H2d*, AI4 mice. Through combined truncation analyses of a B6 derived Chromosome 7 congenic region transferred to the NOD strain and gene expression analyses we identified a hyperexpression variant *Nfkbid* as a strong candidate for an *Idd7* region gene limiting negative selection of diabetogenic CD8 T-cells. *Nfkbid* is a negative regulator of the NF-kB transcription factor that must be expressed at high levels to support the thymic negative selection of CD8, but not CD4 T-cells. Direct CRISPR/Cas9-mediated gene ablation allowed *Nfkbid* expression in NOD mice to be reduced to B6 levels. Both NOD mice homozygous and heterozygous for the ablated *Nfkbid* gene demonstrated significant enhanced thymic deletion of diabetogenic AI4 CD8 T-cells. This important result indicates a hyperexpression *Nfkbid* variant is likely the causal gene within the *Idd7* locus and that strongly contributes defective thymic negative selection of diabetogenic CD8 T-cells in NOD mice.
Identification of Non-HLA Genes Associated with Islet Autoimmunity and Type 1 Diabetes in the prospective TEDDY cohort

Ashok Sharma¹, Xiang Liu², David Hadley²-³, William Hagopian⁴, Wei-Min Chen⁵, Suna Onengut-Gumuscu⁶, Carina Törn⁶, Andrea Steck⁷, Marian Rewers⁷, Anette-G. Ziegler⁸, Åke Lernmark⁶, Juha Mykkänen⁹, Jorma Toppari⁹, Jeffrey P. Krischer², Beena Akolkar¹⁰, Stephen S. Rich⁵, Jin-Xiong She¹, TEDDY Study Group¹⁰

¹Center for Biotechnology and Genomic Medicine, Augusta University, Augusta, GA, USA, ²Pediatric Epidemiology Center, Department of Pediatrics, University of South Florida, Tampa, FL, USA, ³Division of Population Health Sciences and Education, St. George's University of London, London, UK, ⁴Pacific Northwest Diabetes Research Institute, Seattle, WA, USA, ⁵Center for Public Health Genomics, University of Virginia, Charlottesville, VA, USA, ⁶Department of Clinical Sciences, Lund University/CRC, Malmö, Sweden, ⁷Barbara Davis Center for Childhood Diabetes, University of Colorado, Denver, Aurora, CO, USA, ⁸Institute of Diabetes Research, Helmholtz Zentrum München, and Klinikum rechts der Isar, Technische Universität München, and Forschergruppe Diabetes e.V., Munich-Neuherberg, Germany, ⁹Departments of Physiology and Pediatrics, University of Turku and Turku University Hospital, Turku, Finland, ¹⁰National Institutes of Diabetes and Digestive and Kidney Disorders, National Institutes of Health, Bethesda, MD, USA

In addition to the HLA genotypes, a number of non-HLA genes are known to influence risk for islet autoimmunity and type 1 diabetes (T1D). To identify novel T1D genes and/or confirm known genes, we genotyped 5806 subjects from an international prospective cohort, the TEDDY (The Environmental Determinants of Diabetes in the Young) Study. Each individual was typed by ImmunoChip at 176,586 SNPs from regions that may contain autoimmunity genes. Data were analyzed using Cox proportional hazards analyses to discover SNPs influencing the rate and timing of development of islet autoimmunity and/or clinical diabetes. In time to T1D analyses, we found 12 SNPs mapped to 7 previously known regions associated with T1D (PTPN22, CTLA4, CCR5, CENPW, INS, ZFP36L1, and MTMR3; 10⁻⁴>P>1.05x10⁻⁶) and 11 SNPs mapped to 4 novel regions (NME7, KIF26B, REV3L, PPIL2; 10⁻⁴>P>1.08x10⁻⁷). A SNP near PPIL2 (rs428595, P=1.08x10⁻⁷, HR=4.34) reached the genome-wide association threshold. Analyses of time to islet autoimmunity identified 22 SNPs in 4 regions already known to be associated with T1D (PTPN22, MOG, INS and SH2B3; 10⁻⁴>P>8.97x10⁻⁷) and 37 SNPs mapped to 5 novel regions (CTSE, VGLL4, PXK/PDHB, CYLD, PPIL2; 10⁻⁴>P>5.67x10⁻⁶). In conclusion, we identified 4 novel genomic regions associated with T1D and 5 novel regions associated with the development of islet autoimmunity and confirmed 7 regions previously known to be associated with T1D. The novel findings may result from time-to-event analyses as compared to prior cross-sectional studies, or to analysis of autoantibodies rather than T1D, or both.
Adoptive transfer of mRNA-transfected T cells redirected against insulin-reactive T cells can protect NOD mice from diabetes

Gideon Gross¹,⁴, Sigal Fishman¹,², Mark Lewis³, Khai Siew³, Evy De Leenheer³, Dimitri Kakabadse³, Joanne Davies¹, Doron Ziv¹,⁴, Alon Margalit¹,⁴, Nathan Karin², Suasn Wong³
¹MIGAL - Galilee Research Institute, Kiryat Shmona, Israel, ²Technion, Haifa, Israel, ³Cardiff University, Cardiff, UK, ⁴Tel-Hai College, Upper Galilee, Israel

Chimeric antigen receptors (CARs, developed in the late 1980’s by G.G. and Z. Eshhar at the Weizmann Institute of Science, Israel) are widely used today for generating antitumor T-cells and show promising results in the clinic. We have previously shown that chimeric β₂ microglobulin (β₂m) carrying an antigenic peptide at the N-terminus and fused with the intracellular signaling portion of CD3-ζ generated a new type of CAR which could redirect T-cells against antigen-specific CD8 T-cells. Linking the H-2K¹-binding insulin peptide InsB15-23 to the N-terminus of β₂m/CD3-ζ redirected CD8 T-cells against pathogenic CD8 T-cells in a peptide-specific manner and the adoptive transfer of transgenic T-cells expressing this gene prevented diabetes in NOD mice.

Electroporation of in-vitro-transcribed mRNA emerges as a safe and highly efficient strategy for delivering therapeutic genes into T-cells and is currently being evaluated in the clinical setting. Here we used mRNA electroporation for delivering peptide/β₂m/CD3-ζ genes to a reporter T-cell line and primary NOD CD8 T-cells. The chimeric products paired with endogenous MHC-I heavy chains and transmitted strong activation signals upon MHC-I cross-linking. NOD CD8 T-cells transfected with either InsB15-23/β₂m/CD3-ζ or IGRP²⁰⁶-²¹⁴/β₂m/CD3-ζ mRNA killed their respective autoreactive CD8 T-cell targets. Most importantly, adoptive transfer of InsB15-23/β₂m/CD3-ζ CD8 T-cells could protect NOD mice from diabetes.

Our results demonstrate that mRNA encoding chimeric MHC-I receptors is an effective tool for immunotargeting diabetogenic CD8 T-cells in-vivo and recapitulate the critical involvement of insulin-reactive CD8 T-cell clones in the initiation of disease in NOD mice.
Changes in islet tissue-specific microenvironment take place in the course of islet inflammation, which predisposes for islet invasion by immune cells in type 1 diabetes (T1D). Our studies in human T1D pancreata have indicated the presence of greatly altered islet hyaluronan (HA), a major islet ECM component, which is associated with the extent of invasive insulitis and beta cell loss. Our recent studies in tissues from autoantibody-positive donors show that islet ECM is altered in a subset of these donors, and the largest islet HA deposits are the site of accumulation of islet immune cell infiltrates. In addition, we have found that changes take place in the HA-rich ECM in human lymph nodes and spleen in T1D, which suggest a possible involvement of this ECM component in the generation of autoreactive T-cells and B-cells. In the spontaneously diabetic BB DR.lyp/lyp, HA accumulation occurs early in life, and with progression to hyperglycemia, islet HA deposits become larger and the HA-rich areas are the sites of islet immune cell invasion. Increasing islet HA amounts relates to progression from invasive to destructive insulitis and beta cell loss. These novel observations support a key role for the ECM in T1D pathogenesis, and led to the hypothesis that HA guides immune cell migration into the islets and regulates the immune cell phenotype, T-cell activation, and proliferation, and that alterations in islet HA contribute to the increased vulnerability of the beta cells to inflammatory insult.
A hallmark of T1D is insulitis, characterised by the infiltration of T cells into the islets that is predominantly driven by CD8+ T cells. The CD8+ T cells survey the repertoire of antigenic peptide fragments (immunopeptidome) on the surface of insulin-secreting beta cell presented in complex with I HLA molecules. Previous studies have documented the presence of patient-derived autoreactive CD8+ T cells specific towards islet epitopes, however, the human islet beta cell class I immunopeptidome and the natural presentation of these epitopes as T1D progresses have not been shown at a biochemical level.

Here, we have used a reverse immunology strategy in combination with mass spectrometry to confirm and quantify the presentation of key HLA-A2 restricted preproinsulin epitopes, alongside the discovery of novel non-canonical and overlapping chromogranin A peptides, and a novel posttranslationally modified autoantigenic HLA-A2 bound peptide. This study represents the first characterisation of the human islet beta cell immunopeptidome and is also complemented with the proteomic coverage of the same islet cells. Our islet proteomic coverage revealed novel beta cell specific spliced neopeptide species and key posttranslational modifications mediated by beta cell resident enzymes that are known to be induced by inflammation.

Crucially, our current analysis of this complex immunopeptidomic dataset and the correlation with the islet cell proteome will begin to allow us understand the nature of the targeted autoimmune response towards beta cells and provide a better understanding of the aetiology of T1D.
Peripheral blood cell HLA-DQ expression in children with beta cell autoantibodies and increased risk for type 1 diabetes

Agnes Andersson Svärd, Anita Ramelius, Helena Elding Larsson, Åke Lernmark, The DiPiS study group
Department of Clinical Sciences, Lund University/CRC, Skåne University Hospital, Malmö, Sweden

HLA-DQ is strongly associated with the risk not only for type 1 diabetes but also with the first appearing beta cell autoantibody that precedes the clinical diagnosis. Differential expression of HLA-DQ heterodimers on blood mononuclear cell subsets is poorly investigated. It has been hypothesized that the expression of the α and β chains of the HLA-DQ heterodimers may be affected in children with beta cell autoantibodies. HLA-DQ expression in blood cell subsets were therefore studied in children with varying number of islet autoantibodies followed since birth in the Diabetes Prediction in Skåne (DiPiS) study.

Peripheral blood cell subsets were isolated on magnetic microbeads from 69 children (age range x-years, median z) with defined HLA-DQ genetic risk of type 1 diabetes and with or without beta cell autoantibodies alone, or in combination (GADA, IAA, IA-2A, or ZnT8A). Flow cytometry was used to determine sample purity and HLA-DQ frequency. Gene expression of HLA-DQA1/B1/A2/B2 was evaluated with specific TaqMan-probes in the purified cells.

High HLA-DQ cell surface immunofluorescence was observed in B cells (CD19+) and classical monocytes (CD14+CD16-) and decreased with an increased number of beta cell autoantibodies. Only HLA-DQA1 was expressed in both B cells and classical monocytes. Further analysis is necessary to evaluate the expression of HLA-DQB1/A2/B2. Gene expression revealed decreasing relative quantity of HLA-DQA1 in classical monocytes in subjects with increasing number of beta cell autoantibodies. We speculate that HLA-DQ expression on B cells and classical monocytes may be affected by an on-going autoimmunity against the pancreatic islet beta cells.
Shorter T cell receptor beta-chain CDR3 regions in type 1 diabetes result in enhanced repertoire diversity and sharing.

Iria Gomez-Tourino, Yogesh Kamra, Anna Lorenc, Mark Peakman
Department of Immunobiology, Faculty of Life Sciences & Medicine, King’s College London, UK and National Institute for Health Research, Biomedical Research Centre at Guy’s and St Thomas’ Hospital Fou, London, UK

Defects in T cell receptor (TCR) repertoire could predispose to autoimmunity and type 1 diabetes (T1D). To address this hypothesis, we analysed by next-generation sequencing the TCRB repertoire of true naïve(TN), central memory(TCM), regulatory(Treg) and stem-cell like (Tscm) CD4+ T-cells from 8 recently-diagnosed T1D patients and 8 age/sex-matched healthy donors (HD). A total of 58,469,660 cells were examined (mean 1,818,750 TN, 1,306,577 TCM, 379,626 Treg and 155,506 Tscm cells/subject), yielding >155x10^6 total TCRB sequences and >14x10^6 unique clonotypes.

Comparison of T1D and HD TCRB repertoires reveals several key disease-related features. In T1D, TNs and TCMs have higher TCR diversity. TN, TCM and Treg repertoires are highly related in T1D patients, revealing a higher frequency of clonotypes that are shorter as a result of significant bias towards increased deletions/reduced insertions. Diversity, sharing and TCR clonotype length are correlated, suggesting a common, altered underlying pathway. Such alterations are recapitulated in T1D-exclusive clonotypes, which show preferential usage of hydrophobic amino acids at P6 and P7, a feature associated with self-reactive repertoires. Importantly, analysis of unproductive (pre-selection) sequences shows the same deletion/insertion imbalance as well as specific V gene under-representation. We propose a model in which the molecular machinery of TCRB rearrangement in T1D is biased towards the generation of a higher proportion of short TCRB chains, which are further enriched during positive selection and fail deletion during negative selection, yielding a highly diverse TCRB repertoire which could render subjects with T1D more prone to respond against a wider range of targets, including autoantigens.
Multidimensional analysis of GAD65-specific CD4 T cells at single cell resolution shows numerical expansion and distinct type 1 diabetes phenotypes

Iria Gomez-Tourino, Yogesh Kamra, Dietz Sevina, Bonifacio Elio, Eugster Anne, Peakman Mark

Department of Immunobiology, Faculty of Life Sciences & Medicine, King’s College London, and National Institute for Health Research, Biomedical Research Centre at Guy’s and St Thomas’ Hospital Foundation, London, UK, DFG-Center for Regenerative Therapies. Technische Universität Dresden, Dresden, Germany

Autoantigen-specific CD4+ T-cells play a key pathogenic role in type 1 diabetes, but our understanding of their disease contribution is hampered by inability to study them directly ex vivo at the single cell level. We developed a platform process to isolate and phenotype CD4+ T-cells, combined with multidimensional flow cytometry, TCR clonotyping and 48-gene expression analysis by SC-qPCR, in order to define GAD65-specific T-cell fingerprints.

PBMCs from newly diagnosed type 1 diabetes patients (T1D, n=11) and matched healthy donors (HD, n=10) were stimulated for 18 hours in vitro with GAD65/control, stained (lineage, differentiation and live/dead), and cells upregulating CD154 and CD69 index-sorted. GAD65-specific CD4+ T cells were more frequent in patients, especially those with higher autoantibody titres, and mainly displayed central and effector memory (CM, EM) phenotypes.

We found two types of responder cells present in both patients and controls: (i) IL2+ prototypical Th1 cells and (ii) IL2+ Th2 cells co-expressing IL-21 and IL-22. In addition, hierarchical clustering and tSNE identified two disease-related fingerprints: (1) IL2neg cells, amongst which Th1/Th17 phenotypes and FOXP3 expression were notable (2) IL2+ cells of Th2 phenotype co-expressing TNF-α, IL-21, ICOS, Egr2 and GMCSF.

TCRA and TCRB repertoires were private with notable clonal expansions of CM and EM cells bearing both of the disease-related fingerprints. Our findings suggest that type 1 diabetes is characterised by expansion of GAD65-specific CD4+ T-cells into the memory pool. These cells display polyfunctional signatures which are both part of the healthy repertoire and disease related.
Beta cell stress and activation of immune cells in models of type 1 diabetes

Gudrun Marijssen1, Hannelie Korf1, Saurabh Vig1, Inne Crévercoeur1, Conny Gysemans1, Decio Eizirik2, Lut Overbergh1, Chantal Mathieu1

1Laboratory of clinical and experimental endocrinology, KU Leuven, Leuven, Belgium, 2ULB Center for Diabetes Research, ULB, Brussels, Belgium

Macrophages have a specialized molecular machinery to sense and respond to apoptotic cells, but signals from the dying cell itself are also important to determine whether the outcome will be immunogenic or tolerogenic. We explored whether interactions between dying beta-cells and macrophages in a mouse model of type 1 diabetes (T1D) are different as compared to a homeostatic condition, and also evaluated whether the type of beta-cell stress affects immune responses during T1D.

MIN6 cells were exposed to different stress-induced agents, namely thapsigargin (5 µM); cytokines (hIL-1β/mIFN-γ/mTNF-α, 50/250/1000 U/ml); cycloheximide (5 µg/ml) or staurosporin (5µM). The different treatments resulted in approximately 60% beta-cell apoptosis. Stressed or control (non-treated) beta-cells were co-cultured with peritoneal macrophages from 8-week old C57BL/6 or NOD mice.

NOD macrophages exhibited the same ability to clear apoptotic beta-cells as C57BL/6 macrophages from (n=15, p<0.001) irrespective of the mode of apoptosis. Interestingly, NOD macrophages produced higher levels of TNF-α (n= 14, p<0.05) and lower levels of IL-10 (n=14, p<0.05) after interacting with beta-cells, indicating a hyperinflammatory phenotype. Staurosporine-induced beta-cell death led to a remarkable lower cytokine production by macrophages. Clearing of apoptotic cells by C57BL/6 or NOD macrophages (n=3) dampened T-cell activation/proliferation, an effect that was most marked after thapsigargin and staurosporin treatment.

These observations suggest that macrophages obtained from diabetes-prone NOD mice express a more pro-inflammatory profile when confronted with dying beta-cells, as compared with macrophages from control mice. Additionally, the type of beta-cell stress is important for the contribution of immunogenic response of macrophages during T1D.
Macrophage infiltration and extracellular matrix components in the spontaneously diabetic BB DR.lyp/lyp rat were reduced after delaying diabetes onset with the monoclonal Vbeta13 T cell receptor antibody

Linda Faxius1, Marika Bogdani2, Malin Fex1, Anita Ramelius1, Anya Medina1, John Mordes3, Elisabeth P Blankenhorn4, Åke Lernmark1

1Department of Clinical Sciences, Lund University/CRC, Skåne University Hospital SUS, Malmö, Sweden, 2Benaroya Research Institute, Seattle, WA, USA, 3Department of Medicin, University of Massachusetts, Worcester, MA, USA, 4Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA, USA

Abstract

The spontaneously diabetic BB DR.lyp/lyp is often resistant to therapeutic intervention to prevent diabetes. The depleting monoclonal Vbeta13 T cell receptor antibody, 17D5, has been used to prevent both induced and spontaneous rat autoimmune diabetes and was therefore used to treat congenic BBM (M for Malmö) DR.lyp/lyp rats at 40 days of age. All rats injected once a week with saline (n=5; 55-73 days of age) or with 0.1 mg Vbeta16 monoclonal antibody, His42 (n=6; 59-69 days of age) developed diabetes. Rats (n=11) treated weekly with 0.1 mg 17D5 antibody developed diabetes at a variable rate: 3/11 were diagnosed at 60-71 days of age, 5/11 at 74-93 days, and 3/11 were killed at 101-103 days without diabetes. Saline and His42 treated rats had severely distorted islets with loss of insulin positive cells, rearrangement of glucagon positive cells and abundant infiltration within and around the islets of ED1 positive macrophages. Hyaluronic acid (HA) staining was prominent while CD3 positive cells were rare. Treatment with 17D5 reduced ED1 positive cells in the rats that developed diabetes early while rats with delayed onset showed a mix of distorted and normal islets with or without ED1+ macrophage infiltration and HA staining. The rats killed without diabetes had predominantly normal islets, some ED1 positive macrophage infiltration and reduced HA staining. The present data suggest that Vbeta13 T cell receptor antibody treatment may delay the onset of diabetes in the BB DR.lyp/lyp rats in association with reduced HA expression and macrophage infiltration.
Gluten-free Diet during Pregnancy Improves Intestinal Enteropathy and Affects the Immunology in Non-obese Diabetic Mouse Offspring

Martin Haupt-Jørgensen¹, Jesper Larsen¹, Knud Josefsen¹, Tina Z. Jørgensen¹, Julie Christine Antvorskov¹, Axel K. Hansen², Karsten Buschard¹

¹The Bartholin Institute, Rigshospitalet, 2100 Copenhagen, Denmark, ²Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, 1871 Frederiksberg, Denmark

Gluten-free (GF) diet exclusively during pregnancy prevents type 1 diabetes (T1D) in offspring of non-obese diabetic (NOD) mice. Gluten affects the immunology and morphology of the intestine, as well as the immunology of lymphoid tissues. We aimed to elucidate the mechanism behind the preventive effect of T1D in NOD mice that were GF in utero. NOD mice were fed a GF or a gluten-containing standard (STD) diet during pregnancy. Female offspring was lactated by mothers kept on STD diet and fed this diet until 4 and 13 weeks of age, respectively. Insulitis was lower in 13-week-old NOD mouse offspring that were exposed to GF diet in utero compared to those on STD diet in utero. GF diet in utero led to reduced serum anti-tissue transglutaminase titer and improved enteropathy in mice from both age groups. FACS analysis on single cells from 13-week-old mice exposed to GF diet during pregnancy revealed increased proportions of NK cells in spleen (S) and increased expression of NKG2D on NK cells in mesenteric lymph nodes (MLN) and inguinal lymph nodes (ILN). Moreover, GF versus STD diet in utero reduced the expression of interferon gamma (IFNG) in CD4+ T cells and interleukin 22 (IL22) in γδTCR+ T cells from S, but increased the expression of IL6 in CD8+ T cells from ILN and in γδTCR+ T cells from MLN. GF diet in utero improves humoral and histopathological symptoms of celiac disease and leads to immunological changes in lymphoid tissue of NOD mice.
miRNA92a targets KLF2 and PTEN signaling to promote human T follicular helper precursors in T1D islet autoimmunity

Isabelle Serr1,4, Verena B. Ott3,4, Martin G. Scherm1,4, Benno Weigmann5, Anette-Gabriele Ziegler2,4, Carolin Daniel1,4
1Institute for Diabetes Research, Independent Young Investigator Group Immune Tolerance in Type 1 Diabetes, Helmholtz Diabetes Center at Helmholtz Zentrum München, Munich, Bavaria, Germany, 2Institute for Diabetes Research, Helmholtz Diabetes Center at Helmholtz Zentrum München, Klinikum rechts der Isar, Technische Universität München, Munich, Bavaria, Germany, 3Institute for Diabetes and Obesity, Helmholtz Diabetes Center at Helmholtz Zentrum München, Munich, Bavaria, Germany, 4Deutsches Zentrum für Diabetesforschung (DZD), Munich, Bavaria, Germany, 5Department of Medicine 1, University of Erlangen-Nuremberg, Kussmaul Campus for Medical Research, Erlangen, Bavaria, Germany

During the onset of autoimmunity in type 1 diabetes (T1D) the aberrant activation of effector T cell populations is pivotal. T follicular helper (TFH) cells are essential players in the development of high affinity antibodies and their precursor memory compartment circulates in the blood. TFH cells have mainly been studied in clinical T1D, however their role in the onset of islet autoimmunity and the signaling pathways involved in their differentiation are incompletely understood. Here, we demonstrate that the insulin-specific target T cell population is enriched with a CXCR5+CD4+TFH precursor phenotype during the onset of islet autoimmunity. The frequency of these TFH precursors during onset of autoimmunity was controlled by high levels of miRNA92a. Additionally, we identify KLF2 as a novel target of miRNA92a and demonstrate that miRNA92a-mediated TFH precursor induction is regulated by the PTEN-PI3K-KLF2 signaling network. More importantly, we show that a specific miRNA92a antagomir can block TFH induction in vitro and in vivo in NOD mice with IAA+ autoimmunity. Of note, we observed reduced frequencies of insulin-specific CXCR5+TFH precursors in the pancreas of treated NOD mice together with an improvement of insulitis scores. Furthermore, to assess a potential human in vivo relevance, humanized NSG HLA-DQ8 mice were given a miRNA92a antagomir which resulted in decreased levels of polyclonal and insulin-specific CXCR5+TFH cells directly in the pancreas. We therefore propose that the specific targeting of miRNA92a and the PTEN-PI3K-KLF2 signaling network could be used to devise novel precision medicines to limit islet autoimmunity.
A refined molecular signature is predictive of type 1 diabetes disease trajectory both before and after clinical onset

Susanne Cabrera¹, Shuang Jia¹, Mary Kaldunski¹, Carla Greenbaum², Martin Hessner¹
¹Department of Pediatrics and the Max McGee National Research Center for Juvenile Diabetes, Medical College of Wisconsin, Milwaukee, WI, USA, ²Diabetes Clinical Research Program, Benaroya Research Institute at Virginia Mason, Seattle, WA, USA

Novel biomarkers are needed to predict type 1 diabetes (T1D) disease trajectory. We have developed a sensitive array-based bioassay whereby patient plasma is used to induce transcription in healthy "reporter" leukocytes. Previously, transcriptional signatures were scored with an ontology-based composite inflammatory index utilizing 1,374 transcripts (I.I.com1,374). Higher I.I.com reflect increasing inflammatory bias whereas a lower I.I.com reflect decreasing inflammatory bias and are associated with increases in peripheral activated Treg frequencies. Here, using Random Forest Analysis, we report a refined scoring algorithm that requires only 359 transcripts (I.I.com359), affording easier analysis while maintaining accuracy. I.I.com359 was applied to longitudinal samples of local at-risk (n=32) and TrialNet Natural History (n=26) subjects. T1D progressors demonstrated temporal increases in I.I.com359 while plots of non-progressors exhibited negative slopes. We then examined the relationship between I.I.com359 measured at onset and the post-onset disease course. Among 19 placebo-treated subjects participating in the TrialNet CTLA4 Ig trial, a significant inverse relationship between baseline I.I.com359 and C-peptide AUC was observed at all time-points (p<0.05). Further, baseline I.I.com359 negatively correlated with the duration of the partial remission, defined as time to insulin dose-adjusted HbA1c > 9 for 21 local subjects (p=0.0001) and C-peptide AUC < 0.2 nmol/L for TrialNet subjects (p=0.016). In both cohorts, those with I.I.com359 above the median had significantly shorter partial remissions than those below the median (p≤0.01), suggesting that subjects with a higher inflammatory bias at onset will have accelerated β-cell decline. These studies suggest that I.I.com359 has utility in predicting both pre- and post-onset disease trajectory.
The development of intrathymic B cells in young, pre-insulitic NOD mice may abrogate the selection of insulin-reactive T cells.

Ana Pinto, Allison Green
University of York, York, UK

Abnormalities in the NOD thymus, and potentially man, are thought to contribute to Type 1 Diabetes (T1D) progression. Previously, we documented cellular changes in the thymus just prior to T1D development; the accumulation of thymic B cells. Here we show that, in comparison to non-T1D prone mice enhanced thymic B cell accumulation is apparent as early as the first two weeks of life, normalise to that of control mice by 4 weeks, with a second wave of enhanced accumulation at 12 weeks of age. Utilising unique RAG-GFP reporter NOD mice, we show this pattern of thymic B cell accumulation correlates with bimodal intrathymic B cell development, and for the earliest time point, increased homeostatic proliferation. Furthermore, at 4-6 weeks of age thymic B cells are increasingly follicular in phenotype correlating with higher levels of intrathymic IgG1 in comparison to age-matched control mice. Importantly, serum IgG1 levels in NOD mice were decreased with respect to control mice at this age. Histological investigations showed from 4-6 weeks of age, insulin-expressing medullary epithelial cells (mTECS) are detectable in the thymus and confocal microscopy suggests that thymic B cells potentially interact with membrane-bound insulin on the mTECS. Intriguingly, preliminary studies document that insulin-expressing mTECS are greatly reduced, almost undetectable, by 11 weeks of age. We will discuss the functional relevance of these unique thymic B cells in young mice and the potential for targeting them therapeutically to prevent T1D.
RIAM targeting disrupts immune synapses and prevents T cell-mediated diabetes.

Frederic LaGarrigue, Mark Ginsberg, Joseph Cantor
UCSD, La Jolla, CA, USA

Blockade of integrin-ligand binding can ameliorate a variety of autoimmune diseases. Unfortunately, this complete loss of integrin function has led to life-threatening mechanism-based toxicities. Preventing talin-dependent integrin activation is an alternative strategy that could theoretically reduce such toxicities. However, loss of talin-induced integrin activation in all tissues is lethal. RIAM is a Rap1 effector that enables the recruitment of talin to leukocyte integrins and supports activation of β2 integrins in a cell type-specific manner. We thus studied the role of RIAM in an adoptive transfer model for Type I diabetes, and report that RIAM expression in T cells is necessary for diabetes development. Loss of RIAM did not prevent lymphocyte homing to draining lymph nodes 24 hr after transfer, but was required for antigen-driven proliferation and cytotoxic killing. We also observed that RIAM was co-localized with talin and LFA-1 in immune synapses. Loss of RIAM strongly disrupted synapse formation of naïve T cells with APC, and of CTL with specific targets. Our data demonstrate the requirement of RIAM in immunological synapses and in consequent T cell functions in autoimmune disease. Since RIAM-deficient mice are healthy, fertile, and exhibit normal hemostasis, our data identifies RIAM and its regulators as targets for intervention in T cell mediated autoimmunity.
Peripheral Blood Gene Expression Biomarkers of Risk and Progression in Type 1 Diabetes

Linda Yip, Rebecca Fuhlbrigge, C. Garrison Fathman
Stanford University, Stanford, CA, USA

It is difficult to identify individuals who are at risk to develop Type 1 diabetes (T1D) due to the lack of overt symptoms prior to hyperglycemia. And, while T1D does occur in families, ~90% of new patients present without a family history. We examined gene expression of whole blood (PBCs) to ask if gene expression could be used to identify stable biomarkers of risk and/or progression. Samples of PBC RNA from T1D subjects, AA-positive and AA-negative first-degree relatives (FDRs) were provided by TrialNet, and PBC RNA from non-T1D-related controls was obtained locally (Stanford). Gene expression was measured by RNAseq and/or microarray, and candidate biomarker genes were validated by qPCR and NanoString arrays. The PBC gene expression profiles of T1D and T1D-related patients were found to be much more similar to each other than to non-diabetes-related controls, and a subset of these differentially expressed genes could distinguish AA- FDRs from healthy controls. We also identified a panel of genes that could distinguish AA+ FDRs who progressed to hyperglycemia from those who did not >3 years before onset. Many of these genes were similarly changed in a subset of AA- FDRs, suggesting that it may be possible to identify those FDRs who will progress to hyperglycemia years before onset, and possibly prior to the detection of AAs. When validated, these genes may serve as biomarkers of disease risk and progression, and allow T1D to be diagnosed earlier. This may provide additional time for intervention therapy before substantial beta cell damage has occurred.
Combined microarray and proteomic analysis reveals early abnormalities in islets from pre-diabetic NOD mice

Lut Overbergh1, Inne Crèvecoeur1, Valborg Gudmundsdottir2, Wannes D’Hertog1, Ana Carolina Fierro3, Leentje Van Lommel4, Conny Gysemans1, Kathleen Marchal5, Etienne Waelkens5,6, Frans Schuit4, Soren Brunak2,7, Chantal Mathieu1

1Laboratory for Clinical and Experimental Endocrinology, KU Leuven, Leuven, Belgium, 2Department of Bio and Health Informatics, Technical University of Denmark, Lyngby, Denmark, 3Department of Information Technology, IMinds, Faculty of Sciences, Ghent University, Ghent, Belgium, 4Gene Expression Unit, Department of Cellular and Molecular Medicine, KU Leuven, Leuven, Belgium, 5SyBioMa, KU Leuven, Leuven, Belgium, 6Laboratory of Protein Phosphorylation and Proteomics, KU Leuven, Leuven, Belgium, 7The Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, Copenhagen, Denmark

Type 1 diabetes (T1D) is an endocrine disease where a long pre-clinical phase, characterized by immune-cell infiltration in the islets of Langerhans, precedes elevated blood glucose levels and disease onset. Although several studies investigated the role of the immune system in this process called insulitis, the importance of the beta-cells themselves in the initiation of T1D is less understood. The aim of this study was to investigate which intrinsic abnormalities are present in the islets from diabetes-prone non-obese diabetic (NOD) mice before the onset of insulitis. For this purpose, the islet transcriptome and proteome of 2-3 week-old mice was investigated by microarray (N=4) and 2-Dimensional Difference Gel Electrophoresis (N=4), respectively, and subsequently analyzed by sophisticated pathway analysis and ranking of differentially expressed genes/proteins based on their T1D-relevance. We discovered that in the pre-insulitic period, alterations in general pathways related to metabolism and cell-communication are already present. Additionally, an important role for post-translational modifications in islets from NOD mice is suggested, especially citrullination by PAD2, since mRNA levels of this gene were 3.40 and 3.09 fold higher expression in NOD islets compared to NOR and C57Bl/6, respectively (p<0.05). Furthermore, our analyses indicate the susceptibility for protein misfolding in NOD islets because of low expression of protein disulfide isomerases (PDIA3, 4 and 6)(p<0.05 vs C57Bl/6). This might be one of the causative mechanisms to induce beta-cell stress and potential auto-antigen generation. We conclude that the pancreatic islets, irrespective of immune abnormalities, may contribute themselves to the initiation of the auto-immune process.
Glucose-regulated protein of 78kDa (GRP78) acts as a pro-apoptotic receptor on the surface of beta-cells

Saurabh Vig, Gudrun Marijsse, Inne Crèvecoeur, Dieter Rondas, Jef Serré, Chantal Mathieu, Lut Overbergh
Laboratory for Clinical and Experimental Endocrinology, KU Leuven, Leuven, Belgium

Type 1 diabetes (T1D) is characterized by immune infiltration in islets of Langerhans. Proinflammatory cytokines, secreted in the local beta-cell environment, trigger oxidative and endoplasmatic reticulum (ER) stress, with a prominent role for the chaperone GRP78. Our group showed that cytokines induce surface translocation of GRP78 in INS-1E cells, MIN6 cells and mouse islets. Here we aimed to investigate the mechanism of GRP78 translocation and the function of surface GRP78 (sGRP78) in beta-cells.

Incubation of MIN6 cells with golgicide A and brefeldin A inhibited cytokine-induced GRP78 translocation to the membrane (p<0.01), whereas immunohistochemistry showed co-localization of GRP78 with chromogranin A in cytokine-exposed MIN6 cells. Mass spectrometry analysis of sGRP78 interacting partners identified DNAJC3 as a potential binding protein to sGRP78. Its role as co-transporter for sGRP78 was confirmed by the reduced GRP78 translocation upon siRNA silencing of DNAJC3 in MIN6 cells (n=3). Evaluation of the downstream signaling function of sGRP78 was done by co-incubation of cytokine-exposed INS-1E cells with an antibody specifically directed against the COOH terminus of sGRP78. This partially protected INS-1E cells against cytokine-induced beta-cell death (p<0.05), associated with a decrease in pro-apoptotic CHOP, BAX and DP5 (p<0.05 for all) and an increase in anti-apoptotic Mcl1 (p<0.001) and eIF2α (p<0.01). We conclude that cytokines induce surface translocation of GRP78 mainly via the classical secretory pathway and once expressed on the beta-cell surface, GRP78 acts as a pro-apoptotic receptor. The present findings may be important for development of new therapeutics to protect beta-cells against inflammation-mediated destruction.
Field strains of Echovirus 6 infect human exocrine and endocrine pancreatic cells and induce pro-inflammatory innate immune responses

Luis Sarmiento1, Gun Frisk2, Mahesh Anagandula2, Monika Hodik2, Ilaria Barchetta3, Eitan Netanyahu1, Eduardo Cabrera-Rode4, Corrado Cilio1
1Lund University, Malmö, Skåne, Sweden, 2Uppsala University, Uppsala, Sweden, 3Sapienza University of Rome, Rome, Italy, 4National Institute of Endocrinology, Havana, Cuba

Human enteroviruses (HEV), especially Coxsackievirus B and Echovirus (E) have been associated to diseases of both the exocrine and endocrine pancreas, but so far evidence on HEV infection in human pancreas has been reported in islets and ductal cells. This study aimed to investigate the capability of HEV to infect primary human exocrine and endocrine pancreatic cells. Isolated human islets and exocrine cells were either mock-infected or inoculated with seven field isolates of E6. Beta-cell tropic strains of E16 and E30 were assayed in primary exocrine cells. Viral infection, replication, virus-induced cytopathic effect (CPE) and expression of innate immunity genes were measured. Infection of exocrine cells and islets with E6 caused CPE, virus titer increase and production of HEV protein VP1 in both cell types. Virus particles were found in E6-infected acinar cells, both free in cytoplasm and enclosed in vacuoles. Insulin granules accumulation in proximity to virus particles and beta cells functional impairment were demonstrated in E6-infected islets. No CPE or infectious progeny production were observed in exocrine cells exposed to E16 and E30. Exocrine and endocrine cells responded to E6 infection by upregulating the transcription of genes involved in viral recognition (IF1H1), antiviral defense (OAS1, IFN-β) and inflammation (CXCL10, CCL5). This study demonstrates for the first time that exocrine cells are permissive to in vitro HEV infection. Our results indicate that exocrine and endocrine pancreatic cells represent a target for E6 infection, further supporting the potential role of HEV in the etiopathogenesis of pancreatitis and type 1 diabetes.
Post-translational modification of GRP78 in human islets of Langerhans: implications for autoreactive T-cell activation

Inne Crèvecoeur¹, Gabriele Blahnik-Fagan², Ana-Ines Lalanne³, Fernanda Marques Camara Sodre³, David Arribas-Layton², Roberto Mallone³, Lut Overbergh¹, Eddie James², Chantal Mathieu¹

¹Laboratory for Clinical and Experimental Endocrinology, KU Leuven, Leuven, Belgium, ²Benaroya Research Institute, Seattle, WA, USA, ³DeAR Lab, INSERM U1016 Cochin Institute, Paris, France

Beta-cell destruction in islets of Langerhans, leading to the development of type 1 diabetes (T1D), is mediated by infiltrating T-cells. However, beta-cells also play an active role themselves in this process where loss of tolerance against beta-cell antigens can be induced by the generation of post-translational modifications (PTM). Our group previously showed that inflammatory stress induces citrullination of Glucose-regulated-protein 78 (GRP78) in INS-1E cells and mouse islets. Moreover, NOD mice had autoreactive T-cells and auto-antibodies against citrullinated GRP78.

In this study we aimed to translate previous findings to the human situation. Proteome analysis by 2-dimensional gel electrophoresis (2D-DIGE) of cytokine-exposed (IL-1β/IFNγ/TNFα, 50/1000/1000 U/ml) islets from healthy donors revealed post-translational modification of GRP78 in 3 out of 5 islet preparations, suggestive for citrullination. To investigate the presence of CD4+ and CD8+ T-cell responses in the circulation of T1D patients, a range of citrullinated and native GRP78 peptides were designed, with predicted strong binding for T1D predisposing HLA class-II (DR4/DQ8) and HLA class-I (A*0201). First in-vitro tetramer studies showed that one citrullinated GRP78 epitope was recognized by CD4+ T-cells in 3/6 patients and 0/6 healthy controls and GRP78 specific CD4+ T-cells could be visualized directly ex-vivo as well. Although these results need further confirmation, they suggest that human islets of Langerhans are prone to post-translational modification upon inflammation and that citrullinated GRP78 may be an autoantigen in human T1D. These findings may open the road for development of new diagnostic or therapeutic applications for patients with T1D.
Interleukin 27 is essential for the development of type 1 diabetes

Ashley Ciecko, Shamim Khaja, Kevin Mueller, Aron Geurts, Yi-Guang Chen
Medical College of Wisconsin, Milwaukee, WI, USA

Human genome wide association studies have identified a locus on chromosome 16 significantly linked to the development of type I diabetes (T1D). Within this region, IL27 has been proposed as the underlying gene. IL27 encodes the p28 subunit of the heterodimeric cytokine interleukin 27 (IL27), a member of the IL12 family. IL27 has been shown to have contrasting inflammatory and anti-inflammatory activities depending on the cell type, environmental context, and disease state. The unique pleiotropic effect of IL27 makes it an intriguing target in the pathogenesis of T1D. The focus of this study was to test the role of IL27 in the progression of T1D using the non-obese diabetic (NOD) mouse model. We created an IL27 knockout (KO) strain on the NOD background using the zinc-finger nuclease technology. In sharp contrast to wild-type NOD mice, the IL27 KO strain was completely protected from T1D. Additionally, histological examination of pancreatic sections revealed that IL27 KO mice had significantly less insulitis compared to the wild-type NOD control. Adoptive transfer of IL27 KO T cells into NOD.Rag1-/- recipients resulted in diabetes development, indicating that IL27 deficient mice still harbor diabetogenic T cells. We further demonstrated that activation of β-cell autoreactive CD8 T cells in the pancreatic lymph nodes was significantly reduced in IL27 deficient than in wild-type NOD mice. Our observations demonstrate that IL27 is required for diabetes development in the NOD mouse, and provide additional evidence to support its potential role in human T1D.
Metabolic pathways regulate T cell function including the differentiation of effector and regulatory T cells. However, whether there are specific metabolic requirements for tolerance induction has not been determined. The B6.SLE123 mouse is an animal model of lupus in which the autoimmune disease can be dampened by treatment with metabolic modulators, including metformin and 2-deoxyglucose. We have recently established that this animal model is completely resistant to tolerance induction in allogeneic islet transplantation even though it has no underlying anti-islet autoimmunity. We hypothesized that tolerance-inducing therapy with anti-CD45RB acts on key metabolic pathways to promote tolerance and that these pathways are resistant to therapy in B6.SLE123. Treatment with anti-CD45RB induced metabolic changes by downregulating glucose uptake and increasing mitochondrial activity in B6 CD4 T cells, changes that favor CD4 Treg action. SLE123 mice resisted these metabolic changes. We determined the mechanism of anti-CD45RB’s effects by linking treatment to activation of the LKB1/Ampka pathway leading to inhibition of AKT/mTOR signaling, a change that was also resisted by B6.SLE123 mice. We finally demonstrated that tolerance to allogeneic islets is improved in SLE123 mice by targeting both glycolysis and mitochondrial activity with 2-deoxyglucose and metformin in combination with anti-CD45RB. This study highlights the immune metabolism as a critical barrier to tolerance induction and reveals the LKB1/Ampka axis as a novel target for boosting T regulatory function and promoting transplantation tolerance.
Role of peripherally induced regulatory T cells in autoimmune diabetes

Cornelia Schuster, Stephan Kissler
Joslin Diabetes Center/Harvard Medical School, Boston, USA

In recent years, the importance of microbiota in modulating the risk of type 1 diabetes has become increasingly evident. Regulatory T cells (Tregs) are a key component of the immune system and have been implicated in autoimmune diabetes. A majority of Tregs develops in the thymus. However, a subset of Tregs, peripherally induced Tregs (pTregs), is generated in the gut from naïve T cell precursors in response to microbial stimuli and metabolites including short-chain fatty acids (SCFA). Even though microbiota have been shown to both modulate the risk of autoimmune diabetes and affect the frequency of pTregs, a link between pTregs and disease risk has not yet been explored directly.

The generation of pTregs is dependent on CNS1 (conserved non-coding sequence 1), a TGFβ-responsive enhancer in the promoter region for Foxp3, the key transcription factor of Tregs. Deletion of CNS1 in C57BL/6 mice significantly reduced pTregs, while thymus-derived Tregs were unchanged. We generated CNS1-deficient non-obese diabetic (NOD) mice using CRISPR-Cas9 genome editing. As predicted, CNS1 deletion significantly decreased the frequency of pTregs in NOD mice. With this unique model in hand, we are now evaluating the contribution of pTregs to autoimmune diabetes. Conversely, we are using short-chain fatty acids (SCFA) to boost pTreg numbers in NOD animals. Results from these studies will be presented to describe the possible role of pTregs in type 1 diabetes, and to discuss whether pTregs provide a functional link between microbial variation and the risk of autoimmune diabetes.
Using gold nanoparticles for the delivery of auto-antigenic peptide in the NOD mouse model of autoimmune diabetes

RK Singh1, SJ Hanna1, X Zhao2, L Wen3, SA Coulman2, JC Birchall2, MA McAteer4, CM Dayan1, FS Wong1

1Institute of Infection and Immunity, School of Medicine, Cardiff University, Cardiff, UK, 2School of Pharmacy and Pharmaceutical Sciences, Cardiff, UK, 3Section of Endocrinology, Yale School of Medicine, New Haven, USA, 4Midatech Pharma PLC, Milton park, Abingdon, UK

Antigen specific immunotherapy mediated via the sustained generation of regulatory T cells arguably represents the ideal approach to preventing beta cell destruction in type 1 diabetes. However, this approach has proved hard to translate into man in ways that are effective enough to alter the disease process. We therefore sought to optimise this process using ultra-small 3-5nm gold nanoparticles (GNP) to deliver auto-antigenic peptides into mouse skin. Using the non-obese diabetic (NOD) mouse model of autoimmune diabetes, we compared injection of peptide conjugated to GNP to peptide alone.

We showed that local delivery of nanoparticles coated with diabetes-relevant peptides altered peptide distribution in vivo compared to peptide delivered alone. Moreover, peptide-GNP displayed improved antigen presentation in both the skin-draining and distal lymph nodes to adoptively transferred diabetogenic BDC2.5 CD4 transgenic T cells, compared to peptide alone, which was presented only in the skin draining lymph node. Concurrent with this, donor cells displayed an increased activation profile, indicated by higher CD44 and CTLA4 expression. However, no change was noted in the levels of IFNγ or IL10 secreted in vivo. These biological effects appear independent of peptide, as both BDC-2.5 mimotope GNP and hybrid insulin peptides (HIPs) GNP displayed similar results.

Our results have demonstrated that auto-antigenic peptides bound to gold nanoparticles can be successfully delivered using an intradermal route, enhancing distribution and pharmacokinetics as well as peptide presentation. Further experiments will determine HIP GNP effects on diabetes incidence in mice susceptible to autoimmune diabetes.
Analgesic-antipyretic use in young children is not associated with risk for islet autoimmunity in the TEDDY study

Markus Lundgren¹, Leigh Johnson Steed², Roy Tammyra³, Berglind Jonsdottir¹, Patricia Gesualdo⁴, Claire Crouch⁵, Maija Sjöberg⁶, Gertie Hansson¹, William A Hagopian⁵, Anette G Ziegler⁷, Marian J Rewers⁴, Åke Lernmark¹, Jorma Toppari⁶, Jin-Xiong She², Beena Akolkar⁸, Jeffrey P Krascher³, Michael J Haller⁹, Helena Elding Larsson¹

¹Department of Clinical Sciences, Lund University, Malmö, Sweden, ²Center for Biotechnology and Genomic Medicine, Medical College of Georgia, Augusta university, Augusta, GA, USA, ³Health Informatics Institute, Morsani College of Medicine, University of South Florida, Tampa, FL, USA, ⁴Barbara Davis Center for Childhood Diabetes, University of Colorado, Aurora, CO, USA, ⁵Pacific Northwest Diabetes Research Institute, Seattle, WA, USA, ⁶Departments of Physiology and Pediatrics, University of Turku and Turku University Hospital, Turku, Finland, ⁷Institute of Diabetes Research, Helmholtz Zentrum München, and Klinikum rechts der Isar, Technische Universität München, and Forschergruppe Diabetes e.V, Neuherbeg, Germany, ⁸National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, USA, ⁹Department of Pediatrics, University of Florida, Gainesville, FL, USA

Objective: We aimed to determine if analgesic-antipyretic (ANAP) use before 2.5 years of age is associated with risk for islet autoimmunity (IA) at 3 and 6 years of age across the clinical centers of The Environmental Determinants of Diabetes in the Young (TEDDY) study.

Methods: In 8542 children time to antibody seroconversion, in relation to use of ANAP up to 30 months of life, was analyzed using a Cox proportional hazards model with country as stratification factor, number of infections as secondary time dependent covariate, and cumulative analgesic use as primary time dependent covariate of interest. Hazard ratios were estimated for cumulative use of acetaminophen, NSAIDs and total analgesic use with or without concomitant fever.

Results: ANAP use before age 2.5 years was not associated with risk for IA at age 3 years (HR 1.02; 95% CI 0.99-1.05; p=0.130) or at 6 years (HR 1.02; 95% CI 0.99-1.04; p=0.267). However, a small increase in the risk of IA at 3 years of age was seen with use of acetaminophen with concomitant fever (HR 1.05; 95% CI 1.01-1.09; p=0.022), only observed in US when examined in country specific analyses. No other significant correlations with IA could be found for Acetaminophen or NSAID use with or without concomitant fever.

Conclusion: ANAP use in young children is not associated with seroconversion to IA by age 3 and 6 years. The weak correlation between acetaminophen use for fever and IA at 3 years in the US cohort, may be secondary to an infection.
Altered Treg fitness in Type 1 Diabetes – the Treg gene signature as a biomarker for autoimmune activity

Anne M Pesenacker1,2, Jana Gillies1,2, Virginia Chen3, Amrit Singh3,1, Constadina Panagiotopoulos1, Cate Speake4, Scott J Tebbutt1,3, Megan K Levings1,2

1University of British Columbia, Vancouver, Canada, 2BC Children’s Hospital Research Institute, Vancouver, Canada, 3PROOF Centre of Excellence, Vancouver, Canada, 4Benaroya Research Institute, Seattle, USA

Regulatory T cells (Tregs) normally maintain immune tolerance, but fail in type 1 diabetes (T1D) resulting in attack and killing of insulin-producing cells by autoreactive T cells. Many new T1D treatments aim to stop the inflammatory response and boost Treg function; however a major challenge is reliably measuring changes in Treg function before and after treatment. We used an integrative genomic approach to define a Treg gene-signature, which discrimimates between Tregs and conventional T cells regardless of their activation state. We also found that Tregs from the blood of children with new onset T1D have a significantly different gene signature from that of Tregs from healthy age-matched controls. In order to validate these findings, we obtained PBMCs from a cross-sectional cohort of adults with T1D, sorted Tregs as CD4+CD25hiCD127lo cells, and measured the Treg gene signature using nanoString. We have now confirmed that the Treg signature is altered in an adult cohort with T1D and significantly different from age- and sex-matched controls, in particular when incorporating genetic variants linked to T1D and Treg function (CD25, PTPN2, PTPN22). In ongoing bioinformatic analysis, we are developing algorithms that incorporate c-peptide levels, and demographic data to further refine predictive power of the biomarker test. We are assessing data from sorted Tregs and unfractionated peripheral blood mononuclear cells in parallel.

In conclusion, this work might ultimately lead to a clinically applicable biomarker that can be used to monitor changes in Tregs during disease progression and gauge the success of new Treg-targeted treatments.
Herein we characterize the APCs in pancreatic islets during the initiation stage of autoimmune diabetes in the NOD mouse. The resident APC within the islet of all mouse strains is a macrophage. The macrophage resides within the islets since birth and self-replicates throughout the life of the mouse. The islet macrophage in all strains subsists in a state of activation with high expression of MHC-II molecules. Their expression pattern is compatible with an M1 activation profile. In contrast, the macrophages of the pancreatic stroma display an M2-profile. The islet macrophages capture insulin-containing granules exocytosed by Beta cells. They are then capable of presenting peptides derived from insulin to CD4+ T cells. Unique to the islets of the NOD strain is the presence of DCs belonging to the CD103+ lineage dependent upon the Batf3 transcription factor. Although NOD.Rag1-/- mice have a small number of CD103+ DC in islets, their number markedly augments in NODs contemporaneously with the appearance of islet infiltrating CD4+ T cells. This occurs at approximately weaning age. Diabetes initiation and progression is dependent upon the CD103+ DC lineage. The absence of these cells in the NOD.Batf3-/- mouse renders the islet untouched by autoimmune infiltration. Diabetes never develops, the islets are free of T cells, there is complete absence of CD8+ T cell activation, and they exhibit reduced CD4+ T cell priming. Their gene transcripts are very similar to those of the NOD.Rag1-/- mouse. The defect in the knockout mouse can be corrected by replacing the CD103+DC lineage.
Gestational respiratory infections and birth weight show a genetic dependent association with risk of islet autoantibodies in the TEDDY study

Kristian Lynch1, Hye-Seung Lee1, Carina Torn2, Jeffrey Krischer1, Kendra Vehik1, Beena Akolkar3, Marian Rewers4, William Hagopian5, Jin-Xiong She6, Olli Simell7, Jorma Toppari8, Anette Ziegler9, Ake Lernmark2, for the TEDDY Study3

1Health Informatics Institute, Department of Pediatrics, Morsani College of Medicine, University of South Florida, Tampa, FL, USA, 2Department of Clinical Sciences, Lund University, Malmo, Sweden, 3National Institute of Diabetes & Digestive & Kidney Diseases, Bethesda, MD, USA, 4Barbara Davis Center for Childhood Diabetes, University of Colorado, Aurora, CO, USA, 5Pacific Northwest Diabetes Research Institute, Seattle, WA, USA, 6Center for Biotechnology and Genomic Medicine, Medical College of Georgia, Augusta University, Augusta, GA, USA, 7Department of Pediatrics, Turku University Hospital, Turku, Finland, 8Departments of Physiology and Pediatrics, University of Turku and Turku University Hospital, Turku, Finland, 9Institute of Diabetes Research, Helmholtz Zentrum München, and Klinikum rechts der Isar, Technische Universität München, and Forschergruppe Diabetes e.V., Neuherberg, Germany

Birth weight (BW) and gestational respiratory infections (G-RI) are reported to affect the risk of islet autoantibodies (IA) and childhood type 1 diabetes (T1D), however results across studies have been conflicting. We have previously reported that the age and order of the first appearing islet autoantibody (IA) was distinctly characterized by HLA-DQ genotypes. The first appearing IA was predominately IAA with HLA-DQ8 among younger children (<3 years) and GADA with HLA-DQ2 among older. Therefore we aimed to examine whether G-RI and BW were related to IAA-only and GADA-only. Effect modification by PTPN22 (rs2476601), INS (rs689), and CTLA4 (rs231775) were also examined. Characteristics during pregnancy were obtained from a questionnaire to 7301 non-diabetic mothers of singleton infants in TEDDY. Proportional hazard modeling examined factors related to IAA-only and GADA-only up to age 6 years. Overall, G-RI and BW were not associated with IAA-only or GADA-only. However, CTLA4 significantly modified how G-RI related with risk of IAA-only (p=0.004); and BW with risk of GADA-only (p=0.04). Among children with CTLA4-G (n=4414/6407, 69%), G-RI was associated with lower risk of IAA-only (HR=0.64, p=0.02). The risk of GADA-only (HR=1.43, p=0.02) was increased per kg of BW. Among CTLA4-AA children (n=1993/6407, 31%), G-RI and BW showed the opposite trend with risk of IAA-only (HR=1.52, p=0.10) and GADA-only (HR=0.64, p=0.17) respectively. The role of gestational respiratory infections and birth weight on islet autoantibodies in young children may depend on CTLA4 alleles, which is consistent with the possible role of CTLA4 to maintain a normal pregnancy.
Cumulative Life Stress is Associated with Multiple Islet Autoantibodies Appearing Close Together Over Time

Kristian Lynch1, Suzanne B. Johnson2, Ake Lernmark3, Roswith Roth4, Beena Akolkar5, William Hagopian6, Marian Rewers7, Jin-Xiong She8, Anette-G Ziegler10, for the TEDDY Study5

1Health Informatics Institute, Department of Pediatrics, Morsani College of Medicine, University of South Florida, Tampa, FL, USA, 2Department of Behavioral Sciences and Social Medicine, Florida State University College of Medicine, Tallahassee, FL, USA, 3Department of Clinical Sciences, Lund University, Malmö, Sweden, 4Helmholtz Center Munich, Germany; Institute for Psychology, Graz University, Graz, Austria, 5National Institute of Diabetes & Digestive & Kidney Diseases, Bethesda, MD, USA, 6Pacific Northwest Diabetes Research Institute, Seattle, Seattle, WA, USA, 7Barbara Davis Center for Childhood Diabetes, University of Colorado, Aurora, CO, USA, 8Center for Biotechnology and Genomic Medicine, Medical College of Georgia, Augusta University, Augusta, GA, USA, 9Departments of Physiology and Pediatrics, University of Turku and Turku University Hospital, Turku, Finland, 10Institute of Diabetes Research, Helmholtz Zentrum München, and Klinikum rechts der Isar, Technische Universität München, and Forschergruppe Diabetes e.V., Neuherberg, Germany

Stress may initiate or accelerate the development of islet autoantibodies (IA) and type 1 diabetes (T1D). The Environmental Determinants of Diabetes in the Young (TEDDY) study follows the highest genetically at-risk children for these outcomes. The link between negative life events (NLE) and IA in children followed until age 6 years (n=7869) was examined in a Cox regression time dependent analysis. Life stress events in the child were collected by parent report every 3 months until age 4 and biannually thereafter. IA was defined as the appearance of one or more autoantibodies against either insulin, GAD65 or IA-2 persistent at two visits 3 months apart. Previously we reported 10% of children experienced consistently high rate of NLEs from 3 months of age (≥ 1 NLE/year). This high cumulative NLE rate was associated with higher risk for IA compared to children with a lower cumulative NLE rate but only for HLA-DR3/4 children (HR=1.69, p=0.003). We have shown the order of appearance of autoantibodies is related to HLA-DR. Thus, life stress was further examined for association with type of first appearing IA. Of the 540/7869 (6.8%) who seroconverted, 38.5% had GADA-only, 43.3% IAA-only, 1.7% had IA-2A-only and 16.5% had multiple IA. After accounting for factors associated with IA; high cumulative NLE rate was associated with multiple IA (HR=2.36, p=0.004) but not with a single IA (GADA-only, HR=1.21, p=0.41; IAA-only, HR=0.81, p=0.40). Cumulative child life stress appears to increase the risk of multiple IA appearing close together over time near initial seroconversion.
Interim analysis of UST1D: A pilot clinical trial of ustekinumab in recent-onset Type 1 Diabetes Mellitus.

Ashish Marwaha, Tom Elliott, Laura Cook, Sabine Ivison, Annika Sun, Marla Inducil, Megan Levings, Rusung Tan, Jan Dutz
University of British Columbia, Vancouver, Canada

Preclinical studies suggest that T1D auto-inflammation is suppressed by blockade of pro-inflammatory T cells that secrete IL-17/IFN-γ. We assessed the safety and optimal dosing of ustekinumab (a biological targeting the IL-17/IFN-γ pathway) in a phase I/II open-label clinical trial (NCT02117765) in adults with recent-onset T1D.

We enrolled 10 patients within 100 days of T1D diagnosis, aged 18-35 and with a peak C-peptide of 0.2nmol/l or greater on MMTT. Subjects received a loading dose at 1 month followed by either 45mg or 90mg ustekinumab every 3 months. The primary endpoint was safety (rate, frequency and severity of adverse events). We also measured the baseline-adjusted change in 2-h AUC C-peptide response to MMTT at 1 year and performed flow cytometric analyses before and after ustekinumab administration to assess changes in T helper cell subsets.

Ustekinumab-treated patients had 10 adverse events total (1/10 was possibly attributed to study drug). At 1 year, the 90mg cohort had a mean reduction in C-peptide AUC of 0.1pmol/mL and after one dose of ustekinumab demonstrated a mean 50% reduction in circulating Th17.1 cells. The 45mg cohort had a mean C-peptide AUC reduction of 0.26pmol/ml and no changes in Th17.1 cells.

We confirm the safety of ustekinumab in the context T1D and suggest that a 90mg dosing regime is optimal for reducing Th17.1 cells and providing better preservation of C-peptide levels at 12 months. This pilot provides a rationale to proceed to a phase II/III study to test the efficacy of ustekinumab treatment in children with recent-onset T1D.
Increased numbers of CD68+ cells of the exocrine pancreas in slowly progressive type 1 diabetes

Tomoyasu Fukui1,2, Tetsuro Kobayashi2, Erika Jimbo2, Kaoru Aida3, Soroku Yagihashi4, Akira Shimada5
1Division of Diabetes, Metabolism and Endocrinology, Department of Medicine, Showa University School of Medicine, Tokyo, Japan, 2Division of Immunology and Molecular Medicine, Okinaka Memorial Institute for Medical Research, Tokyo, Japan, 3Third Department of Internal Medicine, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Yamanashi, Japan, 4Department of Pathology and Molecular Medicine, Hirosaki University Graduate School of Medicine, Hirosaki, Japan, 5Saitama Medical University of Hospital, Saitama, Japan

It remains unknown how inflammatory infiltration of the exocrine pancreas contributes to the pathogenesis of type 1 diabetes. We examined the correlations between immune cells of exocrine tissue and β-cell dysfunction in slowly progressive type 1 diabetes (SPIDDM). We determined the insulitis frequency, infiltrating leukocyte subtypes, and β-cell mass in pancreata from 1 biopsied and 11 autopsied SPIDDM patients (42-87 years old, 0.3-24 years of disease) and 19 age-matched pancreata from non-diabetic individuals. All SPIDDM cases were positive for GAD autoantibodies (GADAb) by the RIA method. Patients with high and low titers of GADAb (>10 U/ml, <10 U/ml) were assigned to a High-GADAb-Titer Group and Low-GADAb-Titer Group. The pancreatic weight and β-cell weight were significantly lower in SPIDDM than in the non-diabetic individuals. Fasting serum C-peptide levels were significantly correlated with the β-cell weight but negatively correlated with patient age. The SPIDDM exocrine pancreas showed increases in mainly the numbers of CD68+ cells and CD8+ cells, together with a pathological finding of chronic pancreatitis. The numbers of CD68+ cells of the exocrine pancreas were significantly negatively correlated with the β-cell mass. The β-cell mass was significantly lower in the High-GADAb-Titer group than in the Low-GADAb-Titer Group. The insulitis frequency showed no correlations with age, duration of diabetes, numbers of exocrine immune cells, remaining insulin containing islets, or titer of GADAb. Increased numbers of CD68+ cells of the exocrine pancreas, patient age, and islet autoimmunity may be pathogenic factors associated with declining β-cell function in SPIDDM.
Type 1 diabetes (T1D) results from autoimmune destruction of insulin-producing pancreatic β cells, involving CD4+ and CD8+ T cells. Non-obese diabetic (NOD) mice are a model for human T1D. As islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP) is a major diabetogenic antigen in NOD mice, we developed a model to test IGRP antigen-specific immunotherapy. We showed previously that liposomes co-encapsulating antigen and NF-κB inhibitor induced antigen-specific suppression of CD4+ T cells in mice. These liposomes passively target and deliver their payload to dendritic cells in lymphoid organs, suppressing NF-κB activation.

For the current model, we injected NOD mice with liposomes co-delivering IGRP206-214 peptide and NF-κB inhibitor. After adoptive transfer of CTV-labelled IGRP-specific CD8+ T cells, we analyzed proliferation in lymphoid organs, enumerated adoptively-transferred and endogenous antigen-specific T cells using Kd-IGRP tetramers, and measured IFN-γ effector function by intracellular staining ex vivo. To induce antigen-specific T cell activation, mice were immunized with IGRP206-214 peptide and adjuvant. 5 days after immunization, IGRP-specific CD8+ T cells dramatically expanded and production of IFN-γ increased. Twenty four hours after liposome injection to naïve mice, we observed uptake of liposomes specifically by antigen presenting cells (APCs). Delivered peptide was presented by these APCs as CTV-labelled IGRP-specific CD8+ T cells proliferated in draining lymph nodes after subcutaneous delivery of liposomes co-encapsulating IGRP206-214 and NF-κB inhibitor.

These data establish a model in NOD mice for analysis of diabetogenic antigen presentation and response to antigen-specific liposome immunotherapy to complement studies of immunotherapy to prevent or treat diabetes.
Interleukin-22 Therapy in Murine Models of Spontaneous Diabetes

Danielle Borg, Hui Tong, Michael McGuckin, Sumaira Hasnain
Mater Research Institute- The University of Queensland, Translational Research Institute, Brisbane, QLD, Australia

We have shown that IL-22 directly reduces pancreatic β-cell oxidative and endoplasmic reticulum (ER) stress and restores glucose homeostasis in obese hyperglycaemic mice. During the development of autoimmune type 1 diabetes, ER stress/protein misfolding may promote neo-antigen presentation, and subsequently autoimmune T cells produce cytokines that drive oxidative and ER stress contributing substantially to β-cell dysfunction and death. We hypothesised that reducing β-cell stress with IL-22 would prevent or delay the onset of autoimmune diabetes in mice. Six-week old female NOD mice were randomised to receive either vehicle control (n=15; PBS) or recombinant mouse IL-22 (n=10; 200ng/g) i.p. bi-weekly until overt diabetes was confirmed via two consecutive non-fasted blood glucose measurements >11mM. At this time, mice in the vehicle arm were treated with Linbit insulin pellets and randomised to either bi-weekly therapeutic injections of PBS (n=6) or IL-22 (n=5; 200ng/g i.p.) and followed until re-emergence of diabetes (defined as above). Prophylactic IL-22 administration failed to prevent diabetes with 80% of mice in the IL-22 and PBS-treated groups developing overt diabetes, and no delay in time to diabetes (p=0.82, Log-rank test). In the therapeutic intervention arm, IL-22 failed to significantly prolong median time to diabetes progression (vehicle: 35 days, 95%CI 6-47 days vs IL-22: 39 days, 95%CI 6-54 days; p=0.46, Log-rank test). Thus, with this dosing schedule, IL-22 therapy given either pre-diabetes or at diabetes diagnosis could not suppress either the genesis of autoimmunity or mechanisms of autoimmune β-cell dysfunction and destruction involved in NOD autoimmune diabetes.
Development of a novel Treg micro-suppression assay for use in clinical trial monitoring

Jennie Yang1,2, Shereen Sabbah1,2, Timothy Tree1,2

1Department of Immunobiology, Faculty of Life Sciences & Medicine, King’s College London, London, UK, 2National Institute of Health Research Biomedical Research Centre at Guy’s and St. Thomas’ National Health Service Foundation Trust and King’s College London, London, UK

There is strong evidence that defective function of CD4+FOXP3+ regulatory T cells (Treg) may play a role in the development of human type 1 diabetes (T1D) and strengthening immunoregulation by invigorating Treg is a major area of trial activity in T1D.

Although there is an ever-increasing number of assay formats to assess both the frequency and phenotype of Tregs, the in vitro co-culture suppression assay is still considered by many to be the ‘gold standard’ assessment reflecting the key role performed by Tregs in vivo such as suppression of effector T cell (Teff) function. However, this assay typically requires large volumes (>25mL) of fresh blood and is labor intensive, increasing sample variability and reducing the number of samples that can be processed simultaneously, limiting its utility in clinical trial monitoring.

We have developed a robust, high-throughput micro-suppression assay that has low sample requirement (2mL whole blood or <5x10⁶ PBMC) and can measure suppression under a variety of conditions (multiple Teff:Treg ratios and different stimulation conditions). The assay is high throughput (>20 assays/day) and can use both fresh and cryopreserved PBMC. The assay has similar characteristics and gives comparable results to those from standard suppression assays. Using this assay to assess suppression in matched whole blood, fresh and cryopreserved PBMC, we observed a high degree of correlation of suppression between samples from the same individual (P(range)=8x10⁻⁶-0.013, r²(range)=0.67-0.87). We believe this improved method is useful for examining Treg suppression in samples from clinical trials and longitudinal studies where starting material is limited.
STIMULATED URINE C-PEPTIDE CREATININE RATIO VERSUS SERUM C-PEPTIDE FOR MONITORING OF BETA-CELL FUNCTION IN THE FIRST YEAR AFTER DIAGNOSIS OF TYPE 1 DIABETES

Danijela Tatovic¹, Yuk-Fun Liu², Mohammad Alhadj Ali¹, Stephen Luzio³, Gareth Dunseath³, Mark Peakman², Colin Dayan¹
¹Cardiff University, Cardiff, UK, ²King's College London, London, UK, ³Swansea University, Swansea, UK

Background:
Mixed-meal stimulated urine C-peptide/creatinine ratio (UCPCR) has been suggested as a less invasive alternative to standard Mixed-Meal Tolerance Test (MMTT) in assessing beta-cell function. There are limited data comparing these two measures soon after diagnosis of type 1 diabetes (T1D).

Methods:
This randomized, placebo-controlled immunomodulatory trial involved 27 people within 3 months from T1D diagnosis. The participants received intradermal injections of proinsulin peptide C19-A3 every 2 (high-frequency) or 4 weeks (low-frequency) for 6 months. Mixed-meal stimulated UCPCR and serum C-peptide concentration from MMTT (area under the curve) were compared at 0, 3, 6, 9 and 12 months.

Results:
A significant decline in serum (-54.7% after 3 months and onwards) and urine C-peptide (-45.4% after 9 months and onwards) compared to baseline was detected in the placebo group. By contrast, serum C-peptide did not significantly change in the treatment groups, and UCPCR remained unchanged in both treatment groups for 9 months, with a significant decline from baseline at 12 months in the low-frequency group. Serum and urine C-peptide correlated poorly with insulin dose adjusted HbA1c (IDAA1c) and with each other in the first 6 months; the correlations improved in the second half of the follow-up.

Conclusion:
Serial UCPCRs are comparable to standard MMTT in differentiating change in beta-cell function in the first year after T1D diagnosis.

Poor correlation of both methods with IDAA1c in the first 6 months may reflect the effect of beta-cell stress on insulin production and warrants consideration of alternative outcome measures early after diagnosis.
Differences in immune genes expression in the peripheral blood of patients with childhood- and adulthood-onset newly diagnosed type 1 diabetes (T1D)

Raivo Uibo, Katrin Pruul, Kristi Alnek, Kalle Kisand
University of Tartu, Tartu, Estonia

It has been suggested that T1D is a heterogeneous disorder with possible differences in pathogenesis between young-onset and adult-onset cases. In present study we aimed to characterize the activation events in peripheral blood cells collected from recent-onset T1D patients at different ages. For this purpose the expression of immunoregulatory/costimulatory genes in blood cells from 67 T1D patients and 61 controls were analysed in the material collected from Tartu University Hospital, Tallinn Children’s Hospital and from healthy volunteers. The CD25, CD80, CD86, CD226, CTLA4, BTLA, PDCD1, FOXP3, and ICOS expression was evaluated by TaqMan or SYBRGreen assays. In addition, peripheral blood cytokines (n = 20) were studied by the xMAP Technology on Luminex 200. The results were compared with background demographic, genetic polymorphism (HLA, CTLA4, PTPN22, CD226) and immunological (antibodies) data. Our results showed that CD80 gene had higher expression and ICOS gene lower expression in young-onset T1D blood cells, whereas CD86 gene had higher expression in adult-onset T1D blood cells compared to that in age-matched controls. Our study results support the idea that young-onset and adult-onset T1D may have differences in the immunoregulatory/costimulatory pathways. This fact must be taken into account when developing tools for immunomodulatory treatment in patients with T1D.

The study was supported by Estonian Research Council grant IUT20-43 and by European Union Regional Development Fund.
Serum Fatty Acid Composition and Milk Feeding Type in Infancy Are Associated With Risk of Islet Autoimmunity

Sari Niinistö1, Hanna-Mari Takkinen1,2, Iris Erlund1, Suvi Ahonen1,2, Jorma Toppari3,4, Jorma Ilonen3, Riitta Veijola5, Mikael Knip6, Outi Vaarala6, Suvi M. Virtanen1,2

1National Institute for Health and Welfare, Helsinki, Finland, 2University and University Hospital of Tampere and the Science Center of Pirkanmaa Hospital District, Tampere, Finland, 3University of Turku, Turku, Finland, 4Turku University Hospital, Turku, Finland, 5University of Oulu, Oulu, Finland, 6University of Helsinki and Helsinki University Central Hospital and Folkhälsan Research Center, Helsinki, Finland

We analyzed the association between early serum fatty acid composition and milk feeding, and risk of primary insulin autoimmunity in a case-control series nested in the Finnish Type 1 Diabetes Prediction and Prevention (DIPP) birth cohort carrying HLA-conferred susceptibility to type 1 diabetes (n=7782). Primary insulin autoimmunity was defined as the appearance of insulin autoantibodies as the first autoantibody without any other concomitant islet autoantibodies (ICA, GAD, IA-2) and later development of repeated positivity for at least ICA. Serum total fatty acid composition was analyzed at 3 and 6 months of age in 43 cases with primary insulin autoimmunity and in 86 islet autoantibody negative controls matched for sex, HLA-conferred risk, birth time and region. Statistical analysis was done using conditional logistic regression and multiple comparisons considered by false discovery rate (FDR). Serum fatty acid composition differed between breastfed and non-breastfed infants, reflecting differences in fatty acid composition of milk feeding. High serum docosapentanoic (DPA), docosahexaenoic (DHA), palmitoleic, cis-vaccenic and arachidonic acid, and breast milk were associated with decreased and high serum alphalinoelic acid and cow's milk with increased risk for primary insulin autoimmunity. E.g., high serum DPA at 3 and 6 months was associated with decreased risk of the endpoint: OR=0.35 (95% CI 0.17-0.74, p=0.006, FDR=0.030) and OR=0.23 (95% CI 0.09-0.56, p<0.001, FDR=0.030), respectively. In conclusion, early fatty acid status and milk feeding type are associated with the development of primary insulin autoimmunity. High fish fatty acid status (DPA, DHA) in infants may be protective against this endpoint.
MiR-409-3p, an IFN-γ regulating miRNA, is reduced in plasma and in islet infiltrating cells of new-onset diabetic NOD mice and in plasma of recent onset type 1 diabetic patients, representing a candidate circulating biomarker of autoimmune diabetes

Giuliana Ventriglia1, Guido Sebastiani1, Francesca Mancarella1, Dana Cook2, Conny Gysemans2, Chantal Mathieu2, Francesco Dotta1

1Diabetes Unit Department of Medicine, Surgery and Neuroscience, University of Siena, Siena, Italy; Umberto Di Mario Foundation ONLUS, Toscana Life Sciences, Siena, Italy; 2Laboratory of Clinical and Experimental Medicine, KU LEUVEN, Leuven, Belgium

MicroRNAs (miRNAs) are small non-coding RNAs, which negatively regulate post-transcriptionally the expression of their target genes. Despite their intracellular role, miRNAs can also be secreted into the extracellular space, while being stably detected in biofluids like plasma, thus representing a promising class of biomarkers for diagnosis and prognosis. In order to identify potential biomarkers of type 1 diabetes onset we analyzed the expression of 384 miRNAs in the plasma of new-onset diabetic NOD mice (n=5; 12-22 weeks of age) and age-matched normoglycemic NOD mice (n=5). Interestingly, circulating miR-409-3p, a miRNA that regulates the expression of interferon-gamma (IFN-γ), was significantly lower (p<0.001) in new-onset diabetic NOD mice compared to age-matched disease non-progressor normoglycemic mice. Analysis of laser capture microdissected (LCM) islet-endocrine tissue and lymphocytic infiltrates revealed a reduction of miR-409-3p in the insulitic lesion of new-onset diabetic mice compared to normoglycemic mice, suggesting that the differential expression of circulating miR-409-3p mirrors that of islets-infiltrating cells. Moreover, expression levels of IFN-γ, a miR-409-3p target gene, were upregulated in LCM-islet lymphocytic infiltrates from new-onset diabetic mice compared to normoglycemic mice, showing a miR-409-3p/IFN-γ inverse expression pattern, typical of miRNA-target gene relationship. In addition, the analysis of human plasma samples revealed downregulation of miR-409-3p (p<0.05) in recent type 1 diabetic patients versus age-matched control subjects. In conclusion, miR-409-3p may represent a valid circulating candidate biomarker of autoimmune diabetes, possibly mirroring the in-situ conditions and likely to also be involved in the pathogenesis of diabetes as a direct regulator of IFN-γ expression.
Biomarkers for diabetes reversal in NOD mice by clinical-grade pro-insulin and IL10 secreting Lactococcus lactis in combination with low-dose anti-CD3

Dana P Cook¹, Tatiana Takiishi¹, Hannelie Korf¹, Guido Sebastiani², Francesca Mancarella², João Paulo Monteiro Carvalho Mori Cunha¹, Clive Wasserfall³, Noelia Casares⁴, Juan José Lasarte⁴, Lothar Steidler⁵, Pieter Rottiers⁵, Francesco Dotta², Conny Gysemans¹, Chantal Mathieu¹

¹Laboratory of Clinical and Experimental Endocrinology, KULeuven, Leuven, Belgium, ²Diabetes Unit, Department of Internal Medicine, Endocrine and Metabolic Sciences and Biochemistry, University of Siena and Fondazione Umberto Di Mario ONLUS, Toscana Life Science Park, Siena, Italy, ³UF Department of Pathology, Immunology and Laboratory Medicine, College of Medicine, Gainesville, Florida, USA, ⁴Gene Therapy and Hepatology Area, University of Navarra, Pamplona, Spain, ⁵Intrexon ActoBiotics NV, Zwijnaarde, Belgium

Introduction of beta-cell auto-antigens via the gut through genetically-modified Lactococcus lactis (LL) has been demonstrated to be a promising approach for diabetes reversal in NOD mice. In this study we show that a combination of low-dose anti-CD3 with a “clinical-grade” self-containing LL, appropriate for human application, secreting human pro-insulin and IL10 cured 66% of new-onset diabetic NOD mice, comparable to plasmid-driven LL. Diabetes reversal correlated strongly with residual beta-cell mass at therapy initiation and reactivity to the therapeutic autoantigen. Initial blood glucose concentrations below 350 mg/dL and pre-therapy insulin autoantibody (IAA) positivity increased therapy efficacy to 89%, while absence of both biomarkers reduced efficacy to 33%. A decline in IAA positivity was also an immune biomarker of therapeutic outcome. Assessment of the immune changes induced by the LL-based therapy revealed elevated frequencies of CD4⁺Foxp3⁺ T cells in pancreatic draining lymph nodes, pancreas, and peripheral blood of all treated mice, independent of metabolic outcome. Neutralization of CTLA4 and TGF-β, but not IL10, partially abrogated the suppressive function of therapy-induced Tregs. Ablation of FOXP3⁺ Tregs in vivo in therapy-cured NOD.FOXP3.DTR mice broke tolerance and lead to disease recurrence, indicating their necessity for maintenance of tolerance. Furthermore, co-administration of a 15-mer synthetic peptide inhibitor of FOXP3 with therapy at diabetes onset prevented restoration of normoglycemia. In summary, the immune and metabolic biomarkers identified in this study can potentially be used in the future to tailor the LL-based combination therapy for individual type 1 diabetes patients.
Reduction in neutrophil counts are related to the number of islet autoantibodies in TEDDY children at increased genetic risk for type 1 diabetes

Falastin Salami¹, Eva Freyhult², Helena Elding Larsson¹, Åke Lernmark¹, The TEDDY Study group³

¹Department of Clinical Sciences, Lund University/CRC, Skåne University Hospital, Malmö, Sweden, ²Department of Medical Sciences, National Bioinformatics Infrastructure Sweden, Science for Life Laboratory, Uppsala University, Uppsala, Sweden, ³Data Coordinating Center, Tampa, FL, USA

The possible importance of innate immune cells in the pathogenesis of type 1 diabetes (T1D) remains to be clarified. A reduction in peripheral blood neutrophils was previously reported in healthy first degree relatives with islet autoantibodies. Nearly 90% of newly diagnosed T1D patients do not have a first degree relative with the disease. Children enrolled in the TEDDY study have increased genetic risk of T1D and 89% of them are from the general population. In the present study we used complete blood count (CBC) to test if any of the leukocyte populations in the peripheral blood of TEDDY children was statistically related to islet autoantibodies.

CBC was determined for 379 Swedish TEDDY children (age 4-11 years); 324/379 (85%) were negative and 55/379 (15%) positive for one or several of autoantibodies against insulin, GAD65, IA-2, or ZnT8.

The white blood cell count (p=0.043) was reduced in children with autoantibodies due to a reduction in neutrophil count (p=0.006) while lymphocytes (p=0.819), eosinophils (p=0.421), monocytes (p=0.066) and basophils (p=0.767) were unaffected. The reduction in neutrophil count increased with an increasing number of autoantibodies (p=0.008) and it was more common among children with the HLA-DQ2/8 genotype (p=0.015) and among boys (p=0.011).

It is concluded that the reduction in neutrophil counts associated with an increasing number of autoantibodies may contribute to the pathogenesis by increasing the autoimmune attack on the beta cells, by increasing the risk for infection, or by some other process that accelerates the T1D disease process.
Pandemic Influenza A H1N1 Vaccination and subsequent Risk of Type 1 Diabetes in Norway

Lars C Stene¹, Paz LD Ruiz¹,², Hanne L Gulseth¹,², German Tapia¹, Inger J Bakken¹, Siri E Håberg¹
¹Norwegian Institute of Public Health, Oslo, Norway, ²Oslo University Hospital, Oslo, Norway

During the 2009-2010 influenza pandemic, the whole population in Norway was offered an AS03-adjuvanted influenza A(H1N1)pdm09 vaccine (Pandemrix) free of charge, and >30% of the Norwegian population aged under 30 years was vaccinated during a mass vaccination campaign. There have been concerns over a possible increased risk of autoimmune disorders after pandemic influenza vaccination. We aimed to test whether Pandemrix vaccination was associated with increased risk of subsequently developing type 1 diabetes (T1D).

Residents in Norway were identified in the central population registry (n=2,270,045 age <30 years), and incident T1D diagnosed under age 30 years was identified using nationwide registries (insulin use plus T1D diagnosis). Individuals were followed from 1st October 2009 to 30th June 2014. The Norwegian Immunization Registry provided dates of vaccination (registration was mandatory). We used Cox regression with time-dependent exposures to estimate hazard ratios adjusted for age, sex, place of origin, and parental education.

2305 individuals were diagnosed with T1D (incidence rate 26/100,000 person-years). Preliminary analysis showed that the risk of T1D was not significantly increased after vaccination (aHR=0.98, 95%CI:0.89-1.07). Restricting the age-group to <15 years (1632 cases) gave similar results (aHR=1.00,95%CI:0.90-1.10).

We conclude that pandemic vaccination was not associated with risk of T1D.
Deep phenotypic analysis and comparison of islet-reactive CD8 T cells in type 1 diabetes using mass cytometry (CyTOF) and high-dimensional computational approaches

Alice E. Wiedeman, Sara A. Murray, Eddie A. James, Carla Greenbaum, S. Alice Long
Benaroya Research Institute, Seattle, WA, USA

CD8 T cells reactive to islet antigens have been implicated in the pathogenesis of type 1 diabetes (T1D), and phenotypic changes in CD8 T cells have been observed in the transition to clinical disease. While many peptide antigens targeted by autoreactive CD8 T cells have been identified, the phenotype and function of this population remains largely unexplored. We developed a 27-marker mass cytometry (CyTOF) panel to identify and extensively characterize CD8 T cells reactive to pooled class I (HLA-A2) tetramer (Tmr) loaded with peptides of viral or islet antigens in the same sample. Results were confirmed with several smaller flow panels (R²=0.989 versus CyTOF). Nineteen markers on CD8 T cells (Tmr+ and Tmr-) were analyzed by hierarchical clustering, principle component analysis, viSNE, SPADE, and Citrus. Using varied approaches, we consistently found that in PBMCs of three healthy controls and four T1D patients, virus-specific CD8 T cells demonstrated a more memory- and exhausted-like phenotype compared to total CD8 T cells, including increased T effector memory cells (CD45RO+CCR7-), KLRG1, CD57, TIGIT, and PD1. In contrast, islet-specific CD8 T cells (detectable in three T1D patients at >0.2% of total CD8 T cells) did not vary significantly from total CD8 T cells, suggesting that these cells do not have a strongly unique phenotype. This approach of using pooled tetramer for detection of viral- and auto-reactive CD8 T cells in the same sample, in combination with extensive phenotyping by mass cytometry, will be useful in monitoring phenotypic changes during disease progression and response to therapy.
Electrochemiluminescence-based IAA and GADA assays detect the appearance of islet autoimmunity earlier than radioimmunoassay in a significant proportion of children

Liping Yu1, Zhiyuan Zhao1, Dongmei Miao1, Kathy Waugh1, Ling Jiang1, Andrea Steck1, Yu Liu1,2, Kendra Vehik3, David Boulware3, William Hagopian4, Ezio Bonifacio5, Marian Rewers1
1Barbara Davis Center for Childhood Diabetes, University of Colorado School of Medicine, Aurora, Colorado, USA, 2Department of Endocrinology Yifu Hospital Nanjing Medical University, Nanjing, China, 3Health Informatics Institute Morsani College of Medicine University of South Florida, Tampa, Florida, USA, 4Pacific Northwest Diabetes Research Institute, Seattle, Washington, USA, 5Center for Regenerative Therapies Dresden Technische Universität, Dresden, Germany

Defining triggers of islet autoimmunity requires precise determination of timing of islet autoantibody (iAb) appearance. Electrochemiluminescence (ECL)-based assays for iAb to insulin (IAA) and GAD (GADA) detect all iAb isotypes including IgM and may reveal earlier seroconversion since standard radioimmunoassays (RIA) detects primarily IgG.

Age at seroconversion by ECL vs. RIA was compared in all 101 subjects from the Colorado TEDDY site with confirmed persistent RIA-IAA or RIA-GADA. Sera (n=1863) were collected q3 months, from birth, in high-risk participants in The Environmental Determinants of Diabetes in the Young (TEDDY) study. Additionally, 50 age-site-matched RIA-iAb negative controls (761 samples) were tested.

ECL-IAA preceded RIA-IAA in 25/71 children (median: 1.0 year); conversely, RIA-IAA preceded ECL-RIA in only 5/71 (median 0.54 year). ECL-GADA preceded RIA-GADA in 16/76 children (median: 2.8 year); RIA-GADA antedated ECL-GADA in only 5/76 (median: 0.25 year).

The ECL-IAA was present in 71% of RIA-IAA+ children and ECL-GADA in 75% of RIA-GADA+ children. The vast majority of ECL-iAb+ children developed multiple iAbs and/or progressed to T1D (49/63 of ECL-IAA+ and 50/57 of ECL-GADA+). However, 7/8 RIA-IAA+ and 17/19 RIA-GADA+ children without ECL remained single iAb or became iAb negative (p<0.0006 for IAA; p=0.0001 for GADA). ECL assays detected IAA and GADA in, respectively, 14 and 5 children negative by RIA. Most had multiple iAb+ and/or progressed to diabetes. Only 1/761 control samples tested ECL-GADA+ and 2/761 ECL-IAA+.

Measuring ECL-IAA and ECL-GADA in prospective infant studies may enable both improved timing of iAb appearance and improved prognostic accuracy in prediabetes.
Anti-IL-21 combined with liraglutide effectively reverses established hyperglycemia in mouse models of type 1 diabetes

Tamar Boursalian1, Travis Friesen1, Anna Ryden Barrenas1, Nikole Perdue1, Philippe Pagni1, Claire Gibson1, Sowbarnika Sachithanantham2, Malina McClure2, Ken Coppeters1, Matthias von Herrath1,2

1Novo Nordisk, Inc, Seattle, WA, USA, 2La Jolla Institute For Allergy And Immunology, La Jolla, CA, USA

Immunotherapy for type 1 diabetes (T1D) is aimed at halting or deviating the autoimmune response against insulin-producing pancreatic beta cells in order to preserve endogenous insulin production and glucose control. Interleukin-21 (IL-21) is a multifunctional cytokine with several functions described at key checkpoints within the immune system. As such, blockade of IL-21 may be a suitable strategy for immunotherapy in T1D. An attractive approach to enhancing the efficacy of immunotherapies is to combine with a second agent in order to target multiple disease pathways and to allow for safe, long term protection of beta cell mass. Glucagon-like peptide-1 receptor (GLP-1R) agonists may be ideal candidates to combine with immune modulators in treating T1D. Indeed in published preclinical studies, GLP-1R agonists combined with a short course of different immune modulators induced remission in a higher proportion of mice compared to treatment with either agent alone. We have found that blockade of IL-21 with anti-IL-21 antibody is highly effective in delaying diabetes onset in NOD mice, while its effect is variable in reversing already established diabetes. However, combination therapy of anti-IL-21 plus the GLP-1R agonist liraglutide is effective in reversing established disease compared to either monotherapy, and this enhanced efficacy is particularly evident in severely diabetic NOD mice. Moreover, the return to normoglycemia remains stable for the majority of mice even after therapy is withdrawn. Reversal of hyperglycemia with anti-IL-21 plus liraglutide combination therapy is observed in both the NOD model and the RIP-LCMV-GP model of T1D.
Identification of Genetic Variants co-segregated with Islet Autoimmunity in Multiplex Families of Type 1 Diabetes by Genome-wide Exome Analysis

Shinsuke Noso¹, Kazuyoshi Hosomichi², Naru Babaya¹, Yoshihisa Hiromine¹, Hiroyuki Ito¹, Yasunori Taketomo¹, Yumiko Kawabata¹, Hiroshi Ikegami¹
¹Department of Endocrinology, Metabolism and Diabetes, Kindai University Faculty of Medicine, Osaka, Japan, ²Department of Bioinformatics and Genomics, Graduate School of Advanced Preventive Medical Sciences, Graduate School of Medical Sciences, Kanazawa University, Kanazawa, Japan

Backgrounds: Type 1 diabetes in the Japanese population is characterized by markedly high Is (100~200) in multiplex families in spite of very low prevalence (0.014%) in general population, indicating strong familial clustering and the involvement of rare causative genetic variants. We have recruited four rare multiplex families of type 1 diabetes. Present study aimed to identify the genetic causality of familial type 1 diabetes.

Methods: Ten individuals from two families (Six from family I and four from family II) were subjected to whole exome sequencing (Illumina HiSeq). The exome variants were filtered on the basis of variant annotation, functional expectation, allele frequency (<0.01) and LOD score. Linkage was estimated using non-parametric linkage analysis assuming a dominant mode of inheritance.

Results: As for family I, sixteen rare variants from the total of 19,556 exome variants were co-segregated with the phenotype. Among these, five variants were overlapped with three genetic loci with maximal LOD score of 1.8 (6p, 16q and 20p) by linkage analysis. As for family II, 87 rare variants from the total of 19,526 exome variants were co-segregated with the phenotype, but no genetic locus was observed by linkage analysis. Combined linkage analysis of both families suggested two genetic loci with maximal LOD score of 2.41 (14p and 15p).

Conclusion: Candidate genetic loci for familial type 1 diabetes were narrowed down by whole exome linkage analysis of two families. Additional exome data from the other two familial cases will help to identify causal gene.
Loss of IDO1 expression in pancreatic islets from Type 1 diabetic subjects.

Florence Anquetil1, Giada Mondanelli2, Nathaly Gonzalez1, Teresa Rodriguez Calvo1, Jose Zapardiel Gonzalo1, Ursula Grohmann2, Matthias von Herrath1
1La Jolla Institute for Allergy and Immunology, La Jolla, CA, United States Minor Outlying Islands, 2University of Perugia, Perugia, Italy

Indoleamine 2,3-dioxygenase 1 (IDO1) is a metabolic enzyme catalyzing the first rate-limiting step of tryptophan catabolism, which leads to the production of a series of molecules known as kynurenines. IDO1 is considered as a potent immunoregulatory enzyme which catalytic and non-catalytic effects are involved in the regulation of immunity and autoimmune diseases. Current data suggest that the majority of patients with type 1 diabetes (T1D) exhibit an impaired expression and enzymatic activity of IDO1 in whole peripheral blood mononuclear cells (PBMCs).

We examined here, whether there also was a lack of IDO expression directly in the target organ (pancreata) by using tissue specimens obtainable from the Network of Pancreatic Organ Donors (nPOD). Pancreatic sections from pre-diabetic, diabetic and non-diabetic donors were monitored by immunofluorescence in order to assess IDO expression and localization.

Results showed that IDO is mostly expressed in the endocrine tissue, especially in beta cells from healthy organ donors. Interestingly, while IDO was constitutively expressed in non-diabetic and pre-diabetic donors, it was almost completely absent in insulin-containing as well as insulin-deficient islets from donors with T1D. The lack of IDO might contribute to beta cell loss in T1D and might also be an attractive target for future interventions.
IL-6, a major player in Type 2 but not Type 1 diabetes?

Florence Anquetil1, Giada Mondanelli2, Nathaly Gonzalez1, Teresa Rodriguez Calvo1, Jose Zapardiel Gonzalo1, Ciriana Orabona2, Matthias von Herrath1
1La Jolla Institute for Allergy and Immunology, La Jolla, CA, United States Minor Outlying Islands,
2University of Perugia, Perugia, Italy

IL-6 is a pleiotropic cytokine with a key impact on both immune regulation and non-immune events also affecting many cell types and tissues outside the immune system. Under physiological conditions pancreatic islets are known to release IL-6 which has been associated with glucose homeostasis. In type 2 diabetes (T2D), IL-6 was shown to be produced by human isolated islets in response to metabolic stress supporting a paracrine role for IL-6 under these conditions.

Our goal was to assess IL-6 localization and expression, based on the diabetic status (pre-diabetic, diabetic - T1D and T2D - and non-diabetic). We used pancreata obtained from the Network of Pancreatic Organ Donors (nPOD) and performed multiplex immunofluorescence for IL-6 and endocrine hormones.

The presence of physiological IL-6 in islets from non-diabetic donors was confirmed. Moreover, we observed an increase of IL-6 expression in T2D donors. In type 1 diabetes (T1D), lesser differences in expression levels were noted, we observed a slight reduction of IL-6 expression in insulin-deficient islets, but not in insulin-containing islets. Interestingly, IL-6 localization in the endocrine tissue which was found to be mainly in beta cells in non-diabetic donors shifted to non-beta cells in T1D and T2D donors. The precise roles that IL-6 might play in Type 1 versus Type 2 diabetes will need to be further clarified using suitable in vivo models or therapeutic interventions. From our findings it appears that a more prominent role could be expected in T2D.
Paradoxical acceleration of type 1 diabetes when combining IL-2 with effector T cell targeting therapy.

Timothy Bartley, Ekua Brenu, Sachithrani Madugalle, Irina Buckle, Emma Hamilton-Williams
University of Queensland Diamantina Institute, Brisbane, QLD, Australia

Enhancement of regulatory T cell (Treg) function is the goal of many immunotherapies aimed at treating type 1 diabetes. Genetic defects in the IL-2 pathway have led to intensive investigation of IL-2 therapy for in vivo restoration of Treg function. The use of IL-2 is hindered by its effects on other populations such as effector T cells. Combination therapies aimed at suppressing effector T cells while using IL-2 to expand Tregs could be beneficial. We have investigated a combination therapy using IL-2 together with anti-LFA-1 blocking antibody to simultaneously expand Tregs and suppress the activation and migration of autoreactive T cells. When NOD mice were treated with IL-2/anti-IL-2 complexes (IL-2c) and anti-LFA-1, significant Treg expansion occurred in the spleen and was further enhanced within the pancreas compared with IL-2c alone. Activation of islet-specific 8.3 and BDC2.5 cells within the pancreatic LN was dramatically suppressed following IL-2c/anti-LFA-1 treatment along with IFNy production and islet infiltration by 8.3 cells. Short-term IL-2c treatment was found to exacerbate insulitis, which was prevented by combination therapy. We used an accelerated model of T1D transfer in NOD-SCID mice to test whether IL-2c/anti-LFA-1 could prevent diabetes close to disease onset. Paradoxically, combination therapy accelerated diabetes onset compared with single or control therapy. Analysis of non-Treg endogenous IL-2 responsive populations within the pancreas found that combination therapy increased their response to IL-2 despite concomitant Treg expansion. Thus, inhibiting effector T cell migration into the islets may unleash pre-existing islet-resident pathogenic effectors in the presence of exogenous IL-2.
Increase in Pancreatic Proinsulin Area and Preservation of Beta Cell Mass During the Pre-diabetic Phase in Type 1 Diabetes

Teresa Rodriguez-Calvo¹, Jose Zapardiel-Gonzalo¹, Natalie Amirian¹, Ericka Castillo¹, Yasaman Lajevardi¹, Lars Krogvold², Knut Dahl-Jørgensen², Matthias von Herrath¹,³

¹La Jolla Institute for Allergy and Immunology, La Jolla, CA, USA, ²Division of Paediatric and Adolescent Medicine, Oslo University Hospital and Faculty of Medicine, Oslo, Norway, ³Novo Nordisk Diabetes Research & Development Center, Seattle, WA, USA

Type 1 diabetes (T1D) is characterized by loss of insulin production due to beta cell destruction. In this study we challenged the assumption that beta cell loss occurs in a linear fashion beginning early during the pre-diabetic phase and we show, in situ, on pancreas sections that proinsulin is increased while insulin area and beta cell mass are not reduced in autoantibody positive (Ab+) donors. This indicates that beta cells must be lost more precipitously around the time of diagnosis than previously assumed. The increase in pancreatic proinsulin to insulin ratio compared to non-diabetic donors fits well with the previous observation that proinsulinemia and increased proinsulin to C-peptide ratios can be detected in serum from prediabetic individuals. Using high-resolution confocal microscopy we also found a high accumulation of secretory granules containing proinsulin in beta cells from Ab+ donors, which points to a potential defect in proinsulin conversion or to an accumulation of immature vesicles due to a sustained increase in insulin demand. In addition, islets from Ab+ donors had larger sizes and contained a higher number of beta cells per islet, pointing to a possible disruption of the islet microenvironment. Altogether, our data indicates that the normal metabolism of beta cells is altered prior to disease onset but that beta cell mass is maintained until shortly before diagnosis. This suggests that secondary prevention before onset, when beta cell mass remains intact, could yield strong benefits; and therefore, means to detect T1D at this stage should be generally put in place.
Inverse relationship between organ-specific autoantibodies and systemic immune mediators in type 1 diabetes and type 2 diabetes. Action LADA 11

Nanette C Schloot1, Minh Ngyuet Pham1, Mohammed I Hawa2, Paolo Pozzilli3, Werner Scherbaum4, Matthias Schott5, Hubert Kolb5, Steven Hunter6, Guntram Schernthaner7, Charles Thivolet8, Jochen Seissler8, Richard David Leslie2

1German Diabetes Center, Duesseldorf, Germany, 2Blizard Institute, Queen Mary, London, UK, 3Department of Endocrinology and Diabetes, University Campus Bio-Medico, Rome, Italy, 4University of Duesseldorf, Medical Faculty, Duesseldorf, Germany, 5West-German Centre of Diabetes and Health, Verbund Katholischer Kliniken Duesseldorf, Duesseldorf, Germany, 6Regional Centre for Endocrinology and Diabetes, Royal Victoria Hospital, Belfast, Ireland, 7Rudolfstiftung Hospital, Department of Medicine, Vienna, Austria, 8Department of Endocrinology and Diabetes, Lyon-Sud Hospital, Hospices Civils de Lyon, PierreBenit, France, 9Medizinische Klinik und Poliklinik IV, Diabetes Center, Ludwig-Maximilians-University, Munich, Germany

Objective We related organ-specific autoantibodies, including diabetes-associated antibodies (DAA) and non-diabetes-associated autoantibodies (non-DAA) to systemic cytokines/ chemokines in type 1 and type 2 diabetes. Research Design and Methods From the European Action LADA cohort, patients with adult-onset type 1 diabetes (n=80, of which 50 had LADA and 30 had classic type 1 diabetes) and type 2 diabetes (n=626) were analysed for DAA (GADA, IA2A, ICA, ZnT8A), non-DAA (TGA, TPOA, PCA) and 10 immune mediator concentrations (measured by LUMINEX). Results Type 1 diabetes patients (whether classic type 1 diabetes or LADA), apart from their clinical phenotype, could not be distinguished by either autoantibodies (both DAA and non-DAA) or immune mediators. In type 1 diabetes most immune mediators (9/10) were negatively correlated with DAA titres. Type 2 diabetes patients, by definition without DAA, had fewer non-DAA (p<0.0005), but higher levels of pro-inflammatory immune mediators, especially compared with type 1 diabetes patients with high GADA titre; IL-6 (p<0.001), sE-Selectin (p<0.01) and IL-1Ra (p=0.052, trend).

Conclusions Patients with type 1 diabetes had more DAA and non-DAA than type 2 diabetes, while the frequency and nature of these autoantibodies was broadly similar in classic type 1 diabetes and LADA. Systemic immune mediator levels, in the main, were negatively correlated with DAA titres, and, for some, higher in type 2 diabetes, especially when compared with high titre GADA patients. Differences in the clinical classification of diabetes are associated with graded differences in adaptive and innate immune reactivity.
Amelioration of type 1 diabetes in NOD mice with sodium butyrate

NEENU JACOB¹, NEERAJ KHATRI², RAKESH KUMAR¹, NARESH SACHDEVA³
¹Department of Pediatrics, Post Graduate Institute of Medical Education and Research, Chandigarh, India, ²Experimental Animal Facility, Institute of Microbial Technology, Chandigarh, India, ³Department of Endocrinology, Post Graduate Institute of Medical Education and Research, Chandigarh, India

Butyrate is a short chain fatty acid that maintains intestinal homeostasis and is involved in immune regulation. However, its role in amelioration of type 1 diabetes is not sufficiently clarified. The present study aims to investigate the role of sodium butyrate in delaying progression of diabetes in NOD mice. Female NOD mice were administered sodium butyrate (150 mM) in drinking water after the onset of hyperglycemia (blood glucose > 250 mM). We found a significant reduction in hyperglycemia in the treatment group (347.3 ± 20.14 mM, n=9) versus the untreated (control) group (709.4 ± 70.4 mM, n=9) (p=0.002). Sodium butyrate treatment demonstrated a marked prolongation in the survival of treatment group (8 weeks post-hyperglycemia, n=9) compared to the control group (3 weeks post-hyperglycemia, n=9) (p=0.002). There was also complete remission of diabetes in 3/9 mice (33.3%) in the treatment group. Further, there was reduced lymphocytic islet infiltration as determined by immunohistochemistry in the treatment group. However, no significant difference was observed in the percentage of CD4+FoxP3+ intestinal Tregs in the treatment (7.2 ± 2.35 %, n=4) versus the control group (6.23 ± 1.93%, n=4).

The preliminary results indicate that reduction in hyperglycemia in the treatment group could be attributed to reduced migration of Teff cells to the pancreas. However, further experiments with more number of mice and analysis of chemokine receptors on intestinal T cells and their corresponding ligands in the pancreas will provide a better picture on the role of intestinal Tregs in amelioration of hyperglycemia.
Interaction of plasmacytoid dendritic cells and monocytes with DNA-LL37 immune complexes and T cells during initiation of type 1 diabetes.

Darshan Badal¹, Devi Dayal¹, Uma Nahar², Naresh Sachdeva³
¹Department of Pediatrics, Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India, ²Department of Histopathology, Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India, ³Department of Endocrinology, Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India

Plasmacytoid dendritic cells (pDCs) have been proposed to play a role in initiation of type 1 diabetes (T1D) through production of IFN-α and augmentation of Th1 responses. IFN-α producing pDCs have been detected early in T1D subjects and NOD mice, along with increased expression of IFN-α induced genes. Still, there is lack of literature on interaction of pDCs and monocytes with dying β-cells and T cells during initiation of T1D.

We studied the interaction of genomic-DNA released by dying β-cells with antimicrobial peptide, LL37, produced by neutrophils to form immune-complexes. These complexes were used to activate pDCs and monocytes and assessed whether they augment presentation of proinsulin to T cells in an antigenic manner.

DNA-LL37 complexes were generated in-vitro and we observed increased stability of the complexes that protected it from DNAse degradation. Additionally, the uptake of DNA by monocytes was enhanced when bound to LL37, compared to DNA alone. The antigen presentation capacity of monocytes was increased, following uptake of DNA-LL37 complexes as indicated by increased expression of CD80 and CD86. In pDCs, the expression of CD80 was increased following DNA-LL37 uptake. The expression of IFNα and activation of T cells by DNA-LL37 complexes and proinsulin stimulated monocytes and pDC remains to be determined. To understand whether β-cell apoptosis during remodelling could attract pDCs, the infiltration of pDCs in the cadaveric perinatal pancreatic islets is being further investigated. Nonetheless, our initial results suggest that formation of DNA-LL37 complexes can enhance the antigen presentation capacity of monocytes and pDCs.
Environmental factors in early life modify the development of the immune system and the risk of type 1 diabetes (T1D) as demonstrated in remarkable differences in the incidence of T1D between Finnish, Estonian and Russian Karelian children. We studied the maturation of the immune system in infants with HLA-conferred susceptibility to T1D from Finland, Estonia and Russian Karelia. We performed a longitudinal analysis of 33 circulating cytokines with multiplexed Luminex assay in serum samples taken at 3, 6, 12, 18, 24 and 36 months of age from Finnish (56 individuals, 147 samples), Estonian (56 individuals, 148 samples) and Russian Karelian (62 individuals, 149 samples) children matched for HLA-DQ risk genotype and gender. The concentrations of epidermal growth factor (EGF) and soluble CD40 ligand (sCD40L) were consistently higher in Russian children compared to Finnish and Estonian children during the whole study period. After 12 months the concentrations continued to decrease in Finnish children, whereas in Estonian children, a slight increase was observed. During the first 12 months of life, several pro-inflammatory cytokines showed a clear up-regulation. sCD40L and ligands for EGF receptor are known to be regulators of the activity of regulatory T-cells. Thus, we suggest that the decreased expression of EGF and sCD40L in Finnish and Estonian children might contribute to the development of immune dysregulation favoring autoimmunity.
Role of miRNA142a for T cell tolerance in islet autoimmunity

Martin G. Scherm1,2, Isabelle Serr1,2, Adam M. Zahm3, Klaus H. Kaestner3, Anette-Gabriele Ziegler2,4, Carolin Daniel1,2
1Institute for Diabetes Research, Independent Young Investigator Group Immune Tolerance in Type 1 Diabetes, Helmholtz Diabetes Center at Helmholtz Zentrum München, Munich, Germany, 2Deutsches Zentrum für Diabetesforschung (DZD), Munich, Germany, 3Department of Genetics and Institute for Diabetes, Obesity, and Metabolism, University of Pennsylvania, Perelman School of Medicine, Philadelphia, USA, 4Institute for Diabetes Research, Helmholtz Diabetes Center at Helmholtz Zentrum München, Klinikum rechts der Isar, Technische Universität München, Munich, Germany

In type 1 diabetes (T1D) the appearance of multiple islet autoantibodies indicates the onset of islet autoimmunity, in part considerably before clinical symptoms arise. However, the underlying mechanisms triggering onset and progression of the disease remain poorly understood. Regulatory T cells (Tregs) function as key players for the maintenance of immune tolerance and their malfunction is a major contributor to T1D. Recently, various studies highlighted the critical role of miRNAs for the differentiation of CD4+T cells and the complex interplay of immune activation and tolerance. However, the knowledge of specific miRNAs involved in the impaired tolerance regulation in T1D is still limited. Therefore we performed an NGS based pilot screen, to identify miRNAs, differentially expressed in naive and activated CD4+T cells of patients with autoimmunity compared to healthy controls. A significantly higher miRNA142a abundance in CD4+T cells from children with ongoing islet autoimmunity pointed to a potential involvement of miRNA142a in regulating autoimmune activation. To further investigate the role of miRNA142a, we performed in vitro Treg induction experiments under the influence of miRNA142a inhibitors and mimics, showing that the inhibition increases the Treg induction potential in both the human and the mouse system. Using the HITS CLIP technique we could show a high abundance of miRNA142a in the RNA-induced silencing complex (RISC), further underpinning a functional role for tolerance induction and also identifying relevant targets of the miRNA which are implicated in the regulation of Treg cell fate decision.
Hepatic Glycotargeting for Induction of Antigen-Specific Immunological Tolerance

Jeffrey A. Hubbell\textsuperscript{1,2}, D. Scott Wilson\textsuperscript{1,2}, Martina Damo\textsuperscript{1,2}
\textsuperscript{1}Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland, \textsuperscript{2}University of Chicago, Chicago, IL, USA

We are evaluating a novel concept for prophylactic and therapeutic antigen delivery for antigen-specific tolerance induction in T1D models, namely hepatic glycotargeting. We are exploring the hypothesis that specific glycoforms of self-antigens are collected by hepatic cells such as hepatocytes and liver sinusoidal endothelial cells (LSECs) and are there processed tolerogenically. Apoptotic debris is known to be processed tolerogenically; apoptosis is associated with protein desialylation, exposing terminal N-acetyl galactosamine and N-acetyl glucosamine residues. We have developed the chemistry to attach custom-made polymers containing side-chain residues of these sugars to antigens, Ag-pGal and Ag-pGlu, using a linker that provides release in the endosome shortly after endocytosis. We have shown highly efficient collection by both hepatocytes and LSECs in the mouse, leading to highly effective deletion and anergy induction of both CD4 and CD8 T lymphocytes (antigen-specifically) and induction of Tregs, such that approximately one-half of non-deleted antigen-specific CD4 T lymphocytes bear molecular markers of induced Tregs. In the adoptive BDC 2.5 T cell transfer model of T1D in the NOD/scid mouse with activated CD4 T cells that recognize chromogranin A, a beta cell antigen, diabetes induction by the autoreactive T cells was completely abrogated by glycotargeted antigen, whereas no prevention was induced by the free, non-targeted antigen. In this model also, evidence of Treg-mediated tolerance was obtained. Thus, antigens can be effectively targeted for hepatic uptake, into hepatocytes and LSECs, to induce antigen-specific deletion, anergy and regulation.
B lymphocytes invade islets in Type 1 diabetes (T1D) patients and nonobese diabetic (NOD) mice, where they form tertiary lymphoid structures containing germinal centers (GCs). We showed that islet B lymphocytes are enriched for anti-insulin specificities and have undergone somatic hypermutation, indicating T cell-B cell interactions, consistent with their role as antigen-presenting cells. Our work targeting B cell signaling showed that genetic ablation of Bruton's tyrosine kinase (BTK) eliminates anti-insulin, but not normal B cells, and protects against T1D without producing global B cell immunodeficiency. We now report the unexpected discovery that BTK-deficiency also significantly reduces Peyer's Patch B cells and IgA, and alters the microbiome in NOD mice. BTK-deficient NOD mice rederived into a barrier facility develop diabetes at the same rate as their wild type counterparts. This differs strikingly from disease outcomes in a normal specific-pathogen-free facility, where 83% remained healthy, compared with 31% of heterozygotic littermates. Thus, BTK-dependent B lymphocyte alterations that protect against T1D rely upon the presence of a particular microbiome. Because Peyer's Patch GCs generate IgA-producing B cells, and BTK-deficiency impairs GCs, we hypothesize that this results in defective IgA that allows additional microbes to colonize BTK-deficient mice, and that these microbes contribute to disease prevention. We are now working to identify those microbes and to determine whether they are beneficial when used alone, or whether both BTK-targeting and specific microbes are necessary. Importantly, BTK-inhibitors newly approved for humans provide a clinical avenue for this work.
A Role of HHV-6 in the pathogenesis of Type 1 Diabetes?

Somayeh Sabourî, William B. Kiosses, Teresa Rodriguez-Calvo, Jose Zapardiel-Gonzalo, Ericka Castillo, Matthias G. von Herrath

1La Jolla Institute for Allergy and Immunology, La Jolla, USA, 2Core Microscopy, La Jolla Institute for Allergy and Immunology, La Jolla, USA

Type 1 diabetes (T1D) is an inflammatory disease with autoimmune components resulting in the destruction of insulin-producing beta cells in the pancreatic islets. The precise pathogenesis of T1D is still unclear. Genetic predisposition is a critical factor in the development of T1D, however due to the discordance observed in monozygotic twins, other environmental factors such as viruses have been implicated in triggering or precipitating the disease. Human herpesvirus 6 (HHV-6) is a ubiquitous pathogen of the beta-herpesvirus family. HHV-6 has been proposed to play a role in autoimmune disorders of the nervous endocrine systems. GlycoproteinB (gpB) is conserved in all herpesviruses and plays a critical role during membrane fusion and viral entry. To explore the role of HHV-6 in the pathogenesis of T1D, we obtained pancreatic tissue sections from pre/diabetic and non-diabetic donors provided by the Network for Pancreatic Organ Donors with Diabetes (nPOD). The presence of gpB was analyzed at the protein level by indirect immunofluorescence assay. Our data show that gpB is expressed in 4 out of 6 cases in T1D donors whereas only 1 out of 4 cases showed positivity for gpB in the control group. In addition, we could not find any correlation between gpB expression and islet MHC class I expression, which is a hallmark of affected islets in T1D. Our observation provides no clear evidence for the involvement of HHV-6 in the pathogenesis of T1D. Further studies will be necessary to investigate whether HHV-6 infections could play a role as an accelerating factor.
A miRNA181a/NFAT5 axis links impaired T cell tolerance induction with autoimmune Type 1 diabetes

Carolin Daniel¹,², Isabelle Serr¹,², Maike Becker¹,², Benno Weigmann³, Anette-Gabriele Ziegler⁴,²
¹Institute for Diabetes Research, Independent Young Investigator Group Immune Tolerance in Type 1 Diabetes, Helmholtz Diabetes Center at Helmholtz Zentrum München, Munich, Germany, ²Deutsches Zentrum für Diabetesforschung (DZD), Munich, Germany, ³Department of Medicine 1, University of Erlangen-Nuremberg, Kussmaul Campus for Medical Research, Erlangen, Germany, ⁴Institute for Diabetes Research, Helmholtz Diabetes Center at Helmholtz Zentrum München, Klinikum rechts der Isar, Technische Universität München, Munich, Germany

Foxp3⁺ regulatory T (Treg) cells are critical in maintaining immune tolerance. Defects in immune tolerance function as a pivotal pathogenetic trigger of autoimmune disease. To halt the ever-increasing incidence in autoimmune Type 1 diabetes (T1D) and to develop innovative strategies that can interfere with autoimmune destruction a mechanistic understanding of human tolerance induction is urgently needed. However, molecular checkpoints that trigger the onset of autoimmunity in human T1D remain incompletely understood. Here, using T cells from children at an early stage of islet autoimmunity we find that a miRNA181a-mediated increase in signal strength of stimulation and costimulation links Nuclear factor of activated T cells 5 (NFAT5) with impaired tolerance induction and autoimmune activation. We demonstrate that enhancing miRNA181a activity increases NFAT5 expression while inhibiting Foxp3⁺ regulatory T cell (Treg) induction in vitro. In contrast, blocking the binding of miRNA181a to NFAT5 can enhance Treg induction. Accordingly, Treg induction is improved using T cells from NFAT5 ko animals (CD4⁺CD25⁺Foxp3⁺Tregs [% of CD4⁺T cells]: WT: 17.1±2.8 vs. NFAT5 ko: 30.3±1.2, P<0.01) while altering miRNA181a activity does not affect Treg induction in NFAT5 ko T cells. Of note, a specific NFAT5 inhibitor can increase tolerance induction in murine and humanized in vivo models. Moreover, we demonstrate that high costimulatory signals result in PI3 kinase (PI3K)-mediated induction of NFAT5 expression which interferes with Foxp3⁺Treg induction. These findings therefore suggest the miRNA181a/NFAT5 axis as a novel targetable signaling pathway for the development of innovative precision medicines aimed at limiting T1D islet autoimmunity.
Displacement analysis with mutated insulins identifies residues critical to the binding of insulin autoantibodies in type 1 diabetes

Karen Elvers, Debbie Shoemark, Gifty George, David Emery, Shelley Allen, Polly Bingley, Kathleen Gillespie, Alistair Williams
University of Bristol, Bristol, UK

Aims: Insulin autoantibodies (IAA) are important for predicting and monitoring type 1 diabetes (T1D). The major disease-related IAA epitopes are dependent on conservation of amino acids in the A-chain loop and N-terminus of the B chain. More precise mapping would improve understanding of autoreactivity underlying immune dysregulation.

Methods: Sera were available from 38 IAA positive patients with T1D (aged 8.8 years, range 1.9-17.8 years) before 11 days following diagnosis. Native and mutant insulins were expressed in yeast and purified using chromatography. These insulins were added at a single concentration (8.4x10^-9 mol/l) to displace IAA binding in a radiobinding assay with ^125^I-insulin.

Results: Median displacement of binding was slightly greater with native insulin (median 86%, range 63-94%) than with proinsulin (78%, 54-89%) or DesB30 insulin (81%, 65-91%). Further B-chain truncation (DesB23-30 insulin) caused additional loss of immunoreactivity, (71%, 31-88%). Replacement of B1 phenylalanine with alanine and A13 leucine with tryptophan caused large reductions in displacement (31%, 1-69% and 0%, 0-40%, respectively). Moderate reductions by some sera compared with native insulin (88%, 20-97%) were seen with DesB1-5, DesB30 insulin (91%, 9-91%), GluLysine (78%, 29-95%) in which B3 asparagine and B29 lysine are changed to lysine and glutamate respectively, and after changing B2 valine to glycine (83%, 2-86%), while substituting B16 tyrosine with alanine, had little effect (96%, 37-98%). For all comparisons p<0.001.

Summary: Displacement analysis has helped to create a structural model of the major IAA epitope regions. This model will be interrogated further to refine our knowledge of this critical autoantigen.
Autoimmunity Limited by Local Accumulation of Non-Ag-specific CD8+ Effector T Cells

Gustaf Christoffersson, Grzegorz Chodaczek, Sowbarnika Sachithanantham, Matthias von Herrath

La Jolla Institute for Allergy and Immunology, La Jolla, USA, Novo Nordisk Diabetes Research & Development Center, Seattle, USA

The inflammatory lesion at the pancreatic islet in type 1 diabetes (T1D) contains a heterogeneous T cell infiltrate. In studies of human tissue and mouse models, only very low fractions of cytotoxic CD8+ T cells (CTLs) at the islets were specific for known autoantigens. Here, we have studied the non-autoimmune majority of CTLs at the insulitic lesions, mapping their trafficking and function. We used three antigen (Ag)-driven mouse models of autoimmune diabetes; LCMV.RIP-GP, RIP-mOVA, and RIP-OVAlow. By adoptively transferring fluorescent T cell receptor transgenic CD8+ T cells (P14 and OT-I) we were able to track both Ag-specific driver CTLs, and their non-Ag-specific counterparts by flow cytometry and intravital confocal microscopy of the pancreas. As expected, non-Ag-specific CTLs gained access to the islets, but with unexpected anatomic specificity. When we looked into possible synergistic effects to beta cell destruction by their presence, we found that hyperglycemia did not occur at high, but physiologically relevant, levels of infiltrating non-Ag-specific CTLs. The tissue-specific accumulation of high amounts of non-Ag-specific CTLs threw the Ag-specific CTLs into a state of lower activation and proliferation, and ultimately displayed low IFN-γ and high IL-10 production, and upregulation of the inhibitory receptor PD-1. This state of anergy was partly due to ineffective interactions with Ag at the inflammatory lesion, as non-Ag-specific cells were blocking contacts with antigen presenting cells and target beta cells. This non-specific immune suppression by activated, but not autoreactive CTLs may help explain e.g. viral interference in autoimmunity, and provide avenues for future immune interventions.
Nucleotide-binding oligomerization domain-containing protein 2 (Nod2) modulates T1DM susceptibility through the gut microbiota

James Pearson1, Yangyang Li1,2, Chen Chao1,3, Jian Peng1, Lucy Zhang1, Yu Liu2, F. Susan Wong4, Li Wen1

1Section of Endocrinology, School of Medicine, Yale University, New Haven, Connecticut, USA, 2Department of Endocrinology, Jilin University, Changchun, Jilin, China, 3Center of Diabetes and Metabolism, Xiangya 2nd Hospital, Mid-South University, Changsha, Hunan, China, 4Diabetes Research Group, Institute of Infection and Immunity, School of Medicine, Cardiff University, Cardiff, Wales, UK

Increasing evidence suggests that the gut microbiota modify the susceptibility to T1D development through interactions with innate immune receptors like Toll-like receptors (TLRs). Nod2, a nucleotide-binding oligomerization domain-like receptor (NLR), recognizes bacterial-derived muramyl dipeptide, and is strongly associated with Crohn’s disease. To investigate the role of Nod2 in diabetes susceptibility, we generated Nod2-deficient (Nod2-/-) NOD mice.

We found that in Nod2-/- NOD mice, diabetes development was dependent on the gut microbiota. Nod2-/- NOD mice were significantly protected from diabetes only when housed in separate cages from Nod2-sufficient (wild-type, WT) NOD mice, in specific pathogen-free conditions. Further, the composition of the gut microbiota in Nod2-/- mice was very different from WT mice when housed separately. This suggests that T1D susceptibility in Nod2-/- mice is dependent on the alteration of gut microbiota by housing partners. Interestingly, Nod2-deficiency did not affect phenotype or function of dendritic cells and macrophages. However, the presence or absence of Nod2 and the housing conditions influenced the frequency and function of IgA-secreting B cells and T-regulatory (Treg) cells. Furthermore, proinflammatory CD8+IFNγ+ and CD4+IL-17+ populations were reduced upon co-culture with gut bacteria from Nod2-/- mice. Germ-free NOD mice, recolonized with gut microbiota from Nod2-/- mice also had significantly reduced pro-inflammatory cytokine-secreting immune cells but an increased number of Treg cells.

Our data support the effect of gut microbiota on the immune system and T1D susceptibility. Importantly, our study raises a critical question about the housing mode in the interpretation of the phenotype of genetically-modified mouse strains in T1D studies.
Generation of induced pluripotent stem cells from fulminant type 1 diabetes patients and investigation of beta cell destruction

Yoshiya Hosokawa¹, Taro Toyoda², Kenji Fukui¹, Megu Yamaguchi Baden¹, Michinori Funato²,³, Yasushi Kondo²,⁴, Tomomi Sudo², Hiromi Iwahashi¹,⁵, Marina Kishida²,⁶, Akira Watanabe²,⁶, Isao Asaka², Kenji Osafune², Akihisa Imagawa¹, Iichiro Shimomura¹

¹Department of Metabolic Medicine, Osaka University Graduate School of Medicine, Suita, Osaka, Japan, ²Center for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto, Japan, ³Department of Clinical Research, National Hospital Organization, Nagara Medical Center, Gifu, Japan, ⁴Department of Diabetes, Endocrinology and Nutrition, Kyoto University, Kyoto, Japan, ⁵Department of Diabetes Care Medicine, Osaka University Graduate School of Medicine, Suita, Osaka, Japan, ⁶Japan Agency for Medical Research and Development (AMED)-CREST, Tokyo, Japan

Fulminant type 1 diabetes (FT1D) is a subtype of type 1 diabetes mellitus, which is characterized by an abrupt onset of hyperglycemia due to the rapid destruction of pancreatic beta cells. Cytokine-induced apoptosis is assumed as one mechanism causing the beta cell destruction in FT1D, but the etiology has not been fully clarified. One of the reasons is that most pancreatic beta cells are already destroyed when hyperglycemia and ketoacidosis occur. The aim of this study is to generate induced pluripotent stem cells (iPSCs) from FT1D patients and to evaluate cytokine-induced apoptotic reactions of beta-like insulin-producing cells differentiated from the iPSCs. We generated iPSCs from fibroblasts of three FT1D patients by introducing six reprogramming factors. Insulin-producing cells were differentiated from the iPSCs in vitro and confirmed by immunofluorescence analysis and KCl-induced C-peptide secretion. The proportion of cleaved caspase-3- or TUNEL-positive cells in INSULIN (INS)-positive cells derived from FT1D- and control-iPSC clones was evaluated under treatment with TNF-alpha, IL-1-beta and IFN-gamma. INS-positive cells derived from FT1D-iPSCs exhibited higher expression of cleaved caspase-3 than those derived from control-iPSCs. Also, altered expression levels of several apoptosis-related genes were observed in INS-positive cells derived from FT1D-iPSCs by RNA sequencing. We propose that this in vitro disease model can be used to elucidate the disease mechanisms of FT1D.
Hyaluronan content governs pancreatic islet mechanobiology during autoimmune insulitis

Paul L. Bollyky1, Nadine Nagy1, Kenneth H. Hu1, Wenting Zhao1, Yi Cui1, Guadalupe Navarro1, Justin P. Annes1, Pamela Johnson1, Thomas N. Wight2, Manish Butte1

1Stanford University, Stanford, CA, USA, 2Benaroya Research Institute, Seattle, WA, USA

We have identified a novel role for hyaluronan (HA), an extracellular matrix (ECM) polymer, in governing the mechanical properties of inflamed islets. We recently reported that insulitis in type 1 diabetes (T1D) of mice and humans is preceded by intra-islet accumulation of HA, a highly hygroscopic (water-attracting) polymer. We asked whether autoimmune insulitis was associated with changes in the stiffness of islets and whether HA was responsible. To measure islet stiffness, we used atomic force microscopy (AFM) and developed a novel "bed of nails"-like approach using quartz glass nanopillars to anchor islets, solving a long-standing problem of keeping tissue-scale objects from moving while performing AFM. We measured stiffness via AFM nanoindentation with a spherical indenter. We find that insulitis made islets mechanically soft compared to controls. Conversely, treatment with 4-methylumbelliferone (4-MU), an inhibitor of HA synthesis, reduced HA accumulation, altered tissue water content, and restored basal tissue stiffness. These results indicate that HA content governs the mechanical properties of islets. Because tissue mechanotransduction has decisive effects on cellular responses and in light of our recent publication demonstrating that 4-MU treatment halted progression of autoimmune insulitis (Nagy et al., JCI 2015), these findings open up an exciting new avenue for research in understanding the fundamental pathogenesis of tissue-specific autoimmunity.
Distinct inflamed changes of the pancreases of slowly progressive insulin-dependent (type 1) diabetes: comparative analysis with enterovirus-induced fulminant type 1 diabetes

Erika Jimbo1, Tetsuro Kobayashi1, Tomoyasu Fukui1,2, Kaoru Aida3, Souroku Yagihashi4, Akira Shimada5
1Okinaka Memorial Institute For Medical Research, Tokyo, Japan, 2Showa University, Tokyo, Japan, 3Yamanashi University, Yamanashi, Japan, 4Hirosaki University, Aomori, Japan, 5Saitama Medical University Hospital, Saitama, Japan

Aim: The pathological changes of islet inflammation (insulitis) are poorly understood in slowly progressive insulin-dependent (type 1) diabetes (SPIIDDM). We observed immune features of SPIIDDM to compare with that of virus-induced fulminant type 1 diabetes by focusing on insulitis.

Method: Twelve pancreases from SPIIDDM patients, 3 FT1DM patients and 19 non-diabetic controls were recruited. Surface markers for CD45, CD3, CD8, CD4, CD20, CD11c, and CD68 were stained on the pancreatic section.

Results: MNCs positive for CD45, CD3 were infiltrated peri- and intra-islet area in all SPIIDDM pancreases. The inflammation in SPIIDDM islets was less extent in the islets and more prominent in peri-islet area and exocrine pancreas than that in FT1DM pancreas. Numbers of MNCs in peri- and intra-islets area were significantly increased compared with non-diabetic controls in SPIIDDM. Main subsets of MNCs infiltrating to the islets are CD8+ cells and CD68+ macrophages in SPIIDDM. In contrast, main subsets of MNCs observed intra- and peri-islets were CD11c+ dendritic cells (DCs) and CD68+ macrophages in FT1DM. Insulitis were observed even in long standing patients of SPIIDDM. The frequencies of insulitis did not correlated between age of onset of diabetes, titer of GADAAb, preserved beta cell volume and presence or absence of beta cell in SPIIDDM. MHC class I hyper-expression on islet beta cells was observed in SPIIDDM same as reported in FT1DM and AT1DM.

Conclusions: CD3+, CD8+ cell-dominant insulitis were present in SPIIDDM. Insulitis was persisted and beta cells were preserved for long time after the onset of diabetes.
Antigen-Specific CD4 regulation by Liposomes encapsulating calcitriol and ChgA mimotope in NOD mice

Anne-Sophie Bergot, Meghna Talekar, Emma Hamilton-Williams, Ranjeny Thomas
The University of Queensland Diamantina Institute, University of Queensland, Translational Research Institute, Brisbane, Australia

In type 1 diabetes (T1D) insulin-producing pancreatic β cells are destroyed by CD4+ and CD8+ autoreactive T cells. Antigen-specific tolerance is desirable for T1D immunotherapy, to avoid generalised immunosuppression. In the steady state, peripheral regulatory T cells (pTreg) are induced by antigen-bearing lymph node (LN) migratory dendritic cells (DC). In view of liposomes' excellent clinical track record, we encapsulated the autoantigen Chromogranin A (ChgA) BDC2.5 mimotope and the NF-κB inhibitor 1α,25-dihydroxy-vitamin D3 (calcitriol) into liposomes. We delivered them s.c. to target LN antigen presenting cells. NOD mice were injected s.c. with liposomes encapsulating BDC2.5 mimotope, with or without calcitriol, empty liposomes or PBS. DC and T cells were studied 1, 4 or 11 days later. Inguinal (ILN) and pancreatic (PcLN) draining LN and spleens were analysed by FACS, using the BDC2.5 tetramer. At day 1, liposomes were taken up predominantly by CD11c+ CD11b+ migratory DCs in ILN. At day 4 the proportion of BDC2.5-reactive endogenous T cells expanded in ILN, spleen and PcLN in response to liposomes delivering BDC2.5 mimotope with or without calcitriol. By day 11, the T cells recognising BDC2.5 had decreased in all tissues and the residual cells were enriched in PD1+CXCR5-pTreg. These data indicate that endogenous ChgA-specific effector T cells are deleted and Treg induced after a single s.c. administration of liposomes.
Combination therapies of antigen-specific intervention and anti-CD3 treatment in NOD mice

Claudia Selck, Gaurang Jhala, Helen Thomas, Balasubramanian Krishnamurthy, Thomas Kay
St Vincent's Institute, Melbourne, Victoria, Australia

Autoimmune disorders like type 1 diabetes result due to failure of immune tolerance mechanisms. Antigen-specific therapy constitutes an attractive approach to re-establish a tolerant state, but has not been successful in clinical settings. Persistence of antigen-experienced memory cells likely contributes to lack of success in preventing established disease. Thus, antigen-specific therapies may need to be combined with additional immunomodulatory treatments in individuals with ongoing autoimmunity.

We generated non-obese diabetic (NOD) mice with tetracycline-regulated expression of islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP) in antigen presenting cells (TII mice) and tracked IGRP-specific CD8+ T cells using tetramer enrichment. IGRP-specific T cells were absent in TII mice expressing IGRP from birth on, indicating tolerance of naïve cells to this antigen. However, antigen-experienced IGRP-specific T cells were readily detectable in TII mice expressing IGRP from 10 weeks of age, suggesting that they were refractory to peripheral tolerance.

Anti-CD3 (145-2C11) mAb treatment depletes pathogenic T cells in NOD mice. We hypothesized that combining short-term anti-CD3 treatment to delete pre-existing memory T cells followed by IGRP expression may induce effective tolerance in TII mice. Surprisingly, this regimen resulted in a 10-fold increase of antigen-experienced IGRP-specific CD8+ T cells (36,425±23,208 vs 3,614±2,426 cells/mouse in anti-CD3 treated and untreated TII mice, respectively) with up-regulation of the anergy marker PD-1. Therefore, the response to IGRP expression seen in TII mice is dramatically increased by anti-CD3 therapy. The phenotype of these expanded cells, whether they are derived from pre-existing memory cells or the thymus, and diabetes incidence are under investigation.
Serum markers regulating the interleukin-23 / T helper 17 pathway and angiogenesis predict residual beta-cell function in type 1 diabetes

Kerry Buchanan¹, Ian Hughes³, Ahmed Mehdi¹, Tristan Barnes¹, Au Bijin⁴, Bernett Lee⁴, Andrew Cotterill², Kin-Anh Le-Cao¹, John Connolly⁴, Mark Harris²,¹, Ranjeny Thomas¹

¹The University of Queensland Diamantina Institute, Brisbane, Queensland, Australia, ²The Lady Cilento Children’s Hospital, Brisbane, Queensland, Australia, ³Mater Research Institute-University of Queensland, Brisbane, Queensland, Australia, ⁴Singapore Immunology Network, Biopolis, Singapore, Singapore

Autoimmune damage to islet beta-cells impairs glucose-regulated insulin production in type 1 diabetes (T1D). Success of therapeutic immune intervention is confounded by heterogeneity in beta-cell function immediately before and after diagnosis, and prognostic biomarkers identifying pathogenetic pathways are lacking. A prognostic model for predicting residual beta-cell function using non-invasive clinical parameters was determined from AbATE trial data. Stimulated C-peptide could be estimated in children within two years after onset using age, gender, time since diagnosis, BMI-Z score, HbA1c and insulin dose (R² = 0.68). Forty-one serum analytes obtained 3 months after T1D onset were examined in 74 children to assess heterogeneity in residual beta cell function. Reduced estimated C-peptide 1 year after diagnosis was predicted by female gender, low C-peptide and basophil count at diagnosis, higher levels of IL-23 and CRP, and lower CXCL6 and ANGPTL3 measured 3 months after onset (R² = 0.71). In 30 pre-diabetic children participating in the TRIALNET natural history study, female sex, higher BMI-Z score, higher IL-6 and sIL2ra and lower PTH levels measured 12-18 months before diagnosis predicted reduced AUC C-peptide at diagnosis (R² = 0.67). Together these data suggest that dysregulation of the T helper 17 pathway and angiogenesis impair b-cell function in the immediate prodrome and early stages of T1D in children. These biomarkers reveal strategies for patient stratification, immune intervention and monitoring.
Uncovering seroconversion signatures in type-1 diabetes at-risk children

Ahmed Mehdi¹, Ezio Bonifacio², Anette-Gabriele Ziegler³, Kim-Anh Le Cao¹, Mark Harris¹, Ranjeny Thomas¹

¹Diamantina Institute, University of Queensland, Translational Research Institute, The University of Queensland, Brisbane, Australia, ²Center for Regenerative Therapies, Dresden, Germany, ³Institute of Diabetes Research, Helmholtz Zentrum München, München, Germany

Autoimmune-mediated destruction of pancreatic β-cells results in the development of type 1 diabetes mellitus (T1D). The clinical presentation of T1D is preceded by a prodromal period that can last from months to years and is characterised by the production of islet autoantibodies (seroconversion) reflecting loss of immune tolerance to beta cells. Although the presence of autoantibodies and T1D associated HLA genotypes identify high-risk first-degree relatives, biomarkers are needed to predict whether a child will seroconvert and the timing of seroconversion. We hypothesized that time-corrected gene expression data could uncover differentially expressed (DE) genes in a biologically meaningful manner. Specifically we used two large-scale longitudinal datasets; the German BABYDIET and the Finnish T1D prediction and prevention (DIPP), and aligned gene expression with time of seroconversion. Analysis at or near birth uncovered 28 differentially expressed genes that predicted seroconversion. Gene enrichment analysis confirmed that 15 out of these 28 genes are involved in immune-related mechanisms. Moreover, integration of differentially expressed genes with clinical data accurately predicted time of seroconversion (Area under receiver operator characteristic curve of 0.82). This represents a significant advance, not only to understand the mechanisms involved in seroconversion but also to select subjects for ongoing surveillance and for therapeutic interventions aimed at preserving beta cells.
CD8+ T cells specific for the islet autoantigen IGRP are restricted in their T cell receptor chain usage

Yannick Fuchs1,2, Anne Eugster1,2, Sevina Dietz1,2, Christian Sebelefsky4, Denise Kühn1, Carmen Wilhelm1, Annett Lindner1,2, Anita Gavrisan5, Jan Knoop4,5, Anette-G. Ziegler4,6, Ezio Bonifacio1,2
1DFG Center for Regenerative Therapies Dresden, Faculty of Medicine, Technische Universität Dresden, Dresden, Germany, 2Paul Langerhans Institute Dresden of the Helmholtz Center Munich at University Hospital and Faculty of Medicine, Technische Universität Dresden, Dresden, Germany, 3German Center for Diabetes Research (DZD e.V.), Neuherberg, Germany, 4Institute of Diabetes Research, Helmholtz Zentrum München, Neuherberg, Germany, 5Forscherguppe Diabetes e.V, Neuherberg, Germany, 6Klinikum rechts der Isar, Technische Universität München, Munich, Germany

CD8+ T cells directed against beta cell autoantigens are considered relevant for the pathogenesis of type 1 diabetes. Using single cell T cell receptor sequencing of CD8+ T cells specific for the IGRP265-273 epitope, we asked whether there was expansion of clonotypes and sharing of T cell receptor chain usage in the autoreactive CD8+ T cell repertoire. TCR α- and β-chain sequences of 418 multimer-sorted HLA A*0201 patient-derived IGRP265-273-specific CD8+ T cells representing 48 clonotypes were obtained. Three patients had expanded populations of IGRP265-273-specific CD8+ T cells with dominant clonotypes that shared TCR α-chains across patients. The SGGSNYKLTF motif corresponding to TRAJ53 was contained in the TCR α-chain of 384 (91.9%) cells, and in 20 (41.7%) patient-derived clonotypes. TRAJ53 together with TRAV29/DV5 was found in 61 (14.6%) cells and 15 (31.3%) clonotypes. Using next generation TCR α-chain sequencing, we found enrichment of one of these TCR α-chains in the memory CD8+ T cells of patients as compared to healthy controls. CD8+ T cell clones bearing the enriched motifs mediated antigen specific target cell lysis. We provide the first evidence for restriction of T cell receptor motifs in the alpha chain of beta cell antigen specific CD8+ T cells.
Higher eicosapentaenoic (EPA) and docosapentaenoic acid (DPA) status in erythrocyte is associated with reduced risk of islet autoimmunity: The Environmental Determinants of Diabetes in the Young Study

Sari Niinistö1, Iris Erlund1, Hye-Seung Lee2, Ulla Uusitalo2, Irma Salminen1, Carin A. Aronsson3, Sandra Hummel4, Stephen S. Rich5, William Hagopian6, Jorma Toppari7, Jin-Xiong She8, Åke Lernmark3, Anette Ziegler4, Marian Rewers9, Beena Akolkar10, Jeffrey Krischer2, Jill M. Norris11, Suvi M. Virtanen1,12

1National Institute for Health and Welfare, Department of Health, Helsinki, Finland, 2Health Informatics Institute, Morsani College of Medicine, University of South Florida, Tampa, USA, 3Department of Clinical Sciences, Lund University, Malmö, Sweden, 4Institute of Diabetes Research, Helmholtz Zentrum München and Forscherguppe Diabetes, Klinikum rechts der Isar, Technische Universität München and Forscherguppe Diabetes, Munich, Germany, 5University of Virginia School of Medicine, Virginia, USA, 6Pacific Northwest Diabetes Research Institute, Seattle, USA, 7Departments of Physiology and Pediatrics, University of Turku and Turku University Hospital, Turku, USA, 8Center for Biotechnology and Genomic Medicine, Medical College of Georgia, Augusta University, Augusta GA, USA, 9Barbara Davis Center for Childhood Diabetes, University of Colorado School of Medicine, Aurora, USA, 10National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, USA, 11Department of Epidemiology, Colorado School of Public Health, University of Colorado Denver, Aurora, USA, 12School of Health Sciences and Center for Child Health Research and The Science Center of Pirkanmaa Hospital District, University of Tampere and Tampere University Hospital, Tampere, Finland

Fatty acids are involved in immune reactions and inflammatory responses and may have a role in autoimmune inflammation processes leading to type 1 diabetes. Our aim was to investigate the association between erythrocyte fatty acid status and risk of islet autoimmunity in children.

The Environmental Determinants of Diabetes in the Young Study (TEDDY) is a longitudinal cohort study of children at high genetic risk for T1D (n=8676) born between 2004-2010 in the USA, Finland, Sweden and Germany. Islet autoimmunity is defined as repeated positivity for at least one autoantibody out of three measured (IAA, GADA, IA-2A). A nested case-control design contained 398 cases with islet autoimmunity and 1178 sero-negative controls matched for clinical site, family history and gender. Associations between fatty acid status and islet autoimmunity used conditional logistic regression adjusted for HLA risk group.

Higher proportions of eicosapentaenoic acid (20:5n-3) (EPA; OR 0.50; 95% CI 0.27-0.92 at 3 months of age) and docosapentaenoic acid (22:5n-3) (DPA; OR 0.63; 95% CI 0.46-0.87 at 3 months and OR 0.74; 95% CI 0.56-0.97 at 6 months of age) were associated with decreased risk of islet autoimmunity. Higher oleic acid (18:1n-9) proportion at 3 months (OR 1.07; 95% CI 1.01-1.13) and palmitic acid (16:0) at 6 months of age (OR 1.10; 95% CI 1.02-1.19) were associated with increased risk of islet autoimmunity.

These data suggest that fatty acid status during infancy is associated with islet autoimmunity, with higher status of long-chain n-3 fatty acids (EPA and DPA) being protective from islet autoimmunity.
Luminescent Immunoprecipitation System assays with high sensitivity and specificity for insulin autoantibodies

Daniela Liberati1,2, Rebecca Wyatt3, Ilaria Marzinotto1,2, Cristina Brigatti2, Kathleen Gillespie3, Peter Achenbach4,5, Lorenzo Piemonti2, Alistair Williams3, Vito Lampasona1,2
1Division of Genetics and Cell Biology, IRCCS San Raffaele Scientific Institute, Milan, Italy, 2Diabetes Research Institute, IRCCS San Raffaele Scientific Institute, Milan, Italy, 3School of Clinical Sciences, University of Bristol, Bristol, UK, 4Institute of Diabetes Research, Helmholtz Zentrum München, Neuherberg, Germany, 5Forscherguppe Diabetes, Klinikum rechts der Isar, Technische Universität München, Neuherberg, Germany

Aim: Insulin autoantibodies (IAA) are among the first biomarkers of type 1 diabetes (T1D) to appear in the preclinical phase of the disease. While IAA are widely used for screening at-risk individuals and characterizing T1D associated immune responses, the performance of IAA tests has lagged behind other islet autoantibody assays. Our aim is to develop novel IAA assays with greater sensitivity and specificity to improve T1D prediction.

Methods: We have developed several recombinant proinsulin and insulin antigens fused to a luciferase reporter. Alternative placements of the reporter relative to the insulin A and B chain, together with maintenance or removal of the c-peptide, were evaluated for their effect on recombinant protein expression and their performance as tracer antigens in non-radioactive immunoprecipitation LIPS IAA assays compared with an established radiobinding assay (RBA).

Results: We selected two recombinant nanoluciferase-proinsulin and insulin antigens that after testing in LIPS, using sera from 70 new-onset Italian patients and 70 controls, showed high assay performance (ROC-AUC= 0.902 and 0.873 p>0.0001, respectively). Preliminary testing of 10 patients and 15 IAA positive "high-risk" first-degree relatives, with multiple antibodies and/or who progressed to diabetes along with "low-risk" non-progressors (n=39) from the BOX family study, suggests that the new LIPS assays is better than the RBA at discriminating IAA responses associated with future T1D development (ROC-AUC: LIPS=0.946 p<0.0001 vs RBA=0.754 p=0.0007).

Conclusion: IAA measurement using our novel luciferase-(pro)insulins promises improved disease specificity for T1D, suggesting that at-risk subjects will be identified more accurately for recruitment to therapeutic intervention trials.
Detection of tetraspanin-7 antibodies in type 1 diabetes

Daniela Liberati¹,², Ilaria Marzinotto¹,², Carolyn Johnson³, Anya Westwood³, Kerry McLaughlin⁴, Lorenzo Piemonti², Michael Christie³, Vito Lampasona¹,²

¹Division of Genetics and Cell Biology, IRCCS San Raffaele Scientific Institute, Milan, Italy, ²Diabetes Research Institute, IRCCS San Raffaele Scientific Institute, Milan, Italy, ³Joseph Banks Laboratories, Lincoln, UK, ⁴OCDEM university of Oxford, Oxford, UK

Tetraspanin 7 (Tspan7) is a recently identified target of type 1 diabetes (T1D) associated autoantibodies that was formerly detected as a 38kDa radiolabeled autoantigen (Glima38), naturally expressed in neuronal or beta cell lines. Tspan7 has a complex structure that includes four transmembrane domains and two post-translationally modified extracellular domains. Tspan7 autoantibodies have been detected by a Luminescent Immuno-Precipitation System (LIPS), but this has lower specificity than Glima38 antibodies and some Glima38 antibody-positive samples fail to bind Tspan7 in LIPS. The aim of this study was to investigate reasons for the inadequate performance of Tspan7 LIPS. Autoantibodies in T1D failed to bind radiolabeled Tspan7 generated by transcription and translation in vitro, indicating a key role of protein folding and/or post-translational modifications for antibody binding. A nanoluciferase-tagged Tspan7 construct transfected into Expi293 cells was detected by flow cytometry using monoclonal and polyclonal antibodies raised to an ectodomain Tspan7 epitope, compatible with cell surface expression of an at least partially folded recombinant antigen. However, the expressed chimeric Tspan7 did not show the expected glycosylation patterns of Glima38. In LIPS assays, lowering the concentration of antigen eliminated Tspan7 binding of antibodies in non-diabetic control sera whilst maintaining positivity in T1D, suggesting the presence of low-affinity antibodies in some individuals. Whilst the majority of Glima38 antibody positive T1D patients bound nanoluciferase-tagged Tspan7 by the optimized LIPS, several remained negative in the assay. The results demonstrate the importance of maintaining natural structure and post-translational modifications for the binding of Tspan7 autoantibodies in T1D.
Cellular and Subcellular Localization of the Type 1 Diabetes Autoantigen Tetraspanin-7 in Neuroendocrine Cells

Kerry McLaughlin¹, Carolyn Johnson², Anne Clark¹, Michael Christie²
¹University of Oxford, Oxford, UK, ²University of Lincoln, Lincoln, UK

Type 1 diabetes occurs as a result of autoimmune destruction of insulin-producing beta cells of the pancreas. Four established targets of autoimmune responses in diabetes (insulin, IA-2, GAD65 and Zinc Transporter-8) are all associated with dense core or synaptic vesicles in neuroendocrine tissues. Tetraspanin-7 was recently identified as a fifth major target of autoimmunity and we studied the cellular and subcellular localization of tetraspanin-7 in neuroendocrine cells to provide clues on its functional significance. Immunohistochemical labelling demonstrated expression in pancreatic islets, but not in the exocrine pancreas. Tetraspanin-7 was also expressed in regions of the brain and in selected cells in the anterior pituitary, adrenal gland and lung. Multi-color immunofluorescence of formalin-fixed mouse and human islets confirmed that tetraspanin-7 expression colocalized with insulin and glucagon, indicative of beta and alpha cell expression, respectively. While the majority of tetraspanin family members are localized to the plasma membrane, immunogold labelling and electron microscopy of tetraspanin-7 in human and mouse islets indicated high expression in the secretory vesicles and in lysosomes, but only minimal labelling of the plasma membrane. Gold particles were clustered in the vicinity of secretory vesicles undergoing exocytosis at the cell surface indicating a potential role in exocytosis. The results demonstrate that, like other autoantigens in type 1 diabetes, tetraspanin-7 is a component of secretory vesicles in neuroendocrine cells and may play a role in vesicle trafficking, biogenesis or membrane fusion.
Generation of proinsulin-specific regulatory T cells from different sources for cellular therapies in type 1 diabetes

Naresh Sachdeva¹, Mahinder Paul¹, Lakhbir Dhaliwal², Anil Bhansali¹, Devi Dayal³

¹Department of Endocrinology, Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India, ²Department of Obstetrics and Gynaecology, Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India, ³Department of Pediatrics, Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India

Regulatory T cells (Tregs) play an important role in maintaining self-tolerance and protection from type-1 diabetes (T1D). Antigen-specific Tregs are shown to be more specific and effective than polyclonal Tregs. In the current study, we generated proinsulin-specific Tregs (pTregs) from different sources and assessed their efficacy in vitro.

First, we determined the frequency of pTregs in peripheral blood of T1D subjects (n=16) and healthy controls (n=6) using HLA II tetramers loaded with proinsulin derived peptides. Next, we generated pTregs from the CD4+CD25+CD127low/- and CD4+CD25-CD127high/+ fractions of T cells isolated from the peripheral blood of T1D subjects (n=6), healthy controls (n=6) and umbilical cord blood (UCB) (n=6) in an in-vitro culture. Finally, the pTregs were sorted using HLA II tetramers and their efficacy in suppression of effector CD4+ T cells was assessed in presence/absence of proinsulin derived peptides.

We observed that T1D subjects had significantly lower frequency of peripheral pTregs as compared to healthy controls (p<0.05). The frequency of pTregs generated from T1D subjects was also observed to be significantly lower than healthy controls. The frequency of pTregs generated from UCB derived CD4+CD25+CD127low/- T cells (natural Tregs, nTregs) was significantly higher compared to both T1D subjects and healthy controls. pTregs generated from the UCB were also found to be highly suppressive in presence of proinsulin derived peptides. Our initial results thus suggest that UCB can serve as an excellent source of pTregs for cellular therapies in T1D.
Measurement of autoantibodies to glutamate decarboxylase (GAD) using a truncated luciferase tracer offers improved specificity for type 1 diabetes.

Rebecca C. Wyatt¹, Daniela Liberati², Cristina Brigatti², Benjamin T. Gillard¹, Deborah K. Shoemark¹, Kathleen M. Gillespie¹, Peter Achenbach³, Lorenzo Piemonti², Vito Lampasona², Alistair J.K. Williams¹
¹University of Bristol, Bristol, UK, ²IRCCS San Raffaele Scientific Institute, Milan, Italy, ³Technische Universität München, Munich, Germany

Glutamate decarboxylase autoantibodies (GADA) are widely used for predicting Type 1 diabetes (T1D). GADA radiobinding assays (RBAs) using N-terminally truncated GAD₆₅(96-585) radiolabels offer improved disease specificity, but still identify GADA positive individuals who are unlikely to progress to T1D. We investigated whether non-radioactive Luminescence Immunoprecipitation System (LIPS) assays, using full-length GAD₆₅(1-585) or truncated GAD₆₅(188-585) could further improve the specificity of GADA measurement.

Assays were evaluated using “high-risk” and “low-risk” samples, previously measured by RBA with ³⁵S-GADA₆₅(1-585). High-risk samples included 50 recent-onset patients and 47 first-degree relatives (FDRs) who progressed to diabetes or had multiple islet autoantibodies (79% positive with ³⁵S-GADA₆₅(1-585)). Low-risk samples included 110 single GADA positive and 156 GADA negative FDRs who remained diabetes-free. All samples were re-tested by RBA using ³⁵S-labelled GAD₆₅(96-585) and by LIPS using luciferase conjugated (NLuc) GAD₆₅(1-585) and GAD₆₅(188-585). Thresholds were set at the 97.5th percentile of 160 healthy schoolchildren.

Of 97 high-risk samples, 76% tested positive with ³⁵S-GADA₆₅(96-585), 73% with NLuc-GADA₆₅(1-585) (p=0.7) and 72% with NLuc-GADA₆₅(188-585) (p=0.7). Of 266 low-risk samples, 29% tested positive with ³⁵S-GADA₆₅(96-585), 27% with NLuc-GADA₆₅(1-585) (p=0.6) and 20% with NLuc-GADA₆₅(188-585) (p=0.02). The area under the receiver operator characteristics curve for low- and high-risk sera was 0.80 for ³⁵S-GADA₆₅(96-585), 0.81 for NLuc-GADA₆₅(1-585) and 0.83 for NLuc-GADA₆₅(188-585).

GADA measurement using NLuc-GADA₆₅(188-585) offers improved specificity for T1D with similar sensitivity compared with ³⁵S-GADA₆₅(96-585). This suggests deleting the first 187 amino acids of GAD₆₅ does not compromise diabetes specific epitopes and allows individuals at increased T1D risk to be identified more accurately.
Antigen specific immunotherapy is an essential component of strategies for prevention and intervention in type 1 diabetes (T1D). A key example is the trial of GAD-alum immunization, aiming to restore autoreactive T cell balance. While the question of clinical efficacy remains inconclusive, we used samples from the TrialNet intervention study in new-onset T1D to show that in vitro IL-13, IL-4 and IL-5 responsiveness to rhGAD65 is a sensitive and dose-dependent biomarker of GAD-alum treatment. To examine the nature of this response at single cell resolution, we generated CD4 T cell lines and clones using IL-13 capture technology at 3 months post-GAD-alum treatment in subjects with DR3/DQ2, DR3/4/DQ2/8 and DR4/DQ8 HLA haplotypes. GAD65-specific T cell lines were used to screen a panel of 115 overlapping GAD65 peptides to identify epitope targeting and examine cellular phenotype. We identified responses to 10 GAD65 epitope regions, including 4 novel epitopes to which responses were restricted by DR4/DQ8 and DR3/DQ2 haplotypes. Furthermore, we analysed cytokine and T cell lineage transcription factor gene expression by qPCR on single cells from short-term lines sorted as IL-13 responsive to rhGAD65. 20/55 cells showed expression of GATA3/IL-13 and TBX21/IFN-γ, marking them as resembling hybrid Th1/Th2 cells which have not previously been defined in humans or in autoimmunity. These studies indicate that a major outcome of GAD-alum treatment is the generation or expansion of GAD65-specific hybrid Th1/Th2 cells. Functional characterization of these cells will be important as a means of understanding whether GAD-alum based immunotherapy strategies have clinical utility.
Increased Plasmacytoid Dendritic Cell Numbers at Diagnosis or at Start of Treatment of Type 1 Diabetes Predicts Disease Outcome

Martine Boks, Kerry Buchanan, Lisa Nagl, Mark Harris, Ranjeny Thomas
The University of Queensland Diamantina Institute, Brisbane, Queensland, Australia

In type 1 diabetes (T1D) islet beta cell-specific autoimmunity promotes their inflammatory destruction by islet-reactive T-cells and progressive decline in capacity to produce insulin. Autoreactive T-cell activation is driven by antigen presenting cells, such as dendritic cells (DC), presenting islet self-antigens in draining pancreatic lymph nodes. In this study, we analysed the CD1c+ and CD141+ myeloid, and the CD123+ plasmacytoid (p)DC subsets in peripheral blood of 11 children aged 9-13y presenting with T1D within the previous 3 months, 15 age and sex-matched FDR and 10 unrelated HC. The proportion of CD123+ pDC was significantly increased in children with T1D and FDR compared to HC. Presence of islet autoantibodies did not influence the proportion of pDC in FDR. The expression of HLA-DR by pDC of diabetic subjects was significantly higher than that of FDR or HC, indicating increased pDC activation and antigen presenting capacity. This suggests that genetic or environmental factors increase pDC in children at risk, but a specific increase in pDC activity characterizes children who progressed to diabetes. Importantly, CD123+ pDC levels at diagnosis negatively correlated with the estimated C-peptide levels after 12 months. In addition, CD11c- pDC levels at start of Alefacept immunotherapy negatively correlated with C-peptide levels after 24 months. Thus increased pDC frequencies at diagnosis or at start of treatment predicts poor beta cell function 1 year after diagnosis or 2 years after immunotherapy, and this may serve as a prognostic marker for type 1 diabetes disease progression.
Signs of beta-cell autoimmunity in 3-year-old children: A comparison between a cohort with HLA-conferred susceptibility to type 1 diabetes and an unselected cohort

Taina Härkönen\(^1,2\), Heli Siljander\(^1,2\), Anu-Maaria Hämäläinen\(^1,3\), Aleksander Peet\(^4\), Vallo Tillmann\(^4\), Natalya Dorshakova\(^5\), Sergei Mokurov\(^6\), Jorma Ilonen\(^7\), Mikael Knip\(^1,2\)

\(^1\)Children’s Hospital, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland,
\(^2\)Diabetes and Obesity Research Program, University of Helsinki, Helsinki, Finland,
\(^3\)Department of Pediatrics, Jorvi Hospital, Helsinki University Hospital, Helsinki, Finland,
\(^4\)Department of Pediatrics, University of Tartu and Tartu University Hospital, Tartu, Estonia,
\(^5\)Petrozavodsk State University, Department of Family Medicine, Petrozavodsk, Russia,
\(^6\)Ministry of Health and Social Development, Karelian Republic of the Russian Federation, Petrozavodsk, Russia,
\(^7\)Immunogenetics Laboratory, University of Turku, Turku, Finland

The DIABIMMUNE project aimed at assessing the role of hygiene in the development of immune-mediated diseases, particularly type 1 diabetes (T1D), in Finland, Estonia and Russian Karelia. Populations in these countries have relatively similar distribution of predisposing HLA genotypes, but a marked difference in T1D incidence. We analyzed diabetes-associated autoantibodies at the age of 3 years in 302 Finnish, 245 Estonian and 21 Russian Karelian children with HLA-conferred disease susceptibility (the birth cohort, BC) and in 1574 Finnish, 1672 Estonian and 439 Russian Karelian young children from the general population (the young children cohort, YCC) at 3 years of age. Autoantibodies to insulin (IAA), glutamic acid decarboxylase (GADA), islet antigen-2 (IA2A) and zinc transporter 8 (ZnT8A) autoantibodies were analyzed with radiobinding assays. Islet cell autoantibodies (ICA) were analyzed from samples testing positive for at least one of the biochemical autoantibodies. Compared to YCC children, BC children had higher frequency of autoantibodies at the age of 3 years; IAA (2.4 vs 1.4%, p=0.06), GADA (2.3 vs 1.4%, p= 0.09), IA2A (1.2 vs 0.4%, p=0.011), ZnT8A (1.9 vs 0.7 %, p=0.005) and ICA (2.3 vs 0.5%, p=0.002). The frequency of multiple (≥2) autoantibodies was significantly higher in the BC (2.1 vs 0.6%, p<0.001). By the age of 7 years, the incidence of T1D was three-fold in the Finnish BC children compared to YCC, but the difference remained non-significant (1.3 vs 0.4%, p=0.058). These data show that HLA-conferred T1D susceptibility is associated with increased frequency of beta-cell autoimmunity in young children.
Longitudinal 3D visualisation of autoimmune diabetes by functional optical coherence imaging

Anja Schmidt-Christensen¹, David Nguyen², Julia Nilsson¹, Corinne Berclaz², Theo Lasser², Dan Holmberg¹

¹Lund university, EMV, Immunology, Lund, Sweden, ²Laboratoire d’Optique Biomédicale, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

It is generally accepted that structural and functional quantitative imaging of individual islets is beneficial to elucidate the pathogenesis of type 1 diabetes. We previously introduced functional optical coherence imaging (FOCI) for fast, label-free monitoring of beta cell destruction and associated alterations of islet vascularisation.

Therefore, NOD mouse and human islets transplanted into the anterior chamber of the eye (ACE) were imaged with FOCI, in which the optical contrast of FOCI is based on intrinsic variations of the index of refraction resulting in a faster tomographic acquisition. In addition, the phase sensitivity allows simultaneous label-free acquisition of vascularisation.

We demonstrate that FOCI allows longitudinal quantification of progressive autoimmune insulitis, including the three-dimensional quantification of beta cell volume, inflammation and vascularisation. The substantially increased backscattering of islets is dominated by the insulin-zinc nanocrystals in the beta cell granules. This translates into a high specificity for the functional beta cell volume of islets. Applying FOCI to a spontaneous mouse model of type 1 diabetes, we quantify the modifications of the pancreatic microvasculature accompanying the progression of diabetes and reveal a strong correlation between increasing insulitis and density of the vascular network of the islet.

FOCI provides a novel imaging technique for investigating functional and structural diabetes-induced alterations of the islets. The label-free detection of beta cell volume and infiltration together with vascularisation offers a unique extension to study ACE-transplanted human islets. These results are contributing to a deeper understanding of human islet transplant rejection and label-free in vivo monitoring of drug efficacy.
Biodegradable Antigen Associated Nanoparticles Induce Antigen Specific Tolerance in Mouse Models of T1D

Tobias Neef, Dan Xu, Suchitra Prasad, Stephen Miller
Northwestern University Feinberg School of Medicine Department of Microbiology-Immunology, Chicago, IL, USA

Type 1 diabetes [T1D] is an autoimmune disorder characterized by an invasion of immune cells into pancreatic islets and destruction of insulin-secreting beta cells. Previously we have induced antigen-specific tolerance in multiple models of autoimmune disease using antigens coupled to apoptotic splenocytes. However, due to the difficulty of producing coupled splenocytes in a clinical setting, we investigated the use of biodegradable poly(lactic-coglycolicacid) [PLG] nanoparticles as a safe, cost-effective alternative. Here we describe multiple configurations of antigen-associated nanoparticles, including particles coupled to the chromogranin A mimetope P31 via ethylene carbodiimide [ECDI], as well as particles encapsulating P31 [PLG(P31)] or the IGRP epitope NRP-A7 [PLG(NRPA7)]. P31 coupled particles are able to delay disease onset in wild-type NOD mice as well as in an adoptive transfer model in which transgenic CD4+ T cells specific for chromogranin A are infused into immunodeficient Nod.Scid mice. The same tolerance induction is achievable using PLG(P31) or PLG(NRPA7), following a single infusion of either CD4+ or CD8+ T cells. Our data indicates that the tolerance induction is antigen specific and works through multiple mechanisms. We are able to show the treatment results in sequestration of inflammatory T cells in the spleen, but the tolerogenic effect is also dependent on the presence of regulatory T cells. Furthermore, we observe a reduction in inflammatory cytokine production in the pancreas and induction of T cell anergy. The results presented here show the potential of such a platform to serve as a next generation T1D therapy.
Granzyme A-deficiency breaks immune tolerance and promotes organ-specific autoimmune disease through a type I interferon-dependent pathway

Satoru Akazawa1, Zia Mollah1, Hong Quah1,2, Kate Graham1,2, Gaurang Jhala1,2, Balasubramanian Krishnamurthy1,2, Joseph Trapani3, Phillip Bird4, Thomas Brodnicki1,2, Thomas Kay1,2, Helen Thomas1,2

1St. Vincent’s Institute, Fitzroy, Victoria, Australia, 2The University of Melbourne, Department of Medicine, St. Vincent’s Hospital, Fitzroy, Victoria, Australia, 3Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia, 4Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Victoria, Australia

Granzyme A is a protease implicated in the degradation of intracellular DNA. Nucleotide complexes are known triggers of innate immunity and systemic autoimmunity, but a role in organ-specific autoimmune disease has not been demonstrated. To investigate whether such a mechanism could be an endogenous trigger for autoimmunity, we examined the impact of granzyme A deficiency in the NOD mouse model of autoimmune diabetes. NOD.Gzma-/- mice developed diabetes earlier than wild-type NOD mice. In addition, granzyme A deficiency was able to break tolerance in NOD.PI mice that are tolerant to proinsulin and never develop diabetes, resulting in insulin autoantibody production and diabetes. Single-stranded DNA was observed more frequently in the cytoplasm of immune cells in NOD.Gzma-/- mice compared with NOD mice, suggesting that granzyme A is required for efficient degradation of aberrant DNA. Consistent with increased nucleic acid sensing, islets from NOD.Gzma-/- mice had increased expression of IFN-response genes. Diabetes returned to the expected rate in NOD.Gzma-/- mice that also lacked type I IFN receptor expression. Our data suggest a new mechanism for the etiology of autoimmune diabetes, where accumulation of aberrant cytoplasmic DNA in innate immune cells results in excessive IFN production by these cells within islets. This is the first indication of an in vivo role for granzyme A in maintaining immune tolerance.
Development of a multiplex methylation sensitive digital PCR assay to monitor islet autoimmunity in type 1 diabetes

Jody Ye, Fatma Alrashidi, Matthew Suderman, Michelle Curran, Parth Narendran, Chloe Bulwer, Rakesh Amin, Caroline Relton, Colin Dayan, Alistair Williams, Kathleen Gillespie

Diabetes and Metabolism, School of Clinical Sciences, University of Bristol, Bristol, UK, MRC integrative epidemiology unit, School of Social and Community Medicine, University of Bristol, Bristol, UK, Institute of Metabolism and Systems Research, University of Birmingham, Birmingham, UK, Institute of Child Health, University College London, London, UK, Institute of Molecular & Experimental Medicine, University of Cardiff, Cardiff, UK

Methylation specific cell free DNA (cfDNA) offers the potential to monitor the rate of beta cell death, as well as the dynamics of the peripheral immune response. The aim of this study was to develop a novel, high throughput, multiplex assay to quantify beta cell death and immune cell turnover simultaneously in type 1 diabetes.

DNA methylation patterns in islets (n=5) and peripheral blood mononuclear cells (PBMC, n=3) were tested using the genome-wide Illumina EPIC methylation array. Data were analysed using LIMMA analysis in R software. Taqman assays were developed by multiplexing three methylation sensitive markers, two specific for beta cell turnover and one for immune cell turnover. Serum cfDNA extraction methods were compared and protocols from commercialized kits were modified. Each sample was tested in duplicate using droplet digital PCR (dPCR, Bio-Rad).

Comparison of methylation patterns between islets and PBMC identified 3263 individual CpG sites and 49 regions that reached epigenome-wide significance (P<10E-10), but did not include the insulin gene. A modified extraction protocol increased cfDNA yield approximately 7 fold (P=0.0006) compared with current published methods. dPCR amplified the three markers simultaneously without cross-reactivity. Preliminary data suggest that a combination of beta and immune cell targets offers the most sensitivity to discriminate individuals with T1D from control subjects (P=0.0058).

Methylation array has provided multiple novel targets to measure the rate of beta cell death. Multiplex dPCR that quantifies both beta and immune cell turnover offers the potential for a novel, efficient and high-throughput assay to monitor islet autoimmunity.
Is there regulation of the autoimmune response in slow progressors to type 1 diabetes?

Anna E. Long¹, Claire Williams¹, Dorothy J. Becker², Ingrid M. Libman², F. Susan Wong³, Rosaura Casas⁴, Johnny Ludvigsson⁴, Andrea K. Steck⁵, Marian J. Rewers⁵, Peter Achenbach⁶, Alistair J.K. Williams¹, Kathleen M. Gillespie¹

¹University of Bristol, Bristol, UK, ²University of Pittsburgh, Pittsburgh, PA, USA, ³Cardiff University, Cardiff, UK, ⁴Linköping University, Linköping, Sweden, ⁵University of Colorado, Colorado, CO, USA, ⁶Technische Universität München, Munich, Germany

Multiple islet autoimmunity increases risk of diabetes but not all individuals positive for two or more islet autoantibodies progress to disease within a decade. The SNAIL study seeks to harmonise data from longitudinal studies to identify the characteristics of slow progression to type 1 diabetes. 157 individuals with multiple islet autoantibodies (IAA, GADA, IA-2A and ZnT8A) followed for more than 10 years without progression were identified from six studies (BOX; BABYDIAB; DAISY; Pittsburgh Diabetes; ABIS; TRIALNET). Individuals enrolled in BOX provided “Rapid Progressor” (diagnosed < age 5yrs) and samples near diagnosis. HLA Class I and II were analysed by PCR-SSP. Islet autoantibody profile was analysed by radioimmunoassay and/or ECL. Intermediate HLA-Class II risk was more frequent in Slow (60%, n=104) than Rapid Progressors (42%, n=352) with a reciprocal reduction in high risk genotypes (24% vs. 48%; pCorr=0.005); only one carried protective HLA DQ6. Slow Progressors carried fewer HLA - Class I B risk alleles (48%, BOX) than Rapid Progressors (86%; pCorr<0.001). Of 35 Slow Progressors with longitudinal data available, 13 (37%) retained multiple autoantibodies after 10 years (p<0.001). Reduced positivity for IAA and GADA and levels of IA-2A and ZnT8A was observed (p<0.05 for all). Slow Progressors also had lower levels of all IA-2A IgG (1-4) subclasses than patients sampled near diagnosis (p<0.05). Multiple autoantibody positivity is not maintained in some Slow Progressors suggesting regulation of the autoimmune response. Immuno-phenotyping of B-cells and CD4+/CD8+/regulatory T-cells in these individuals is ongoing to elucidate the mechanisms underlying a decreased humoral response and delayed progression.
Type 1 diabetes is an autoimmune disease in which the insulin-producing β cells in pancreatic islets are destroyed. Human studies suggest cytotoxic T lymphocytes (CTL) are the major cells responsible for β cell lysis, and a direct role for CTL in β cell death has been demonstrated in the non-obese diabetic (NOD) mouse model of spontaneous autoimmune diabetes. The NK group 2 member D (NKG2D) immune receptor is implicated in the diabetogenic CTL response. However, the mechanisms by which NKG2D affects diabetes pathogenesis are not clear. Utilizing NKG2D-deficient NOD mice, we demonstrate that NKG2D expression affects diabetes development by at least two previously undescribed mechanisms. First, we found NKG2D influences NOD diabetes development via interaction with the microbiota composition. Second, when depleted of microbiota, NKG2D-deficient mice had enhanced diabetes development. This demonstrated a surprising protective role for NKG2D in NOD diabetes pathogenesis. We show that the NKG2D ligand H60a is expressed on activated NOD T cells both in vivo and in vitro. During in vitro CD8+ T cell differentiation into CTLs, we found NKG2D-H60a interaction generally decreased the subsequent CTL effector cytokine response. Taken together, these findings demonstrate there is a protective role for NKG2D during diabetes development that is likely mediated by NKG2D-ligand interaction during diabetogenic CTL differentiation.
Multiple autoimmunity in children and young adults type 1 diabetes

Aizhan Kozhakhmetova¹, Rebecca Wyatt¹, Claire Caygill¹, Rachel Aitken¹, Janet Wenzlau², Claire Williams¹, Kathleen Gillespie¹, Alistair Williams¹
¹University of Bristol, Bristol, UK, ²University of Colorado, Denver, USA

It is well established that individuals with type 1 diabetes (T1D) are at increased risk of other autoimmune diseases but the absolute risks are unclear. The aim of this study was to determine the frequency of autoantibodies to thyroid peroxidase (TPOA), tissue transglutaminase (TgA), and gastric ATPase (ATPase4A) in a well-characterised population based cohort with T1D and to identify the genetic characteristics of multiple autoimmunity.

Samples were analysed from individuals with T1D [n=1061; 464 male; median age 11.8yrs (range 0.7-28yrs), median age at diagnosis 10.9 yrs (range 0.4-21yrs)] participating in the population-based Bart’s Oxford (BOX) family study. Autoantibodies to TPO, Tg and ATPase were measured by radioimmunoassay. HLA class II and non-HLA SNPs (rs3087243 in CTLA-4, rs12935413 in KIAA0350 and rs1893217 in PTPN2) analysis was carried out by PCR-SSP and Taqman genotyping respectively.

Overall, 22.7% of individuals with T1D were positive for at least one non-islet autoantibody. The prevalence of TPOA, TgA and ATPase4A in patients was 9.2%, 9.1%, and 8.4% respectively. Two autoantibodies were observed in 2.8% and all three autoantibodies in 0.3% of the cohort. TgA was associated with younger age and TPOA with older age (p<0.001 for both) but no age effect was observed for ATPase4A. All autoantibodies were associated with female gender (p<0.005). Risk of multiple autoimmunity is modulated by different HLA class II DRB1*03,*04 and non-HLA SNP combinations.

Over one fifth of children with T1D will develop gut, thyroid or gastric autoimmunity. HLA and non-HLA genes modulate risk, supporting evidence for common pathways of autoimmunity.
Preproinsulin signal peptide epitopes are loaded into HLA class I molecules via a non-canonical processing pathway dependent on signal peptide peptidase

Martin Eichmann¹, Beate Hehn², Norkhairin Yusuf¹, Marius K. Lemberg², Mark Peakman¹, Deborah Kronenberg-Versteeg¹

¹Peter Gorer Department of Immunobiology, Faculty of Life Sciences and Medicine, King's College London, London, UK, ²Zentrum für Molekulare Biologie der Universität Heidelberg (ZMBH), DKFZ-ZMBH Allianz, Heidelberg, Germany

The signal peptide (SP) region of preproinsulin (PPI) is a hotspot for generating epitopes presented by type 1 diabetes (T1D) associated HLA class I molecules. During translational translocation of PPI, the SP is cleaved and retained within the endoplasmic reticulum (ER) membrane, implying that it is processed for immune recognition outside of the canonical, proteasome-directed pathway. To identify PPI SP processing pathway(s), we used the requirement for transporter associated with antigen processing (TAP) for epitope generation as a probe, and identified cytoplasm-proximal (TAP-dependent) and ER-luminal (TAP-independent) regions of SP, presented by different HLA class I molecules (PPI3-11 on HLA-A*24:02 and PPI15-24 on HLA-A*02:01, respectively). This implied that enzymatic cleavage of SP within the ER membrane releases peptide fragments both into the cytosol and ER-lumen for further processing and presentation. We used in vitro translation and translocation with specific inhibitors and gene knockout to characterise the intramembrane protease responsible. We find that the signal peptide peptidase (SPP), a member of the presenilin-like intra-membrane aspartyl protease family, cleaves the SP of PPI in vitro. In live cells, CRISPR-guided SPP KO modulates cytoplasm-proximal and ER-luminal epitope generation differently (increased and decreased, respectively). These data point towards the existence of intramembrane protease redundancy and altered processing in the absence of SPP function. It is conceivable that disturbance of this processing pathway could arise under non-physiological conditions (e.g. ER stress) and lead to increased presentation of autoantigen-derived epitopes on beta-cells, enhancing autoimmune responses.
Clinical heterogeneity of type 1 diabetes (T1D) found in Asia

Yongsoo Park
Kosair Children’s Hospital Research Institute, U of Louisville, Louisville, USA

Diabetes among young patients in Asia is caused by a complex set of factors. In addition to typical autoimmune type 1 diabetes and non-autoimmune types of T1D associated with insulin deficiency, latent autoimmune diabetes in adults (LADA) is a form of autoimmune-mediated diabetes, usually diagnosed during insulin-dependent stage after certain period of non-insulin requiring phase, which can be diagnosed earlier based on anti-islet autoantibody positivity. Although many epidemiological surveys of LADA have been conducted in Caucasian and Asian populations, their reported prevalence rates vary due to the use of different diagnostic criteria. In a recent study with a comparable design and valid methodology to those in Caucasians, the prevalence of LADA in Korea using GAD autoantibody positivity as the diagnostic criterion was higher (4.4%) than the previously reported prevalence of 1.7% in a population-based T2D survey. However, after 60 months of follow-up, only 3 of the 39 patients initially diagnosed with LADA had become insulin-dependent, and they were all positive for multiple anti-islet autoantibodies (GAD, IA-2 and ZnT8 antibody). This demonstrates that true insulin dependency, which was initially indicated by multiple antibody positivity, has not increased in the Korean population. Despite etiological heterogeneity, in the usual clinical setting, early diagnosis and classification of patients with diabetes relying on clinical grounds including measuring autoantibodies and fasting plasma c-peptide could be a possible viable method to minimize complications.
Combinatorial ex vivo tetramer analysis of beta cell specific CD4 T-cell responses

Gabriele Blahnik-Fagan¹, Hannes Uchtenhagen¹, I-Ting Chow¹, William Kwok¹,², Eddie James¹
¹Benaroya Research Institute, Seattle, WA, USA, ²University of Washington Department of Medicine, Seattle, WA, USA

Auto-reactive T cell responses are thought to play a key role in type 1 diabetes (T1D). However, effective monitoring and characterization of T cells that respond to beta cell epitopes in subjects with T1D has been limited by technical obstacles, including their inherently low frequencies variable responsiveness of individual subjects to single epitopes. We have recently implemented a combinatorial staining approach that allows direct ex vivo characterization of multiple CD4+ T-cell specificities in a single sample. We applied this combinatorial approach to directly measure the frequency and phenotype of beta cell specific CD4+ T cells in subjects with established T1D. For this work we utilized five peptides that were previously identified as naturally processed DRB1*04:01 restricted epitopes from proinsulin, GAD65, IA-2, and IGRP. Responses to each of these peptides can be readily detected after in vitro expansion culture. However, our results indicated that proinsulin specific T cells were consistently present at higher frequencies than GAD65, IA-2, and IGRP specific T cells. Phenotypic analysis of beta cell specific CD4+ T cells revealed that subjects with T1D had a higher frequency of proinsulin specific T cells with a memory phenotype than HLA matched controls. The majority of these memory cells were CXCR3 positive and an increased percentage were CCR7 negative, suggesting Th1-like effector memory responses in subjects with T1D. Finally, we demonstrated that this approach is compatible with combinatorial class I multimer analysis, facilitating the characterization of self-reactive CD4+ and CD8+ T cells using a single sample.
Selective recognition of self-proteins that have undergone enzymatic post-translational modification (PTM) by CD4+ T cells is increasingly implicated as a means of potentiating the loss of tolerance in autoimmune diseases such as type 1 diabetes (T1D). In particular, enzymatic deamidation and citrullination of beta cell derived peptides at HLA anchor residues enhances their presentation by high risk alleles (DR4 and DQ8). Modification of residues outside of binding pockets alter T cell recognition, resulting in enhanced proliferation and increased cytokine production. Our work demonstrates that CD4+ T cells specific for PTM epitopes are present in the peripheral blood of patients with T1D at significantly higher frequencies *ex vivo* than in HLA matched controls. Upon re-activation, PTM specific T cells from T1D patients produce elevated levels of effector cytokines, most notably interferon gamma. Among T1D patients, we observed heterogeneous frequencies of PTM specific T cells with a range of functional profiles. This led us to hypothesize that activation of a PTM specific T cell response is of particular significance in a subset of T1D patients. To test this hypothesis we compared the *ex vivo* frequency and functional profiles of PTM specific T cells in a cross sectional cohort of patients selected to include diverse levels of residual c-peptide. Our findings implicate a mechanistic role for PTMs in exacerbating autoreactive T cell responses in T1D.
Reduced serum immunoreactive trypsin in type 1 diabetes

Thato Phuthego, Anna E Long, Kyla Chandler, Ben Gillard, Kathleen M Gillespie, Alistair J.K Williams
University of Bristol, Bristol, UK

Type 1 diabetes (T1D) is caused by T-cell mediated destruction of insulin-secreting pancreatic beta cells, but many T1D patients have altered exocrine function and morphology. Levels of serum exocrine enzymes may therefore represent useful biomarkers of disease.

The aim of this study was to assess whether levels of a marker of pancreatic exocrine function, immunoreactive trypsin (IRT) was different between T1D patients and relatives at high or low risk of progression to diabetes.

A DELFIA sandwich ELISA was used to measure IRT levels in random sera from 70 T1D patients (31 males) and age and gender-matched healthy relatives. For patients, the median age at diagnosis was 10 years (range 1-37) and samples were available a median of 4 years from diagnosis (range 0-15). Of these, 34 (16 males) had samples available less than 2 years from diagnosis.

The median IRT level was 25 (range, 19-44) ng/ml in T1D patients and 32 (19-55) ng/ml in relatives (p<0.0001). IRT concentration was also reduced in patients <2 years from diagnosis compared with age and gender-matched relatives. The median IRT level in patients <2 years from diagnosis was 24 (20-44) ng/ml compared with 28 (19-52) ng/ml in relatives (p<0.0001). IRT was associated with age in patients (r=0.379, p=0.001) and relatives (r=0.256, p=0.032) but not gender.

IRT levels are decreased in T1D patients compared to low-risk relatives even shortly after diagnosis, suggesting that biomarkers of reduced exocrine function will be useful for following disease progression in at risk individuals.
Association of autoreactive memory CD4 T cells expressing the chemokine receptor CXCR3 in the peripheral blood of pancreas-kidney transplant recipients with type 1 diabetes recurrence.

Helena Reijonen¹, Gwen Werra¹, Ben Falk¹, Sahil Virdi², Francesco Vendrame³, Isaac Snowhite², Gloria Allende², George Burke⁴, Alberto Pugliese²,³
¹Benaroya Research Institute, Seattle, WA, USA, ²Diabetes Research Institute, Miller School of Medicine, University of Miami, Miami, FL, USA, ³Department of Medicine, Miller School of Medicine, University of Miami, Miami, FL, USA, ⁴Department of Surgery, Miller School of Medicine, University of Miami, Miami, FL, USA

Patients with T1D may become recipients of simultaneous pancreas-kidney (SPK) transplants to restore insulin secretion and kidney function; 5-6% of SPK recipients develop T1D recurrence (T1DR) on follow-up (Diabetes, 2010) despite immunosuppression that prevents rejection. T1DR is preceded by seroconversion for multiple autoantibodies and these are risk factors for T1DR (AJT, 2016). We are investigating the role of memory T cells in T1DR. We report data from 5 SPK recipients with T1DR and 11 with normal glucose tolerance (NGT). We studied autoreactive T cells using a pool of MHC class II tetramers loaded with T1D-associated peptides from multiple autoantigens. T1DR patients had higher frequency of autoreactive CD4 T cells than NGT patients (p=0.0018). Regardless of diabetes status, patients with autoantibody conversions had higher frequency of autoreactive CD4 T cells than patients who lacked or had persisting autoantibodies from prior to transplant (stable) (p=0.0326). These increased frequencies were observed in the memory compartment and not in the naïve compartment. Our results support an association of memory autoreactive CD4 T cells with T1DR and autoantibody conversion. Most converters in the NGT and T1DR groups (all T1DR patients were converters) had higher proportions of CXCR3+, autoreactive CD4 T memory T cells than stable SPK patients (p=0.0099). Significant increases in memory autoreactive T cells appear more likely near diagnosis, perhaps representing a later event that precipitates beta cell destruction. If validated by more extensive studies, CXCR3 may be a potentially suitable therapeutic target to antagonize islet autoimmunity.
Characterization of a Novel Pre-Diabetic Murine Model for Type 1 Diabetes

Emily Esakov¹, Nirdesh Gupta¹, Brigid Gregg², Neha Nandedkar¹, Ali Al-Dieri¹, Marica McInerney¹
¹University of Toledo, Toledo, Ohio, USA, ²University of Michigan, Ann Arbor, MI, USA

In the non-obese diabetic (NOD) mouse model, T1D is an autoimmune disease characterized by insulitis and T cell-mediated destruction of pancreatic islet beta cells. Insulin receptor (IR) is a chemotactic receptor capable of driving T cell movement in response to insulin. In published work, purified T cells, obtained from diabetic mice, and sorted into IR positive and negative cells were adoptively transferred into irradiated nondiabetic NOD mice. Only recipients that received IR positive T cells had insulitis and diabetes. If the IR expressing cells are moving into the pancreas based on an insulin gradient, then artificially making T cells with high IR expression should allow for movement into the pancreas in mice that are not susceptible to T1D.

A novel transgenic mouse model was made that has a FLAG tagged mouse insulin receptor behind a CD3 promoter and enhancer for expression on T cells in a mouse not susceptible to T1D development (C57BL/6). Transgenic BL/6-CD3FLAGmIR mice show evidence of T cell trafficking into the pancreas. Although insulitis occurs, no diabetes has developed. However, metabolic abnormalities have been observed based on intraperitoneal glucose tolerance testing, insulin response to glucose challenge, and staining of pancreatic islets. Characterizing IR expression on T cells may be of key importance for understanding the pathogenesis of diabetes. If IR expression is established as a mechanism to move T cells into islets, then blocking chemotaxis, via the IR, would provide a new therapeutic target to block cell movement into the pancreas, thus preventing T1D.
Infiltration of identical T cell repertoires in multiple organs with autoimmunity in NOD mice

Sydney Look, Laurie Landry, Theodore Williams, Thomas Delong, Kathryn Haskins, Maki Nakayama
University of Colorado Denver, Aurora, CO, USA

Patients with one autoimmune disease, such as type 1 diabetes, are at a higher risk for developing additional autoimmune disease(s) than healthy individuals. Similarly, NOD mice spontaneously develop autoimmune diabetes and Sjögren's syndrome. In addition to a genetic predisposition, we hypothesized that T cells activated in a primary organ may play a role in the development of secondary autoimmune diseases. To determine if and how often identical T cells infiltrate multiple targeted organs and whether the infiltration is antigen-specific, we analyzed T cell receptor (TCR) repertoires in pancreatic islets, salivary glands, and peripheral blood of NOD mice and tested reactivity of dual-infiltrating TCRs to these tissues. Salivary glands contained a higher frequency of overlapping TCR sequences with islets than peripheral blood (32.4 ± 11.3% with islets v.s. 13.1 ± 2.7% with peripheral blood, p=0.03). In addition, the single cell TCR analysis identified identical TCR pairs at the nucleotide level including a second alpha/beta sequence in both islets and salivary glands, indicating the presence of T cells derived from same monoclonal T cells in these organs. Six out of 7 TCRs found in both islets and salivary glands responded to islets but not to salivary glands. In conclusion, we demonstrated the significant proportion of identical T cells infiltrating pancreatic islets and salivary glands in NOD mice. However, the current study does not support the concept that T cells in an affected organ migrate to another due to antigen specificity.
Possible Link Between Maternal Mid-Pregnancy Macrophage Chemoattractants and Childhood Type 1 Diabetes

Karl Mårild1,7, Maria Vistnes2,7, German Tapia1,7, Øyvind Midttun3, Per M Ueland4, Torild Skrivarhaug5, Pål R Njølstad6, Geir Joner5, Ketil Størdal1, Lars C Stene1

1Norwegian Institute of Public Health, Oslo, Norway, 2Diakonhjemmet Hospital, Department of Internal Medicine, Oslo, Norway, 3Bevital AS, Bergen, Norway, 4University of Bergen, Department of Clinical Science and Haukeland University Hospital, Laboratory of Clinical Biochemistry, Bergen, Norway, 5Oslo University Hospital, Department of Paediatric and Adolescent Medicine and University of Oslo, Institute of Clinical Medicine, Oslo, Norway, 6University of Bergen, KG Jeepsen Center for Diabetes Research and Haukeland University Hospital, Department of Paediatrics, Bergen, Norway, 7These authors contributed, equally, Norway

Background: Elevated levels of circulating cytokines are found in patients with type 1 diabetes (T1D), and the immunological status in utero is suspected to contribute to the development of the disease. This study therefore aimed to test whether immunological biomarkers in mid-pregnancy or at birth predicted increased risk of childhood T1D.

Methods: In total 20 immunological biomarkers were quantified in maternal mid-pregnancy plasma samples (CCL2, CCL3, CCL4, CXCL10, granulocyte-macrophage colony stimulating factor (GM-CSF), interferon (IFN)-γ, interleukin (IL)-1β, -2, -4, -5, -6, -10, -12p70, -13, -17A, IL-1 receptor antagonist, IL-2 receptor-α, tumor necrosis factor and umbilical-cord plasma at birth (neopterin and kynurenine-to-tryptophan ratio), of 188 children with T1D and 571 randomly selected controls included in the Norwegian Mother and Child Cohort Study.

Results: Although none of the other immunological markers were associated with T1D at the predefined significance level 0.01, a tendency (p-values <0.05) towards higher levels of CCL2, CCL3, CCL4, kynurenine-to-tryptophan ratio in cases than in control subjects was found. For CCL2, this tendency remained after adjusting for potential confounders, including gender, premature birth, birth weight, parity, infections and smoking during pregnancy, as well as maternal age, T1D, HLA type, age, and pregestational body mass index.

Conclusion: While mid-pregnancy plasma cytokine levels reflecting macrophage activation tended to be associated with an enhanced risk of T1D development in the offspring, biomarkers associated with other branches of the maternal immune system were not.
Blocking allele specific antigen presentation with methyldopa in type 1 diabetes

Aimon Alkanani¹, Kristen McDaniel¹, Scott Brackett¹, David Ostrov², Peter Gottlieb¹, Aaron Michels¹
¹University of Colorado, Aurora, CO, USA, ²University of Florida, Gainesville, FL, USA

Specific HLA genes confer type 1 diabetes (T1D) risk and protection. HLA-DQ8 (DQB*0302) provides an odds ratio for disease development of 6.5-11, while DQ6 (DQB*0602) is 0.03. The dichotomy of risk highlights the importance of antigen presentation in T1D. HLA-DQ8 is present in ~60% of T1D patients, making it an attractive therapeutic target. Using in silico molecular modeling and docking, FDA approved drugs were docked into each of 4 pockets along the DQ8 peptide binding groove. Methyldopa, a clinically well-established antihypertensive drug, was identified as a lead candidate. Methyldopa inhibited DQ8 antigen presentation and T-cell responses to natural and post-translational modified peptides in vitro and in DQ8 transgenic mice. We evaluated methyldopa treatment in an open-label phase 1b dose escalation study with 20 DQ8+ T1D subjects, ages 18-46 years (mean 25) with diabetes ≤2 years (mean 133-days). All subjects tolerated low (500mg BID) and moderate (500mg TID) doses, while 18/20 tolerated the high dose (2-3g/day). There were no serious adverse events. There was a reduction from baseline in DQ8 antigen presentation by PBMCs with a low dose (p=0.001), moderate dose (p<0.001) and high dose (p=0.02), which returned to normal 6 weeks after stopping therapy. The response was specific as DR4 and DQ2 responses were not affected. Patients had good glycemic control and 2-hour AUC for C-peptide following a MMTT at 12 weeks was similar to baseline levels. Long-term randomized double-blinded placebo controlled trials are needed to evaluate the exact role of methyldopa in potentially preserving residual beta-cell function in T1D.
Impaired β-cell antigen expression in the pancreatic lymph nodes contributes to gender bias in diabetes onset in NOD mice

Martina Damo, Jeffrey Hubbell
Institute for Molecular Engineering, University of Chicago, Chicago, IL, USA

The onset of autoimmune diabetes is associated to reduced expression of β cell antigens in the pancreatic lymph node stromal cells (pLNSCs) of female NOD mice as compared to diabetes-free female NOD-B10 mice. Such reduction leads to impaired immune tolerance towards pancreatic antigens and subsequent increased risk of diabetes.

70-80% of NOD females turn diabetic by the age of 12-16 weeks as opposed to only 20-30% of male NOD mice, although the reasons for such bias remain poorly investigated. Our working hypothesis is that the expression of pancreatic antigens by pLNSCs is reduced in female NOD mice as compared to age-matched male NOD mice.

We have sequenced the RNA from the pLNs of 6, 10 and 14 week old female and male NOD mice. The expression level of β cell antigens is significantly reduced in females as compared to age-matched males and it is especially reduced in older mice. Co-culture of NOD pLNSCs with insulin-specific G9C8 CD8+ T lymphocytes has confirmed that female pLNSCs have reduced insulin presentation capacity as compared to age-matched male pLNSCs and that insulin presentation is reduced in the pLNSCs from NOD mice at the age of diabetes onset (14 weeks). Our data confirm that differences in the gene expression levels and in the antigen presentation capacity of β cell antigens by pLNSCs exist between diabetes-prone female and diabetes-resistant male NOD mice and that such differences could contribute to the higher risk of autoimmune diabetes in NOD females.
Identification of an HLA-A3 restricted ZnT8-reactive islet-infiltrating CD8 T cell line derived from a donor with long-term type 1 diabetes

Jenny Aurielle Babon1, Megan DeNicola1, David Harlan1, Rita Bottino2, Alvin Powers3, Roberto Mallone4, Sally Kent1

1Department of Medicine, Division of Diabetes, Diabetes Center of Excellence, University of Massachusetts Medical School, Worcester, MA, USA, 2Institute of Cellular Therapeutics, Allegheny-Singer Research Institute, Pittsburgh, PA, USA, 3Diabetes, Endocrinology and Metabolism, Vanderbilt University Medical Center, Nashville, TN, USA, 4INSERM, U1016, Institut Cochin, DeAR Lab, Paris, France

The zinc transporter 8 (ZnT8) was previously reported to be a major type 1 diabetes antigen. An HLA-A2 restricted immunodominant epitope in the transmembrane domain of ZnT8, ZnT8186-194, was recently identified and ZnT8186-194-specific CD8 T cell responses were detected in recent onset HLA-A2+ T1D patients. An epitope-binding algorithm (SYFPEITHI) predicted a binding score of 23 for HLA-A3 to a 10-mer containing the HLA-A2 restricted peptide sequence. We have previously isolated and expanded CD8 T cells from the islets of an HLA-A3+, 30 year old male donor with 20 years of T1D (HLA A1, A3, DR1, DR4) by culturing handpicked islets on matrigel with media supplemented with T cell growth factors and cytokines. By intracellular cytokine staining, we identified a CD8 T cell line that produced IFNγ and TNFα in the presence of autologous targets that were pulsed with either the ZnT8185-194 or ZnT8186-194 peptide but not to other peptide nonamers. This ZnT8-specific CD8 T cell line is restricted by HLA-A3 as it responds to ZnT8 peptide pulsed-autologous BLCLs and to BLCLs that express HLA-A3, but not to targets that do not have HLA-A3 expression. This suggests a novel ZnT8-CD8 T cell epitope that is restricted by HLA-A3 as identified from an islet-infiltrating T cell.
Times of Peak Innate and Adaptive Immunity in Type 1 Diabetes (T1D) when Sampling from Peripheral Blood Subsets - Potential Implications for Study Design and Assessing Immune Function and Therapeutic Interventions

McKenzie Akers¹, Heather Rauch¹, Alyssa Woodwyk¹, Thomas Blok¹, Patrice Mason¹, Daniel Perry², Todd Brusko², Mark Anderson², Clive Wasserfall², Craig Beam¹

¹Western Michigan University Homer Stryker M.D. School of Medicine, Michigan, USA, ²University of Florida, Florida, USA

Our current understanding of the potential influence of circadian rhythms on cellular immune populations thought key to T1D development is quite limited. Hence our objective for this study was to address normal cellular fluctuations in both innate and adaptive immune subsets. We hypothesized such efforts could lead to an improved understanding of the influence of time of collection on studies seeking to understand the pathogenesis of T1D and its treatment. **METHODS:** Venous blood samples (10 cc) were drawn from 9 healthy volunteers every 4 hours over a 24 hour inpatient period, followed by extensive flow cytometric phenotyping. Timing of peak peripheral blood frequencies were determined with a statistical method for fitting a cosine curve to 24 hr data (COSINOR). **RESULTS:** We observed many major cell populations with significant (p<0.05) circadian patterns. Such cell populations and their time of peak (military) were:

- CD4Tcell(00:30)
- Classical Monocytes(02:00)
- Treg(02:00)
- CD4+CD8+(02:00)
- CD56bright NK(03:00)
- CD8Tnaive(03:00)
- CD8T(05.30)
- CD4+CD8-(11:00)
- Monocytes(12:00)
- CD4Temra(12:00)
- CD8Temra(12:30)
- NK(13:00)
- CD4Tem(13:00)
- CD8Tem(13:00)
- DC(14:00)
- CD56dim NK (15:00)
- NK-T(15:00)
- CD4Tcm(20:00)
- CD8Tcm(21:00)
- B-Cells(22:00)
- Monocytes(23:00)

**CONCLUSIONS:** There is appreciable heterogeneity in the timing of peak circulating immune cell frequencies measured by PBMC sampling. This heterogeneity suggests that T1D autoimmunity studies need to carefully consider PBMC timing as an important part of study design, and suggests the idea of multiple sampling throughout the day. These findings are also expected to have important implications for transcriptional gene profiles and immune function.
Population-based screening for early stage type 1 diabetes in children aged 2 to 5 years: results from the Fr1da study

Peter Achenbach¹, Florian Haupt¹, Christiane Winkler¹, Andreas Beyerlein¹, Robin Assfalg¹, Stephanie Zillmer¹, Nicole Maison¹, Melanie Heinrich¹, Katharina Warncke¹, Karin Lange², Michael Powell³, Jadwiga Furmaniak⁴, Bernard Rees Smith³, Ezio Bonifacio⁴, Anette-G. Ziegler¹

¹Institute of Diabetes Research, Helmholtz Zentrum München, Munich, Germany, ²Department of Medical Psychology, Hannover Medical School, Hannover, Germany, ³FIRS Laboratories, RSR Ltd, Cardiff, UK, ⁴Center for Regenerative Therapies Dresden, Technische Universität Dresden, Dresden, Germany

Type 1 diabetes (T1D) can be diagnosed at an early pre-symptomatic stage by detection of beta cell autoantibodies. The Fr1da study aims to assess whether early staging of T1D (1) is feasible at a population-based level, (2) prevents severe metabolic decompensation at clinical manifestation of T1D, and (3) reduces psychological distress through preventive teaching and care.

Fr1da will screen 100,000 children from Bavaria aged 2-5 years for the presence of multiple beta cell autoantibodies. Capillary blood, obtained by primary care pediatricians, is tested for antibodies to GAD, IA-2, and ZnT8 using a multiplex 3-screen ELISA. Samples with ELISA results >98th centile are re-tested in separate GAD, IA-2, ZnT8, and insulin autoantibody radiobinding assays. Multiple autoantibody-positive children, confirmed in a second blood sample, are diagnosed with early stage T1D and families are invited to participate in an education and counselling program. Depression, anxiety and burden of early diagnosis are assessed.

After 75 weeks of screening, 144 (0.33%) of 43,547 children have been diagnosed with early stage T1D. Of those, 7 already had manifest T1D based on random blood glucose. An oral glucose tolerance test was done in 105 children (32 pending); 3 had manifest T1D based on 2-hour blood glucose, 6 were dysglycemic and 96 normoglycemic. Of the latter, 4 developed manifest T1D on follow-up. None of 14 children experienced ketoacidosis at clinical onset of T1D.

In conclusion, staging for early T1D within a public health setting appears to be feasible. Data on 100 weeks of Fr1da screening will be presented.
Virus-induced molecular signatures in the human pancreatic tissue at the onset of type 1 diabetes and upon *in-vitro* infection

Henna Kallionpää¹, Juhi Somani², Mahesh Anagandula³, Soile Tuomela¹, Niina Lietzén¹, Lingjia Kong¹, Sami Oikarinen⁴, Riikka Lund¹, Omid Rasool¹, Heikki Hyöty⁴, Gun Frisk³, Harri Lähdesmäki¹,², Knut Dahl-Jørgensen⁵, Lars Krogvold⁵, Riitta Lahesmaa¹

¹Turku Centre for Biotechnology, University of Turku and Åbo Akademi University, Turku, Finland, ²Aalto University, Espoo, Finland, ³Uppsala University, Uppsala, Sweden, ⁴University of Tampere, Tampere, Finland, ⁵Oslo University Hospital, Oslo, Norway

Recent studies support the role of Enteroviruses, and more specifically, of Coxsackievirus B1 (CBV) infections, in the pathogenesis of type 1 diabetes. To further address the role of CBV we have studied pancreatic tissue from living T1D patients and how islet cells respond to CBV infection.

We have performed RNAseq analysis on four pancreatic tail resections obtained by laparoscopy in the Norwegian Diabetes Virus Detection study (DiViD) from live, young adult patients recently diagnosed with T1D. Enteroviral capsid protein 1 (VP1) was detected in the islets of all these patients by immunohistochemistry. The analysis revealed approximately 400 genes to be differentially regulated when compared to healthy organ donors. Moreover, analysis of serum proteomics from the DiViD patients is in progress.

We have also investigated the transcriptional response of hand-picked human pancreatic islets *in-vitro* infected with CBV-1 (two strains) and CBV-4 (model of non-lytic infection). CBV-1 strains caused a strong transcriptional response (on average 1367 differentially genes at day 4 post-infection) compared to effects induced by CBV-4 (157 differentially regulated genes at day 4). For example, insulin, islet amyloid polypeptide and somatostatin were downregulated with CBV-1 strains but not with CBV-4.

We will integrate the data produced at different measurement levels, correlate it to our previous findings on peripheral blood RNA and proteome data of prediabetic children, as well as with published data on virus responses in different tissues. Our results on CBV-induced host response provide new insights into their role in T1D.
Functional Analysis of Islet Antigen Specific-CD8+ T Cells in Type 1 Diabetes Using T Cell Libraries

Hideki Ogura¹, Paula Preston-Hurlburt¹, Cate Speake², Carla Greenbaum², Kevan Herold¹
¹Department of Immunobiology, Yale University, New Haven, CT, USA, ²Diabetes Clinical Research Program, Benaroya Research Institute, Seattle, WA, USA

Recent studies identified pancreatic islet antigen-specific CD8+ T cells in patients with type 1 diabetes (T1D) and healthy subjects with comparable frequencies, however the difference in function of these cells in the immune response was not fully understood. Other groups have used T cell library to assess the reactivity of CD4+ T cells to pathogens or myelin autoantigens, but CD8+ T cell libraries have not been previously studied. We generated approximately 6,000 CD8+ T cell libraries from peripheral blood mononuclear cells of 11 patients and 8 healthy subjects and studied the reactivity against islet antigen peptides including preproinsulin, IGRP, IA-2, and ZnT8. The frequency of T cell libraries having islet antigen-specific CD8+ T cells with robust IFNg production was significantly higher in the libraries from CD45RO+ fraction of T1D patients compared to healthy subjects. The frequency declined over time after onset of the disease ($r^2=0.36$, $p=0.011$). Besides IFNg, these cells produced higher levels of inflammatory cytokines such as TNF-a, IL-6 and IL-2. The libraries were further expanded and responses to islet antigen peptides were interrogated. ZnT8 was a major antigen recognized by these cells. High dimensional analysis by CyTOF with multi-channel MHC tetramer staining revealed phenotypic characteristics of the islet antigen-specific CD8+ T cells in the T1D patients. These data imply the functional difference of islet antigen specific CD8+ T cells between T1D patients and healthy subjects, and suggest possible prognostic/therapeutic targets.
Maternal Use of Dietary Supplements During Pregnancy and Risk of Celiac Disease in the Offspring

Jimin Yang¹, Roy Tamura¹, Carin Aronsson², Ulla Uusitalo¹, Åke Lernmark², Marian Rewers³, William Hagopian⁴, Jin-Xiong She⁵, Jorma Toppari⁶, Anette Ziegler⁷, Beena Akolkar⁸, Jeffrey Krischer¹, Jill Norris³, Suvi Virtanen⁹, Daniel Agardh²

¹University of South Florida, Tampa, FL, USA, ²Lund University, Malmö, Sweden, ³University of Colorado, Aurora, CO, USA, ⁴Pacific Northwest Diabetes Research Institute, Seattle, WA, USA, ⁵Augusta University, Augusta, GA, USA, ⁶University of Turku and Turku University Hospital, Turku, Finland, ⁷Institute of Diabetes Research, Technische Universität München, Neuherberg, Germany, ⁸National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, USA, ⁹National Institute for Health and Welfare, Helsinki, Finland

Perinatal exposure to nutrients and dietary components may affect the risk of celiac disease (CD). We investigated the association between maternal use of vitamin D, omega-3 fatty acids (n-3 FAs), and iron supplements during pregnancy and risk of CD autoimmunity (CDA) and CD in the offspring. Children at increased genetic risk were prospectively followed from birth in The Environmental Determinants of Diabetes in The Young (TEDDY) Study. CDA was defined as having persistently positive tissue transglutaminase autoantibodies (tTGA). Diagnosis of CD was either biopsy-confirmed or considered likely if having persistently elevated levels of tTGA >100 AU. Of 6627 enrolled children, 1136 developed CDA at median 3.1 years of age (range 0.9-10) and 409 celiac disease at median 3.9 years of age (range 1.2-11). Use of supplements containing vitamin D, n-3 FAs, and iron was recalled by 66%, 17%, 94% of the mothers, respectively, at 3-4 months postpartum. The mean cumulative intake over the entire pregnancy was 2014 mcg vitamin D (SD 2045 mcg), 111 g n-3 FAs (SD 303 g), and 8806 mg iron (SD 7017 mg). After adjusting for country, child's HLA-genotype, sex, family history of celiac disease, exclusive breastfeeding duration, and household crowding, Cox proportional hazard ratios did not suggest statistically significant association between the intake of vitamin D, n-3 FAs, or iron and risk of CDA or CD. Dietary supplementation during pregnancy may help boost nutrient intake, but it is not likely to modify the risk of the disease in the offspring.
Genetic and pharmacological disruption of the AICDA-RAD51 axis results in the accumulation of CD73+ B-lymphocytes with T1D suppressive capacities

Jeremy Ratiu1, Muneer Hasham1, Jeremy Racine1, Jane Brance1, Qiming Wang1, Harold Chapman1, Caroline Leeth2, Kevin Mills3, Dave Serreze1

1The Jackson Laboratory, Bar Harbor, ME, USA, 2Department of Animal and Poultry Sciences, Virginia Polytechnic and State University, Blacksburg, VA, USA, 3Cyteir Therapeutics, Cambridge, MA, USA

We assessed the role of AICDA mediating B-lymphocyte class switching (CSR) and somatic hypermutation (SHM) in T1D development. CRISPR-Cas9 methodology was used to create Aicda deficient NOD mice that proved to be strongly T1D resistant, but displayed similar characteristics of pre-clinical pathogenesis. While unable to undergo SHM and CSR, B-lymphocyte numbers were not decreased within spleens, pancreatic lymph nodes (PLN) and islets of NOD.Aicda-/- mice. Adoptive transfer studies to NOD-scid recipients indicated NOD.Aicda-/- B-lymphocytes play an active T1D suppressive role. Such protection was mediated by the increased numbers of CD73+ B-lymphocytes observed within the spleen, PLN and islets of NOD.Aicda-/- mice. We found both in vivo and in vitro that B-lymphocyte expressed CD73 molecules mediating hydrolysis of AMP to adenosine exert immunosuppressive effects upon T-lymphocyte activation. In addition to those within immunoglobulin gene sequences required to induce CSR and SHM, AICDA also induces DNA strand breaks throughout the genome that if not repaired in a RAD51 complex dependent manner result in B-lymphocyte death. Thus, we also tested if T1D development in NOD mice was inhibited by treatment with the RAD51-inhibitory 4,4’-disothiocyanatostilbene-2,2’-disulfonic acid (DIDS) small molecule targeting B-lymphocytes undergoing SHM and CSR. Pharmacological inhibition of RAD51 in NOD mice recapitulated the T1D protective effects of Aicda gene ablation including an expansion of CD73+ B-lymphocytes. These results indicated targeting of the AICDA-RAD51 axis in maturing autoreactive B-lymphocytes has immunomodulatory effects resulting in the expansion of populations with regulatory activity, and can be utilized as a therapeutic strategy for T1D prevention.
Maternal and Neonatal Vitamin D Status and Risk of Type 1 Diabetes in Norway and Denmark

Karl Mårild1,3, Steffen U Thorsen2,3, Sjurdf F Olsen4, Klaus K Holst5, German Tapia1, Charlotte Granström6, Thorhallur I Halldorsson6, Arieh Cohen4, Margaretha Haugen1, Marika Lundquist4, Torild Skrivarhaug6, Pål R Njølstad7, Geir Joner6, Per Magnus1, Ketil Størdal1, Alberto Ascherio8, Jannet Svensson2,9, Lars C Stene1,9

1Norwegian Institute of Public Health, Oslo, Norway, 2Herlev University Hospital, Herlev, Denmark, 3These authors contributed, equally, Denmark, 4Statens Seruminstitut, Copenhagen, Denmark, 5University of Copenhagen, Department of Biostatistics, Copenhagen, Denmark, 6Oslo University Hospital and University of Oslo, Oslo, Norway, 7Haukeland University Hospital and University of Bergen, Bergen, Norway, 8Harvard University, Boston, MA, USA, 9These authors contributed, equally, Norway

Perinatal vitamin D status has been proposed to influence the risk of type 1 diabetes (T1D), but the few available studies have shown mixed results. We aimed to study this potential association in two case-control studies nested within the Norwegian Mother and Child Cohort Study (MoBa) and the Danish National Birth Cohort (DNBC). We identified T1D from nationwide diabetes registries. Plasma samples were collected from the first trimester of pregnancy (DNBC only), second trimester, cord blood, and from the mother soon after delivery (MoBa only). A total of 3966 samples from 448 cases and 1172 randomly selected controls were assayed for 25-hydroxyvitamin D (25OHD) using liquid chromatography followed by tandem mass spectrometry. 25OHD was substantially lower in cord blood than maternal pregnancy blood. The average, season adjusted plasma 25OHD during pregnancy based on all available measurements were estimated from a random intercept linear regression model, and then used as the exposure in a logistic regression model. The average 25OHD in pregnancy (modelled for week 25 of pregnancy) was 57nM in DNBC and 64nM in MoBa. The overall odds ratio per 10nM increase in average 25OHD in pregnancy after adjustment for country, was 0.925, 95%CI: 0.84, 1.02. There were no significant curvilinear dose-response relationship. Cord blood 25OHD alone was also not significantly associated with T1D. Based on this preliminary analysis, we conclude that the average plasma 25-hydroxyvitamin D concentration during pregnancy was not significantly related to risk of childhood onset T1D.
Systems Analyses of Large, Cross-Sectional Cohorts Highlight the Prominence of Both Memory T Cell Activation and Early B Cell Maturation in T1D

S. Alice Long¹, Sara Murray², Scott Presnell², Karen Cerosaletti³, Jerill Thorpe¹, Katharine Schwedhelm¹, Charlie Quinn², Cate Speake³, Carla J Greenbaum³, Jane Buckner¹
¹Translational Research Program, Benaroya Research Institute, Seattle, WA, USA, ²Systems Immunology Program, Benaroya Research Institute, Seattle, WA, USA, ³Diabetes Clinical Research Program, Benaroya Research Institute, Seattle, WA, USA

Cellular phenotypes associated with T1D likely include multiple cell types, which together, may offer clues to pathogenesis and guide therapeutic choices. However, defining and ranking these phenotypes has been challenging due to patient heterogeneity, cellular variability, and limited global cellular analyses. Thus, we designed a large (n=100/group), age and gender matched, cross-sectional study of control and T1D subjects including approximately 300 reproducible (intra-assay CV; median 0.13) traits of B and T cells using 3 steady-state and 4 cytokine stimulation flow panels. Clustering immunological traits and subject traits within each cell type in a heatmap clearly identified cellular traits as the defining features, not disease, age, or gender. Comparing individual immunological features, we found that memory Treg and Teff activation state (HLA-DR, CD95, CD25), transitional B-cell IgD and CD95 expression and plasmablast traits were enriched in T1D. These results were confirmed using machine learning based feature selection as an exploratory tool to identify traits that define T1D. Using an alternative approach, we first correlated all pairs of traits within controls and T1D. Then, these correlations were compared between cohorts, revealing a network of altered relationships in T1D. IgD, CD40, IgM on B-cells and IL-2, CD38, CCR4, HLA-DR, and CD71 on T cells were enriched in correlations altered in T1D. Together, our findings offer a more comprehensive understanding of the phenotypes associated with T1D highlighting activation state of memory T cells and early B-cell traits, with implications for selection and stratification of subjects for treatment of T1D with immune-mediated therapies.
Zinc Transporter-8 Autoantibodies are a Late Biomarker in Risk of Clinical Onset in Genetically Type 1 Diabetes High-Risk Children: The TEDDY Study

Kendra Vehik1, Beena Akolkar2, William Hagopian3, Marian Rewers4, Jorma Toppari5, Anette G. Ziegler6, Jin-Xiong She7, Ezio Bonifacio6,9, Ake Lernmark10, Jeffrey Krischer1, TEDDY Study Group2
1Health Informatics Institute, Morsani College of Medicine, University of South Florida, Tampa, FL, USA, 2National Institute of Diabetes & Digestive & Kidney Diseases, Bethesda, MD, USA, 3Pacific Northwest Diabetes Research Institute, Seattle, WA, USA, 4Barbara Davis Center for Childhood Diabetes, University of Colorado, Aurora, CO, USA, 5Departments of Physiology and Pediatrics, Turku University Hospital, Turku, Finland, 6Institute of Diabetes Research, Helmholtz Zentrum München, and Klinikum rechts der Isar, Technische Universität München, and Forschergruppe Diabetes e.V., Neuherberg, Germany, 7Center for Biotechnology and Genomic Medicine, Medical College of Georgia, Augusta University, Augusta, GA, USA, 8Center for Regenerative Therapies, University of Technology, Dresden, Germany, 9Paul Langerhans Institute Dresden, German Center for Diabetes Research (DZD), Dresden, Germany, 10Department of Clinical Sciences, Lund University/CRC, Skane University Hospital, Malmö, Sweden

Previous studies suggest ZnT8A may increase risk of T1D independent of other autoantibodies. TEDDY prospectively evaluated ZnT8A as a contributor of T1D risk. Children at genetic high-risk for T1D (n=8676) were followed until age 10 years for development of GAD65, insulin and IA-2 autoantibodies (AAb) (n=667) defined as positive in two samples 3 months apart and confirmed in another lab. ZnT8A was similarly tested in samples of children with at least one non-ZnT8 AAb. Time-varying Cox regression analysis adjusting for HLA, gender, country of residence and T1D family history examined whether ZnT8A was associated with risk of T1D (n=200) separately for children with a single or multiple non-ZnT8 AAb. At time of initial seroconversion ZnT8A was found in 5% (29/554) with a single non-ZnT8 AAb+. When a second non-ZnT8 AAb+ appeared, 21% (n=84/395) had ZnT8A, of which 57/84(68%) with and 27/84(32%) without ZnT8A had IA-2A, which is rarely a first appearing AAb. Of those who developed T1D, 55%(n=109/200) were ZnT8A+, of whom 97% had multiple non-ZnT8 AAb. Regardless if the child had only a single non-ZnT8 AAb (HR=0.80, p=0.24) or multiple non-ZnT8 AAb (HR = 0.80, p<0.0001), the appearance of ZnT8A was associated with a reduced risk for T1D. ZnT8A does not contributed to increased risk of T1D when accounting prospectively for whether or not child had multiple non-ZnT8 AAb. Time dependent adjustment for other autoantibodies needs to be considered when examining ZnT8A as a risk factor for T1D as ZnT8A appears more frequently in children with multiple non-ZnT8A autoantibodies.
Cloning and expression of IA-2 reactive antibody fragments from single human B cells

Carolyn Johnson1, Kerry McLaughlin2, Richard Fettbower3, Michael Christie1
1University of Lincoln, Lincoln, UK, 2University of Oxford, Oxford, UK, 3University of Leeds, Leeds, UK

B-cells likely play a role in Type 1 diabetes by acting as efficient antigen presenting cells. Further characterization of the role that B-cells play in disease has been hampered by the limited availability of cloned autoantigen-specific B-cells from diabetic patients, largely as a consequence of their low frequency in the circulation. The aim of this study was to determine whether autoantibodies could be cloned by FACS sorting of single B-cells labelled with fluorescence-tagged antigen, followed by amplification, cloning and expression of immunoglobulin variable region genes from the individual sorted cells. IA-2 was used as the target autoantigen. CD19+/CD27+ memory B-cells were magnetically purified from blood mononuclear cells of IA-2 antibody positive Type 1 diabetic patients and incubated with FITC-labelled IA-2. Cells were washed and single labelled cells sorted by FACS. Of the B-cell population, 0.01% were labelled with the IA-2 construct and nine FACS-purified single B-cells were selected for amplification of heavy and light chain V-regions of IgG transcripts in nested PCR reactions using pooled V region primers. Four of these cells generated heavy chain sequence and two produced light chains. The two paired heavy and light chains were assembled as single chain Fv fragments and expressed in E coli. Both were shown to specifically bind the target IA-2 antigen by radioligand binding assay. The results demonstrate that FACS purification of autoantigen specific B-cells followed by amplification and expression of B-cell receptors is an efficient approach to increase the repertoire of cloned islet autoantibodies for studies in Type 1 diabetes.
The Hierarchy of Energy Demanding Processes in human CD4+ T cells

Lisa Holthaus¹, Daniel Lamp¹, Jan Knoop², Anette-Gabriele Ziegler²,³, Martin Jastroch¹,⁴
¹Institute for Diabetes and Obesity, Helmholtz Zentrum Munich, Munich, Germany, ²Institute of Diabetes Research, Helmholtz Zentrum Munich, Munich, Germany, ³Forscherguppe Diabetes e.V., Munich, Germany, ⁴Center for Regenerative Therapies, Dresden, Germany

Upon activation by stimulation, T cells execute effector functions by increased proliferation and cytokine production. The increased energy required for these functions is via production of ATP from glycolysis and oxidative phosphorylation. Here, we examined the sensitivity of T cell functions to energy supply and hypothesized that there is a hierarchical order of energy-demanding processes during the activation of human CD4+ T cells. We measured functional readouts including early activation status, proliferative capacity and cytokine production of bulk human CD4+ T cells. In parallel, real-time measurements of the respiratory and glycolytic flux was monitored. To restrict mitochondrial ATP production, low affinity inhibitors of mitochondrial respiration, rotenone and oligomycin, were added. At various stages of T cell activation and function, we found distinctive requirements for mitochondrial ATP. We consistently found that T cell cytokine secretion was most susceptible to ATP restriction, followed by proliferation and early activation status, thus revealing a hierarchy of energy demand in activated T cells. This hierarchy was similar for Th1, Th2, Th17, and Th17 associated cytokine production. Thus, conditions of mitochondrial stress have inhibitory functions on T cell function. We are currently exploring whether the hierarchy is the effects of various mitochondrial inhibitors with different targets in T cell energy metabolism, and how they relate to T cell function.
Predictors of Progression from Dysglycemia to Diabetes in the TrialNet Pathway to Prevention Study

Diane Wherrett1, Polly Bingley2, David Boulware4, Michael Haller5, Jennifer Marks5, Henry Rodriguez4, Jerry Palmer2

1Hospital for Sick Children/University of Toronto, Toronto, Ontario, Canada, 2University of Washington and VA Puget Sound Health Care System, Seattle, Washington, USA, 3University of Bristol, Bristol, UK, 4University of South Florida, Tampa, Florida, USA, 5University of Miami, Miami, Florida, USA, 6University of Florida, Gainsville, Florida, USA

Dysglycemia, defined as fasting glucose 110-125 mg/dL, 2 hour glucose 140-199 mg/dL or 30, 60, or 90 minute value on oral glucose tolerance testing (OGTT) ≥ 200mg/dL, is highly predictive of progression to type 1 diabetes (T1D), particularly in children. The TrialNet Pathway to Prevention study monitors high risk family members of those with T1D every 6 months with OGTTs. We analyzed data from 1127 participants (271 developed T1D) to find factors associated with progression to T1D using Cox proportional regression analysis with univariate and multivariable stepwise models. In the multivariable analysis, significant hazard ratios (HR) were found for ICA512/IA2 Ab positivity (2.03, p=0.004), # of antibodies (1.21, p=0.040), DPT1 risk score (DPTRS) (2.79, p<0.0001) and HbA1c (2.99, p<0.0001). Examining those ≥18 y.o. (n= 437, 48 developed T1D), significant HR were found for ICA+ve (4.76, p=0.005), DPTRS score (1.46, p=0.05), Index60 (2.46, p=0.02) and HbA1c (15.9, p=0.0005). In those <18 y.o. (n=688, T1D in 223), significant HR were found for ICA512/IA2 Ab positivity (2.68, p<0.0001), DPTRS (1.37, p=0.0007), Index60 (2.15, p<0.001) and HbA1c (4.24, p<0.0001). Recursive partitioning analysis was used to identify optimal cut points for significant variables. DPTRS≥7, then ≥3 antibodies (c-statistic 0.63) were cut points in the full population, in those ≥18, ICA+ve with Index60 ≥1.0 and ICA-ve with Index60 ≥0.5 (c-statistic 0.66) were cut points and in those <18, DPTRS ≥8 and HbA1c ≥5.4 (c-statistic 0.69) were cut points. Consideration of these factors in design of trials allows identification of lower and higher risk populations.
Cytokine profiles in memory CD4+ T cells at onset of type 1 diabetes

Jan Knoop1, Anita Gavrisan1, Denise Kuehn4, Julia Reinhardt4, Anette-Gabriele Ziegler1,2, Ezio Bonifacio4,5

1Institute of Diabetes Research, Helmholtz Zentrum Munich, Germany, Munich, Germany, 2Forschergruppe Diabetes e.V., Munich, Germany, 3Klinikum rechts der Isar, Munich, Germany, 4University of Technology Dresden, Dresden, Germany, 5Institute for Diabetes and Obesity, Helmholtz Zentrum Munich, Munich, Germany

The phenotype of autoreactive T cells in type 1 diabetes (T1D) directed against beta cell autoantigens is described as TH1 and/or TH17 and/or TH21, but is largely uncharacterized. We developed multiparameter cytokine profiling combined with proliferation to describe the phenotype of beta cell-responsive CD4+ T cells. The hierarchy of cytokine production by total CD4+ T cells from children at onset of T1D (n=18) was IL-2 (median, 35.4% positive CD4+ T cells), TNFα (19.2%), GM-CSF (5.5%), IFNγ (4.8%), IL-4 (1.1%), IL-22 (0.6%), IL-17A (0.5%), IL-21 (0.4%), and was not different to age-matched controls (n=23). In contrast, memory CD4+ T cells from patients in two independent data sets showed increased proliferative responses to GAD65 (data set 1, p=0.0007; data set 2, p=0.0088) and proinsulin (data set 1, p=0.0101; data set 2, p=0.0007) as compared to matched control subjects. The cytokine profiles of GAD65, proinsulin and tetanus toxoid responsive CD4+ T cells were predominantly characterised by GM-CSF, IFNγ, and IL-21. An increased proportion of cytokine producing proinsulin-responsive memory CD4+ T cells in patients was observed for IL-17A (data set 1, p=0.007; data set 2, p=0.022) and IL-22 (data set 1, p=0.015; data set 2, p=0.028), and additionally, an increase in responsive CD4+ T cells with a GM-CSF+IL-17A+IL-22-IFNγ/+ phenotype was shown in the patients (data set 1, p=0.023; data set 2, p=0.005). Using a unique combination of in vitro antigen stimulation, proliferation, and multiparameter cytokine profiling, we have been able to identify a phenotype of autoreactive T cells that is raised in T1D patients.
The genetic basis for the HLA-DR3/4 excess in type 1 diabetes patients

Charles E. Larsen1,2, Michael R. Trautwein1, Dennis R. Alford1, Zareen Vadva1, Chester A. Alper1,3
1Program in Cellular and Molecular Medicine, Boston Children's Hospital, Boston, MA, USA, 2Department of Medicine, Harvard Medical School, Boston, MA, USA, 3Department of Pediatrics, Harvard Medical School, Boston, MA, USA

We developed a stochastic epigenetically-triggered Mendelian oligogenic (SEMO) model for type 1 diabetes (T1D). The model's genetic component includes recessive inheritance for the major histocompatibility complex (MHC) susceptibility gene(s) based on MHC haplotype sharing by affected sib pairs and the fit in some populations of MHC alleles/haplotypes to the Hardy-Weinberg equilibrium (HWE). However, many patient populations exhibit an HLA-DR3/4 heterozygote excess. Many investigators believe MHC susceptibility recessive inheritance is unlikely due to this lack of MHC HWE. We previously provided evidence for greater parental subpopulation mixing in T1D families (54%) compared to control families (27%), thus explaining their higher offspring T1D incidence and rising population incidence. We postulate specific HLA-DRB1*03 and DRB1*04 haplotypes mark previously isolated populations that had selected against different causal T1D non-HLA loci. There are two HLA-DR3 and at least five HLA-DR4 T1D susceptibility conserved extended haplotypes (CEHs) that presumably arose in different European subpopulations. Our model suggests the HLA-DR3/DR4 excess involves only some susceptibility CEHs. We therefore analyzed Type 1 Diabetes Genetics Consortium data for patients from several thousand T1D families organized into nine geographic cohorts. The distribution of HLA-DRB1/-DQB1 haplotype combinations varied significantly between cohorts. Some HLA haplotypes fit the HWE among many patient cohorts while others did not. Specific haplotypes appear to explain most of the lack of DR3/DR4 HWE in many patient cohorts. Thus, it was not HLA-DR3 or DR4 specificities that increased T1D "risk" but specific MHC haplotype combinations that distorted (but did not negate) the HWE expected from Mendelian recessive inheritance.
A stochastic epigenetically-triggered Mendelian oligogenic (SEMO) disease model for type 1 diabetes

Chester A. Alper, Charles E. Larsen, Michael R. Trautwein, Dennis R. Alford

Program in Cellular and Molecular Medicine, Boston Children's Hospital, Boston, MA, USA, Department of Pediatrics, Harvard Medical School, Boston, MA, USA, Department of Medicine, Harvard Medical School, Boston, MA, USA

The incidence of type 1 diabetes (T1D) is increasing 3-5%/year. The cause has been attributed to an undefined changing environment. However, there is much evidence against the environment (or any changing non-genetic mechanism) in causing the rising incidence: 1) If the environment causes both the failure of all identical twins of patients to have T1D and the rising incidence, the concordance rate among these twins should be rising, but it is not; 2) Migrants from high- to low-incidence countries continue to have high-incidence children; 3) T1D concordance among fraternal twins is the same as siblings of patients in general; 4) T1D incidence in the offspring of two T1D parents is identical to the identical twin rate. These observations argue strongly against the environmental hypothesis. Additionally, T1D genetic association studies show strong susceptibility in the major histocompatibility complex but many optional additive genes of small effect increasing T1D risk with little to no genetic linkage. We developed an alternative model to genetic "risk" and environmental influence involving three recessive interacting causal genes, all located on human chromosome 6, and a stable stochastic epigenetic trigger. The model yields testable predictions and explains many puzzling T1D features, including its rising incidence, the high risk of HLA-DR3/DR4 heterozygotes, the rarity of affected relatives of patients, and T1D incidence among first-degree relatives of patients. Since selection against any causal gene could prevent T1D, we postulate the rising incidence results from increasing mixing of parents from previously isolated populations that had selected against different causal genes.
Inhibition of Interleukin-6 Trans-signaling Attenuates Inflammation Associated with Diabetic Retinopathy

Maria Valle¹, Janine Dworshak¹, Ahmed Ibrahim³, Mohamed Al-Shabrawey³, Ashok Sharma¹, Ruth Caldwell⁴, Sylvia Smith², Shruti Sharma¹,5

¹Center for Biotechnology and Genomic Medicine, Augusta University, Augusta, GA, USA, ²Cellular Biology and Anatomy, Augusta University, Augusta, GA, USA, ³Dental College of Georgia, Augusta, GA, USA, ⁴Vascular Biology Center, Augusta University, Augusta, GA, USA, ⁵Dept of Ophthalmology, Augusta University, Augusta, GA, USA

Interleukin-6 (IL-6) is a major mediator of inflammation and increasing evidence suggests that the IL-6 pathway plays a prominent role in the pathogenesis of diabetic retinopathy (DR). Interestingly, even though retinal endothelial cells lack membrane bound IL-6 receptor, IL-6 mediated signaling is observed in these cells via the trans-signaling pathway regulated by soluble IL-6 receptor (sIL-6R). Recent studies suggest that IL-6 trans-signaling is crucially involved in several inflammatory diseases.

To determine the role of IL-6 trans-signaling in DR, we used soluble glycoprotein 130 fused chimera protein (sgp130Fc), a selective inhibitor of IL-6 trans-signaling. For in-vitro studies, retinal endothelial cells were exposed to IL-6 (10ng/mL) and sIL6R (150ng/mL) to activate IL-6 trans-signaling and sgp130Fc (10ug/mL) was used to selectively inhibit IL-6 trans-signaling. For in-vivo studies, diabetes was induced in C57BL/6 mice by streptozotocin (STZ, 75 mg/kg). After 6 weeks of diabetes, mice were intraperitoneally injected twice weekly with sgp130Fc (0.5mg/kg body weight) or vehicle (PBS) for 2 weeks. Post-treatment, the retinal phenotype was characterized by fundoscopy, fluorescein angiography (FA) and OCT measurements.

We found that IL-6 trans-signaling is sufficient to cause barrier disruption in endothelial cells. Blockade of IL-6 trans-signaling using sgp130Fc, maintained normal endothelial barrier function. In a diabetic mouse model, specific inhibition of IL-6 trans-signaling by sgp130Fc fusion protein suppressed diabetes induced ocular inflammation. These data indicate that a pathway driven by IL6 and sIL6R contributes to vascular inflammation in diabetic retina. Specific targeting of this pathway may be a promising new approach for the treatment of DR.
Importance of Lipid-Signaling from Immune- and β-cells in the Development of Type 1 Diabetes

Sasanka Ramanadham¹, Alexander Nelson¹, Ying Gai¹, Robert Bone²
¹University of Alabama at Birmingham, Birmingham, AL, USA, ²Indiana University School of Medicine, Indianapolis, IN, USA

Type 1 diabetes (T1D) is a consequence of islet β-cell destruction and little is known about how lipid signals generated by immune- and β-cells contribute to this process. We previously identified Ca²⁺-independent phospholipase A₂β (iPLA₂β) expression in islet β-cells and found that iPLA₂β activation promotes β-cell apoptosis. PLA₂s hydrolyze the sn-2 substituent from membrane phospholipids to release a free fatty acid (i.e. arachidonic acid) that can be metabolized to oxidized lipids (eicosanoids) by cyclo- and lipo-oxygenases (COX and LO). Several eicosanoids are inflammatory and because immune cells also express iPLA₂β, we considered the possibility that iPLA₂β-derived lipids from these cells impact T1D development. We find that diabetes-prone NOD mice express higher iPLA₂β in β-cells and immune cells and that selective inhibition of iPLA₂β with FKGK18 reduces insulitis, preserves β-cell mass, and ameliorates T1D. These observations caused us to further explore the role of iPLA₂β in immune cells and we found that TNFα production from CD4⁺ T-cells and antibody production from B-cells are reduced by iPLA₂β inhibition. Reduced TNFα production with inhibitors of COX (indomethacin) or 12-LO (CDC) suggested that products of these eicosanoid arms promote proinflammatory cytokine generation. Further, adoptive transfer of diabetes by CD4⁺ T-cells to immunodeficient NOD.scid mice, pretreated with FKGK18 to inhibit endogenous iPLA₂β, was reduced by 50%. Collectively, these findings lead us to suggest that iPLA₂β activity in immune- and β-cells together provide critical lipid signals that impact onset and progression of T1D. This work was supported by grants (SR) from Iacocca Family Foundation & NIH/NIDDK.
Protection from T1D development in TRIF-deficient NOD mice is dependent on altered gut microbiota influencing immune cell functions

Elke Guelden¹,², Chen Chao¹, Ningwen Tai¹, Susan Wong³, Li Wen¹
¹Department of Internal Medicine, School of Medicine, Yale University, New Haven, USA, ²Department of Immunobiology, School of Medicine, Yale University, New Haven, USA, ³Institute of Infection and Immunity, School of Medicine, Cardiff University, Wales, UK

We previously showed that Toll-like-receptors (TLRs) and their signaling adaptor molecule MyD88 are key regulators of T1D development in the NOD mouse. TRIF is a major signaling molecule down-stream of TLR3 and TLR4. The aim of our current study was to investigate the role of TRIF in the immunopathogenesis of T1D development.

We generated TRIF-deficient (TRIF-/-) NOD mice and monitored diabetes development in these mice and TRIF-sufficient (TRIF+/+) littermates in specific-pathogen-free (SPF) housing conditions. Interestingly, we found that diabetes development was not significantly affected by the presence or absence of TRIF if they were co-housed. However, TRIF-/- mice exhibit a significantly delayed and reduced diabetes development when they were housed separately from TRIF+/+ mice (p<0.01). To test if the disease protection is mediated by the gut microbiota, we transferred fecal materials from TRIF+/+ mice to TRIF-/- NOD mice. The fecal transfer significantly diminished diabetes protection (p<0.01). 16S rRNA sequencing of fecal samples revealed a marked reduction of Proteobacteria and significantly lower abundance of Campylobacterales, Heliobacter and Sutterella (p<0.01) in TRIF-/- NOD mice housed separately from TRIF+/+ mice. Principal coordinate (PCoA) analysis showed two distinct clusters of the gut microbiota when housed separately. However, the difference was reduced by co-housing and/or fecal transfer. Furthermore, we found that the functions of antigen-presenting-cells (APC) were significantly compromised in the absence of TRIF compared to APCs from TRIF+/+ NOD mice.

In conclusion, our results suggest that TRIF shapes the gut microbiota, which in turn alter immune cell functions modulating diabetes development.
Combining IL-7Rα Blockade with Islet Autoantigen Vaccination to Prevent and Reverse Type 1 Diabetes

Cristina Vazquez Mateo, Sarah Goldberg, Justin Collins, Katherine Modzelewski, Devin Steenkamp, Barbara Nikolajczyk, Hans Dooms
Boston University School of Medicine, Boston, MA, USA

IL-7Rα blocking antibodies have the capacity to prevent and reverse Type 1 Diabetes (T1D) in NOD mice by modulating various immunoregulatory mechanisms. However, long-term antibody treatment was required to maintain efficacy, raising concerns about attrition of protective T cell memory and immunosuppression. Hence, we combined short-term anti-IL7Rα antibody administration with islet autoantigen-specific vaccination in an effort to limit the treatment duration while focusing on targeting the pathogenic T cells. Combining anti-IL-7Rα antibodies with islet peptides and alum led to increased Treg expansion and a delay in T1D onset, suggesting that blocking IL-7 during an autoimmune response enhances immunoregulation. In a second approach, we developed a conjugated vaccine to activate islet autoantigen-specific T cells in the presence of IL-7Rα blocking antibodies. Treatment of pre-diabetic NOD mice with this combination resulted in increased IL-2 and IL-10 production and Treg expansion. Preliminary T1D incidence studies indicate that this combinatorial vaccine has the potential to prevent and reverse T1D. Importantly, we have developed humanized NSG mice engrafted with PBMCs from T1D patients to evaluate the impact of our combinatorial vaccine on the numbers, phenotype and function of human islet autoantigen-specific T cells. ELISPOT assays revealed that the conjugated vaccine increased the number of cytokine-producing T cells and Tregs in the spleen of the engrafted mice, confirming that the targeted human islet-specific T cells are present and sensitive to immunization. Together, our data suggest that combining anti-IL-7Rα antibodies with an autoantigen-specific approach may reduce the risk of long-term immunosuppression while maintaining or improving therapeutic efficacy.
A novel population of β cells that are resistant to immunological attack occur during the progression of autoimmune diabetes in NOD mice

Jinxiu Rui1, Songyan Deng1, Arnon Arazi2, Ana Luisa Perdigoto1, Kevan Herold1
1Yale University, New Haven, CT, USA, 2Broad Institute of MIT and Harvard, Cambridge, MA, USA

Type 1 diabetes (T1D) is a chronic autoimmune disease thought to involve the destruction of insulin producing β cells by diabetes antigen specific T cells. However, the ways in which β cells response to immune attack are not known but may account for the disease course and even persistence of β cells after diagnosis. Here, we report a novel population of β cells identified during the progression of T1D in NOD mice that can survive immune attack. This population develops from normal β cells in response to islet immune infiltrates and can be prevented with anti-CD3 mAb therapy. RNA-seq analysis identified activated pathways in the new population involving cell movement, growth and proliferation, immune responses, and cell death and survival. qRT-PCR showed reduced expression of diabetes antigens (insulin, IGRP, GAD) and increased expression of inhibitory immune markers (PD-L1 and Qa-2). The new subpopulation shows higher rates of cell proliferation measured by BrdU incorporation compared to the parent β cells. This new subpopulation is more resistant to killing when diabetes is precipitated in NOD mice with cyclosphosphamide. These studies identify a novel mechanism of cell protection from immunologic destruction in which β cells lose features of their normal identity and increase expression of immune inhibitory molecules and proliferation. This mechanism may lead to long term survival of a subpopulation of β cells as has been described in many individuals with long standing T1D.
Unveiling the specificity of islet-antigen specific regulatory T cells in T1D patient using single cell deep-sequencing

Dagi Xu¹, Shen Dong¹, Marc Martinez-Llordella², Gaurav Chopra³, Dmytro Lituiev¹, Chun Ye¹, Jeffrey Bluestone¹
¹UCSF, San Francisco, CA, USA, ²King’s College London, London, UK, ³Purdue University, West Lafayette, IN, USA

CD4⁺CD25hiCD127loFoxp3⁺ T Regulatory cells (Tregs) are critical in controlling autoreactive T cells. The deficiency of functional Tregs is associated with Type 1 diabetes (T1D) and many other autoimmune diseases. In addition, antigen-specific Tregs suppresses more efficiently compared to polyclonal Tregs in mouse models. Thus, identifying the antigen specificity of Tregs, especially at the site of inflammation, is critical to improve our understanding of Treg deficiencies in T1D and may lead to clinically applicable advances. However, the Treg TCRs recognizing self-antigens in autoimmune diseases are less clarified due to the rare frequency and the anergic status of Treg in vitro. In this study, by the combination of sub-culturing, polyclonal stimulation and antigen stimulation, we can identify islet-antigen specific Tregs from both PBMC and pancreatic draining lymph node of T1D patients using antigen-driven Treg proliferation assay we developed in our lab. The majority of islet antigen-specific Tregs is CD45RO⁺Tregs compared to the CD45RA⁺ counterpart. Importantly, we confirmed the enrichment and identified the TCR sequence using single cell deep-sequencing. In the future, we will further characterize the uncovered Treg TCR and the phenotype of the identified islet antigen-specific Tregs via comparison between healthy and T1D subjects.
Islet amyloid accelerates diabetes onset in NOD mice and enhances islet allograft rejection

Heather C. Denroche, Imelda W. Suen, Derek L. Dai, Galina Soukhatcheva, C. Bruce Verchere
BC Children’s Hospital, University of British Columbia, Vancouver, BC, Canada

The deposition of islet amyloid from aggregates of islet amyloid polypeptide (IAPP) is thought to contribute to the development of beta cell dysfunction in type 2 diabetes. IAPP aggregates are also a potent proinflammatory stimulus for macrophages, and in human IAPP transgenic (hIAPP Tg/0) mice (a model in which islet amyloid forms rapidly, as rodent IAPP does not aggregate) the deposition of islet amyloid induces islet inflammation. We hypothesized that by activating innate proinflammatory pathways, IAPP aggregates may accelerate allo- and auto-immune mediated beta cell destruction. To test the role of islet amyloid in allograft rejection, we transplanted islets from hIAPP Tg/0 mice or hIAPP 0/0 controls into genetically mismatched, streptozotocin-induced diabetic recipient mice. Balb/c recipients of hIAPP Tg/0 islets had accelerated graft failure (median failure time of 26.5 days vs > 80 days in controls, p=0.06), corresponding with extensive islet graft destruction and infiltration of CD45-positive cells. In C57Bl/6 recipients (a more stringent model of allograft rejection), although hIAPP expression did not significantly potentiate rejection rates, hIAPP Tg/0 islet allografts displayed increased immune infiltration prior to rejection. To examine the role of IAPP aggregation in beta cell autoimmunity, we backcrossed the hIAPP transgene onto the NOD background. We found that hIAPP Tg/0 mice backcrossed ≥6 times (>97±1% NOD via single nucleotide polymorphism analysis) have accelerated diabetes onset relative to littermate controls (p=0.012). Collectively, these data suggest that IAPP aggregation and islet amyloid may be a currently unrecognized accelerant of allo- and autoimmune-attack of beta cells.
JAK1/2 inhibitor ruxolitinib inhibits diabetogenic immune responses and delays diabetes in NOD mice

YiQun Zhang¹,², Jan Dutz¹,²
¹University of British Columbia, Vancouver, Canada, ²BC Children's Hospital, Vancouver, Canada

In Type 1 diabetes, pancreatic islet β cells are destroyed by diabetogenic CD8 T cells. Interferons (IFNs) and IL2 play pathogenetic roles in the activation of diabetogenic T cells. Ruxolitinib, a Janus Kinase (JAK) 1/2 inhibitor, suppresses receptor signaling induced by IFNs and γ chain cytokines. The aim of this study was to investigate effects of ruxolitinib on diabetogenic immune responses and diabetes development in non-obese diabetic (NOD) mice. Female NOD mice were treated with oral ruxolitinib (50 mg/kg) or vehicle daily for 10 days from 6 weeks of age. Pancreatic LNs (PLN) and pancreas were examined for presence of insulitis, IFNγ and NKG2D expression, cytotoxic function and mice were followed for diabetes development.

Insulitis scores were lower in ruxolitinib-treated NOD pancreas than in control treated mice. The proportion of IFNγ⁺CD4⁺, IFNγ⁺CD8⁺ and NKG2D⁺CD8⁺ T cells were decreased in NOD pancreas after ruxolitinib treatment. Ruxolitinib prevented the proliferation of adoptively transferred diabetogenic CD8 T cells. In vivo killing of islet peptide–pulsed CFSE-labeled splenocytes was reduced in ruxolitinib-treated NOD PLNs. Diabetes development was delayed after ruxolitinib treatment. Ruxolitinib inhibited pSTAT1 and pSTAT5 induction in PLN cells stimulated by IFNα and IL-2 respectively in vitro. The number of pSTAT3⁺ and pSTAT5⁺ immune cells in the pancreas was reduced after ruxolitinib treatment.

Taken together, ruxolitinib treatment inhibits insulitis, Th1 immune response, CD8 T cell activation, cytotoxicity and diabetes development through inhibition of the JAK-STAT pathways. Thus, JAK1/2 inhibition may provide a novel treatment for autoimmune diabetes.
Detection of Autoreactive CD4+ T cells in T1D Using Standardized HLA-matched Reagents

Tomasz Sosinowski1,2, Jonathan Dekermanjian1,2, MyLinh Dang1,2, William W. Kwok3, Howard W. Davidson1,2
1Barbara Davis Center for Diabetes, Aurora/Colorado, USA, 2University of Colorado Anschutz Medical Campus, Aurora/Colorado, USA, 3Benaroya Research Institute, Seattle/Washington, USA

The MHC class II region is the most important locus regulating genetic risk for the development of type 1 diabetes (T1D). Numerous studies in mice and humans have culminated in a mechanistic model in which the products of MHC class II risk-alleles bind self-derived antigenic peptides leading to activation of cognate CD4+ T cells and eventual destruction of pancreatic beta cells. Consequently such T cells are key targets for T1D antigen-specific immunotherapies.

A key requirement for the development of effective antigen-specific immunotherapies is the availability of robust mechanistic biomarkers. However, current methods for the detection of autoreactive CD4+ T cells directly ex vivo in humans remain sub-optimal, most likely because of their low number in the peripheral blood. ELISPOT assays have the necessary sensitivity, but in their “classical” format can exhibit significant variability caused by heterogeneity in antigen processing and presentation.

To eliminate non-T-cell dependent variables we have replaced the natural antigen presenting cells (APCs) and soluble peptides present in the standard ELISPOT with a panel of cellular “artificial APCs” (aAPCs) that express only defined covalently linked MHC class II-peptide complexes, together with appropriate co-stimulatory and adhesion molecules. Our results indicate that aAPC-based assays exhibit both increased sensitivity (lower background and higher signals), and specificity (absence of “off-target” signals) relative to the traditional format. The aAPC-based assays also perform reliably using cryopreserved samples.

We propose that these improved assays should be suitable for functional analysis of autoreactive CD4+ T cells for monitoring therapeutic efficacy in future antigen-specific immunotherapy trials.
A Pipeline for Discovery of Biomarkers Predicting C-peptide Decline

Cate Speake1, Elizabeth Whalen2, Raphael Gottardo3, Frans K. Gorus4, Martin J. Hessner5, Eddie A. James1, Megan K. Levings6, Peter S. Linsley2, Alberto Pugliese7, Simi Ahmed8, Jared M. Odegard1, Gerald T. Nepom1,9, S. Alice Long10, JDRF Biomarker Working Group Core for Assay Validation1

1Diabetes Research Program, Benaroya Research Institute, Seattle, WA, USA, 2Systems Immunology Program, Benaroya Research Institute, Seattle, WA, USA, 3Vaccines and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA, 4Diabetes Research Center, Brussels Free University, Brussels, Belgium, 5Department of Pediatrics and the Max McGee National Research Center for Juvenile Diabetes, Medical College of Wisconsin, Milwaukee, WI, USA, 6Department of Surgery, The University of British Columbia, and Child & Family Research Institute, Vancouver, BC, Canada, 7Diabetes Research Institute, University of Miami Miller School of Medicine, Miami, FL, USA, 8JDRF, Research Department, New York, NY, USA, 9Immune Tolerance Network, Bethesda, MD, USA, 10Translational Research Program, Benaroya Research Institute, Seattle, WA, USA

Individual or composite biomarkers that can predict C-peptide decline after diagnosis may be useful for patient stratification for clinical trial enrollment or for therapeutic selection. From ITN, we obtained longitudinal sample sets from patients who have been clinically and metabolically well-characterized to apply to biomarker discovery projects. We also established an assay development pipeline to collaboratively identify and test assays. We prioritized assays that would cover a wide breadth of immunological and metabolic readouts, and developed statistical analysis plans to appropriately combine disparate data types. 9 assays from 8 independent laboratories are currently being tested using this framework, with the goal of identifying individual and composite biomarkers of C-peptide decline. The 9 assays were each tested for technical reproducibility in the lab where the assay was developed. Analytes from each assay with sufficient technical precision (CV<20%) were then used to test biological variability in a cross-sectional set of T1D samples. After completing analysis of the first 5 assays, 20 analytes have been identified that are associated with disease duration, C-peptide at draw, and/or age at diagnosis. Using this data as a framework, our bioinformatics team is developing combinatorial models to cross-compare assay results and classify markers that predict clinical features of samples from recent-onset subjects enrolled in the control arms of 3 ITN-sponsored trials. From this pipeline, we have identified a wide breadth of technically reproducible, biologically relevant assays that can now be applied to prediction of C-peptide decline.
Reduced response to IL-2 by CD4 memory T effector cells in T1D subjects is dependent on activation state and negative regulation

Jerill Z. Thorpe¹, Katharine Schwedhelm¹, Carla J. Greenbaum², Cate Speake², Karen Cerosaletti¹, Jane H. Buckner¹, S. Alice Long¹
¹Translational Research Program, Benaroya Research Institute, Seattle, WA, USA, ²Diabetes Clinical Research Program, Benaroya Research Institute, Seattle, WA, USA

To best apply IL-2-based therapies in T1D, it is important to understand factors that regulate IL-2 response. We have shown that some T1D subjects display defective IL-2 signaling and this defect is more prominent in effector (Teff) than regulatory (Treg) T cells. Known IL2RA and PTPN2 risk alleles accounted for some, but not all, of the reduced response. To determine additional factors involved in reduced IL-2 signaling, we assayed memory CD4 Teff cells of control and T1D subjects held constant for known risk alleles. In T1D subjects, IL-2 signaling remained significantly lower after in vitro activation and proliferation, suggesting involvement of cell intrinsic factors. Reduced pSTAT5 in response to IL-2 was not due to decreased IL-2R expression or divergence to the MAPK or AKT pathways, as measured by western blot. However, analysis by flow cytometry showed a correlation between basal activation state and reduced IL-2 signaling. To further explore the role of activation on IL-2R signaling, we performed targeted qPCR analysis of IL-2R pathway genes, and 2 TRAF3 isoforms, recently shown by others to be induced by activation and regulate IL-2R signaling. Enhanced negative regulation (SOCS3, PIM1, and SHP2) and increased levels of a truncated TRAF3 isoform were found in T1D subjects with low IL-2R signaling. Experiments to better understand the relationship between activation state, negative regulation, and IL-2R signaling are on-going. Together, these findings suggest that genetics and activation state may contribute to reduce IL-2R signaling in T1D and have implications for development of targeted IL-2-based therapies.
Longitudinal assessment of inherent immune biomarker variation in Type 1 Diabetes: Making sense of the noise to understand the disease

JD Wesley1, C Speake2, B Sehested Hansen3, N Perdue1, E Lanxon-Cookson1, D Friedrich1, M Pham1, J Grainger1, J Cracraft1, C Gibson1, WW Kwok2, A Gorst-Rasmussen3, MG von Herrath1, CJ Greenbaum2

1Novo Nordisk Research Center, Seattle, WA, USA, 2Benaroya Research Institute, Diabetes Clinical Research Program, Seattle, WA, USA, 3Novo Nordisk A/S, Søborg, Denmark

Immune biomarkers are widely used to understand disease mechanisms, progression, risk, and therapeutic response in Type 1 diabetes (T1D). The utility of immune biomarkers depends on understanding their technical and biological variability. Therefore, to assess the variation of key T1D immune biomarkers, we performed a longitudinal observational study comprising 30 T1D subjects, 15 subjects with Type 2 diabetes (T2D), and 15 healthy subjects (HS). Subjects provided samples 9-10 times over a year, including 2 visits that were 1-week apart. Autoantibodies, autoantigen-specific CD8 T-cell frequency, autoreactive CD4 and CD8 T-cell cytokine production, and natural killer cell (NK) and monocyte subsets were evaluated. Autoantibodies and NK and monocyte subsets were fairly stable over time based on coefficient of variation (CV); notably, anti-insulin antibodies were detectable in a subset of insulin-treated T2D subjects. Antigen-specific CD8 T-cells were detected in T2D and HS as well as in T1D subjects. These T-cells, however, had a high CV as did autoantigen-driven cytokine production. The inherent variation associated with immune biomarkers, especially those present at low levels, makes the use and interpretation of such markers challenging. Our results highlight the need to carefully consider sample volume, frequency, and timing with respect to the frequency and expression of the biomarker of interest—more rare populations will require greater sample volume and, potentially, more frequent sampling. These findings underscore the complexity of measuring immune biomarkers in human populations and the challenge of using rare peripheral populations to understand an organ-specific disease.
An Insulin-IAPP Hybrid Peptide is an Autoantigen in the NOD Mouse Model of Autoimmune Diabetes

Timothy Aaron Wiles¹, Thomas Delong¹, Rocky Baker¹, Brenda Bradley¹, Gene Barbour¹, Roger Powell², Nichole Reisdorph², Kathryn Haskins¹
¹Department of Immunology and Microbiology, University of Colorado School of Medicine, Aurora, CO, USA, ²Pharmaceutical Science, University of Colorado School of Medicine, Aurora, CO, USA

We recently reported that several diabetogenic CD4 T cell clones from the non-obese diabetic (NOD) mouse recognize hybrid insulin peptides (HIPs). These novel autoantigens are formed by post-translational fusion of insulin C-peptide fragments to other peptides present in pancreatic beta cells. An insulin-IAPP hybrid peptide (6.9HIP) is highly antigenic for the clone BDC-6.9. We report here that the precise epitope for BDC-6.9 is centered on the hybrid peptide junction. Residues in both the insulin and IAPP portions of the molecule are critical for antigenicity. Furthermore, we expand upon our previous mass spectrometric analysis of beta cells to confirm that the insulin-IAPP hybrid peptide is the natural antigen for BDC-6.9. Using an I-Ag7:6.9HIP tetramer, we demonstrate that CD4 T cells specific for 6.9HIP are prevalent in the pancreas of diabetic NOD mice, suggesting that 6.9HIP is an important target of autoreactive CD4 T cells in the NOD mouse.
Islet Antigen Specific CD4 Memory T cells Express Expanded TCR Pairs and Unique Transcriptional Profiles in Type 1 Diabetes

Karen Cerosaletti, Faris Whitman, Junbao Yang, William Kwok, Peter Linsley
Benaroya Research Institute at Virginia Mason, Seattle, WA, USA

Islet antigen reactive T cells in peripheral blood track with β-cell destruction in type 1 diabetes (T1D). To identify novel characteristics of islet specific T cells we performed an unbiased assessment using novel single cell RNA sequencing methods. PBMC from T1D patients or matched healthy control subjects (HC) were stimulated with a pool of immunodominant DR4 restricted islet peptides and activated T cells were isolated using CD154 enrichment. CD154+CD69+ CD4 memory T cells were sorted directly into a Fluidigm C1 Single-Cell chip for Nextera library generation and RNA sequencing. RNAseq reads were processed in parallel to capture the transcriptome by alignment to the human reference genome and tabulation of transcript levels, and identify the V(D)J CDR3 regions of productive TCR α/β pairs by assembly of de novo contigs using Trinity coupled with IMGT/HIGHV-QUEST. We detected private TCR clonotypes in islet reactive CD4 memory T cells in all subjects. However, T1D patients expressed identical TCR pairs in multiple cells, suggesting prior in vivo expansion. Expanded TCR pairs were present in longitudinal samples from the same T1D subject and were specific for a single islet peptide present in the stimulating peptide pool. T1D cells with expanded clonotypes had a gene expression profile that was distinct from cells with non-expanded TCRs and HC cells, characterized by a unique subset of T cell activation/differentiation genes. Our results illuminate TCR clonotype/phenotype relationships of islet specific CD4 T cells that may impact the immunopathology of subjects with T1D, and serve as biomarkers and therapeutic targets.
Antigen discovery for regulatory T cells (Tregs) in type-1 diabetes (T1D)

Shen Dong¹, Daqi Xu¹, Morvarid Mehdizadeh¹, K. Chirstopher Garcia², Jeffrey A. Bluestone¹
¹UCSF, CA, USA, ²Stanford, CA, USA

Antigen specific Tregs are more efficient than polyclonal Tregs in their suppressive capacity, which makes them attractive tools for further targeted tolerogenic therapy. Autoreactive T cells specificity has been well characterized in type 1-diabetes but little is known about autoreactive Treg specificity. Whether Tregs recognize the same tissue-specific self-antigens as autoreactive T cell, or they recognize different self-antigens but regulate local immune responses via bystander suppression is unclear.

Our goal is to identify pancreatic islet antigen-specific Tregs and interrogate their antigen recognition. We first analyzed Treg TCR usage by performing single cell TCR sequencing on Tregs from the pancreatic islet of 12 weeks NOD mice. Data show an abundance of the TCR allele TRAV5D4 usage in islet infiltrated Tregs that has been characterized to target a primary insulin peptide. We are currently analyzing islet Tregs TCRs of NOD mice at different ages in order to identify TCRs expanded in islet inflamed tissues. Moreover, we will analyze the TCR usage of pancreas infiltrated or draining lymph node Tregs from T1D patients from the nPOD network. Our final goal is to identify enriched TCRs clones antigen specificity by using a peptide-major histocompatibility complex (pMHC) library displayed on yeast. We have validated the DR4 pMHC yeast library for human TCRs and we are currently developing the I-A⁰ pMHC yeast libraries for the mice Treg TCRs.

New antigen identification for pancreatic islet-infiltrated Tregs will allow us to improve our understanding of Treg deficiencies in T1D and may lead us to design new immunosuppressive therapies.
Appearance of Antibodies to 5 Human Herpesviruses and Coxsackie B4 Virus (CoxB) at the Time of Islet Antibody Seroconversion (IA-SC) or Type 1 Diabetes (T1D) Onset

Corbin Johnson¹, John Willis¹, Michael Killian¹, Angela Wolf¹, Rachel Hervey¹, Peter Burbelo², William Hagopian¹,³
¹Pacific Northwest Diabetes Research Institute, Seattle, WA, USA, ²NIDCR, NIH, Bethesda, MD, USA, ³University of Washington, Seattle, WA, USA

Viral infections could trigger IA-SC or T1D onset. Virus identification might enable vaccine-based prevention. Like enteroviruses, herpesviruses infect during childhood, exhibit tissue trophism and establish chronic infections. We tested association of viral infection with IA-SC or T1D, using sequential samples from the Diabetes Evaluation in Washington (DEW-IT) study. Of 4,106 infants with high-risk HLA, 564 developed IA. Of these, 42 IA-SC cases had ≥1 sample drawn >6 months before IA-SC, one ≤6 months before IA-SC, and one at IA-SC. Each IA-SC child was matched to one without IA. Validated, luciferase-based assays measured viral antibodies to HSV1, HSV2, EBV, CMV, HHV6B, and to CoxB. Viral antibodies appearing <6 months before IA-SC was considered a match. Among 42 IA-SC cases/42 controls, we observed 0/0 matches for HSV1, 0/0 for HSV2, 1/1 for EBV, 1/1 for CMV, 3/5 for HHV6B, and 4/6 for CoxB. Among all individuals, 5%, 6%, 87%, 38%, 94% and 83% developed HSV1, HSV2, EBV, CMV, HHV6B, and to CoxB. Viral antibodies appearing <6 months before IA-SC was considered a match. Among 42 IA-SC cases/42 controls, we observed 0/0 matches for HSV1, 0/0 for HSV2, 1/1 for EBV, 1/1 for CMV, 3/5 for HHV6B, and 4/6 for CoxB. Among all individuals, 5%, 6%, 87%, 38%, 94% and 83% developed HSV1, HSV2, EBV, CMV, HHV6B and CoxB antibodies during observation, respectively. Thus only EBV, HHV6B and CoxB were prevalent, but did not differ between IA-SC cases and controls. Separately, 25 DEW-IT children developing T1D, and matched IA children without T1D, were tested for viral antibodies. Viral antibodies appearing <6 months pre-onset was considered a match. EBV, CMV and HHV6B matches occurred in 1/22, 0/25 and 0/22 T1D cases, and in 1/22, 0/25 and 1/22 controls, respectively. Not even a trend associated new viral infection with IA-SC or with T1D onset, but we are testing more individuals to verify.
Assessing fear of hypoglycaemia in new-onset type 1 diabetes

Mohammad Alhadj Ali1, Yuk-Fun Liu2,3, Rachel Stenson1, Laura Adams3, Nicola Leech4, Rob Andrews5, Sunil Nair6, Mark Peakman2, Colin Dayan1

1Cardiff University School of Medicine, Cardiff, UK, 2King’s College London, London, UK, 3Guy’s and St. Thomas’ Foundation Trust, London, UK, 4Victoria Royal Infirmary, Newcastle upon Tyne, UK, 5Bristol Royal Infirmary, Bristol, UK, 6Countess of Chester Hospital NHS Foundation trust, Chester, UK

Introduction: In type 1 diabetes (T1D), hypoglycaemia is the most common side effect of insulin therapy and a major barrier to achieving optimal glycaemic control. Hypoglycaemia is defined by low levels of blood glucose that can trigger multiple negative physiological outcomes. Here, we describe assessment of fear of hypoglycaemia (FoH) in new-onset T1D by using the Hypoglycaemia Fear Survey (HFS-II) in a Phase 1b trial of peptide immunotherapy.

Methods: This was a multi-centre, randomized; double-blind, placebo-controlled trial, of 10μg of PI C19-A3 peptide administered intradermally every 14 or every 28 days, with follow-up for 48 weeks. 27 patients aged 18-45 with HLA-DRB1*0401 genotype, positive islet autoantibody status and a stimulated C-peptide >0.2 pmol/mL were recruited within 100 days of diagnosis.

Results: A significant reduction in the total HFS-II score from baseline was seen at 12 months in the placebo group (P=0.0001) and combined treatment groups (P=0.01). In particular, the worry subscale score was significantly reduced in the placebo and combined treatment groups (P=0.0007 and P=0.04 respectively), but not the behavior subscale.

Conclusion: Fear of hypoglycaemia falls in the first year of T1D. Assessing HFS-II can provide a practical tool to study the impact of newly developed therapies on hypoglycaemia and identifying patients at risk who may require additional clinical support in managing hypoglycaemia. More trials are required to increase our knowledge of the natural history of FoH in T1D, particularly early in disease onset within clinical trials.
Proinsulin peptide C19-A3 immunotherapy in new-onset type 1 diabetes is well tolerated and associated with a reduction in total daily insulin usage

Mohammad Alhadi Ali1, Yuk-Fun Liu2,3, Rachel Stenson1, Laura Adams3, Nicola Leech4, Rob Andrews5, Sunil Nair6, Mark Peakman2, Colin Dayan1
1Cardiff University School of Medicine, Cardiff, UK, 2King’s College London, Lonon, UK, 3Guy’s and St. Thomas’ Foundation Trust, London, UK, 4Victoria Royal Infirmary, Newcastle upon Tyne, UK, 5Bristol Royal Infirmary, Bristol, UK, 6Countess of Chester Hospital NHS Foundation trust, Chester, UK

Introduction: In type 1 diabetes (T1D), nonclinical studies have shown that antigen specific therapy (ASI) strategies have been effective in disease prevention, and some of them have worked in more stringent setting close to, or at, disease onset. Clinical trials of ASI as applied to T1D have predominantly focused on insulin as an autoantigen.

Aims and objectives: We aimed to examine the safety of the naturally processed proinsulin peptide C19-A3 (PI C19-A3) in new-onset T1D, as well as, its metabolic effects.

Methods: This was a multi-centre, randomized; double-blind, placebo-controlled trial, of 10μg of PI C19-A3 administered intradermally every 14 or every 28 days for 24 weeks. 27 patients aged 18- 45 with HLA-DRB1*0401 genotype, positive islet autoantibody status and a stimulated C-peptide >0.2 pmol/mL were recruited within 100 days of diagnosis.

Results: PI C19-A3 was generally well tolerated and safe. No systemic hypersensitivity or severe adverse events were reported. The mean change in daily insulin use was significantly lower in the high frequency arm at 6, 9 and 12 months (p=0.03; p=0.04; p=0.01, respectively) and significantly lower in the low frequency arm at 12 months (p=0.009) compared with placebo. There was a trend for increased HbA1c in the placebo group, whereas change in the treatment groups was minimal or values declined.

Conclusion: The study demonstrates that treatment with PI C19-A3 appears to be safe and well tolerated when administered over extended periods. It was also associated with reduced or stable daily insulin use, and was not associated with poorer glycaemic control.
Interleukin-35 (IL-35) is a cytokine expressed by T regulatory and B cells that suppresses conventional T cell proliferation and effector functions. Transgenic beta-cell expression of IL-35 protects non-obese diabetic (NOD) mice from autoimmune diabetes. We sought to determine whether expression of IL-35 could similarly protect against autoimmune diabetes and islet allograft rejection. We generated an adeno-associated virus driving IL-35 expression downstream of the rat insulin promoter (AAV6-RIP-IL-35) to express IL-35 in beta cells in vivo following injection of virus via the pancreatic duct. Expression of IL-35 was confirmed by qPCR and immunofluorescence. NOD mice with beta cell IL-35 expression from 6 weeks of age had reduced diabetes compared to controls that received empty vector (29±11 vs. 75±12%; p<0.05). To assess IL-35 protection from allograft rejection, mouse islets were isolated 3 weeks following in vivo transduction with AAV6-RIP-IL-35, and transplanted into streptozotocin (STZ)-diabetic Bl/6 recipients. Lack of rejection was observed in a number of recipients of untreated islets, leading us to reassess islet allograft rejection across different strains and genders in our animal facility. We found transplantation of male Balb/c islets into female Bl/6 recipients to be the most stringent combination for allograft rejection for our experiments. At 60 days post-transplantation, 33% of IL-35-expressing islets were protected from allograft rejection, whereas all empty virus controls were rejected (p<0.05). These data demonstrate that beta-cell expression of IL-35 reduces diabetes incidence in NOD mice and protects against islet allograft rejection, suggesting that IL-35 may have therapeutic potential in autoimmune diabetes and islet transplantation.
Glucokinase gene seems to be a more suitable target then insulin for the detection of beta cell death

Jana Sklenarova, Lenka Petruzelkova, Stanislava Kolouskova, Jan Lebl, Zdenek Sumnik, Ondrej Cinek
Paediatric Dpt. of 2nd Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic

Detection and quantification of free circulating unmethylated INS DNA presumably released from beta cells has been previously used for assessing the beta cell destruction rate. As the targets within the INS gene suffer from suboptimal specificity, we sought to improve the assay parameters by using the glucokinase gene (GCK) tissue-specific pancreatic promoter.

The amount of methylated and unmethylated GCK promoter DNA was measured using a dual fluorescent probe droplet PCR assay, and compared with the previously published droplet PCR assay for insulin gene. The method was fine-tuned using a synthetic methylated and unmethylated target sequence and DNA from pancreatic islets and blood cells. Circulating DNA from serum was obtained from recent-onset T1D children (n=25), autoantibody positive first-degree relatives of T1D patients (n=14) and healthy controls (n=20).

The specificity and sensitivity of the GCK assay was higher than that of the INS assay: the target sequence in blood was 99.6% methylated in GCK as compared to 86% methylation in INS. Islet DNA was shown to be 89% unmethylated in GCK comparing to 68% in INS. The unmethylated/methylated DNA ratio did not differ between recent-onset patients and healthy controls, but was borderline-significantly increased in the autoantibody-positive first-degree relatives compared to controls (p= 0.06) and to recent-onset T1D children (p=0.04).

In conclusion, the data indicates that GCK may be a more efficient target for assessment of specific beta-cell methylation patterns as compared to the INS gene. This enhancement, however, has not improved the clinical utility of the assay.
Polarization of islet-specific T-cell responses is altered following allogeneic islet transplantation and correlates with declining metabolic graft function

Shereen Sabbah¹, Aaron Liew², Augustin Brooks², Rhiannon Kundu¹, James Reading¹, Pratik Choudhary², Shareen Forbes³, Miranda Rosenthal⁴, Martin Rutter⁵, Paul Johnson⁶, Mark Peakman¹, James Shaw², Timothy Tree¹


Following allogenic islet transplantation, declining graft function has been associated with inflammation, chronic allograft rejection and/or recurrent islet autoimmunity. We examined the phenotype and frequency of islet-specific T-cells before and after transplant, and their relationship with graft function.

Islet-specific T-cell responses were examined in 58 patients (110 samples total), using IFN-γ & IL-10 ELISpots at various time-points pre- and post-transplant. In parallel, graft function was assessed by MMTT.

Using cross-sectional cohorts of patients, we observed a gradual decline in graft function over time post-transplant (p=0.03). In spite of the long duration of disease in islet recipients and induction therapy with T-cell depleting agents, islet-specific T-cells were detectable pre-transplant and ≤6 months post-transplant. Notably, the phenotype of responses was altered from a predominantly IFN-γ-dominated response pre-transplant, to an IL-10-dominated response immediately post-transplant, with a gradual reversion to an IFN-γ response >24 months post-transplant. Unbiased hierarchical cluster analysis of islet-specific T-cell responses post-transplant revealed two main agglomerations; characterized by IFN-g or IL-10. Interestingly, patients within the IL-10+ cluster had significantly lower levels of C-peptide compared to those in the IFN-g+ cluster (p=0.008). However, longitudinal analysis revealed a significant decline in the C-peptide levels in the period preceding the IL-10 response, but stable graft function following the IL-10 response. In contrast, whereas levels of C-peptide were higher at the time of an IFN-g response, they declined significantly following this response. These results indicate that following transplantation, islet-specific T-cell responses rapidly reemerge and the phenotype of responding cells is associated with islet graft function.
T Cells of Multiple Autoreactivities Derived Directly from Islets of Donors with Type 1 Diabetes (T1D)

Sally Kent1, Jenny Aurielle Babon1, Megan DeNicola1, René Maehr1, Rita Bottino6, Ali Naji5, John Kaddis6, Wassim Elyaman3, Eddie James7, Marcela Brissova6, Lut Overbergh2, Chantal Mathieu2, Thomas Delong10, Kathryn Haskins10, Alberto Pugliese11, Martha Campbel Thompson12, Clayton Mathews12, Mark Atkinson12, Alvin Powers8,9, David Harlan1

1University of Massachusetts Medical School, Worcester, MA, USA, 2KU Leuven, Leuven, Belgium, 3Brigham and Women’s Hospital and Harvard Medical School, Boston, MA, USA, 4Allegheny-Singer Research Institute, Pittsburgh, PA, USA, 5University of Pennsylvania School of Medicine, Philadelphia, PA, USA, 6Beckman Research Institute, City of Hope, Duarte, CA, USA, 7Benaroya Research Institute at Virginia Mason, Seattle, WA, USA, 8Vanderbilt University, Nashville, TN, USA, 9Vanderbilt University Medical Center, Nashville, TN, USA, 10University of Colorado School of Medicine, Aurora, CO, USA, 11University of Miami, Miami, FL, USA, 12University of Florida, Gainesville, FL, USA

A significant knowledge gap exists in understanding the interactions between the islet infiltrating, islet-autoantigen reactive T cells and their targets in individuals with T1D. From the largest cohort to date of islets from pancreata of donors with T1D, we isolated, by direct growth from islets or by FACS-sorting of T cells from enzyme-dispersed islets, 236 T cell clones/lines from islets from 9 donors with T1D, 5 T cell lines from 1/7 donors without T1D, and no lines from 2 donors with type 2 diabetes (T2D). CD4+ and CD8+ T cell lines/clones were assayed for reactivity with known islet-derived peptide targets and panels of peptides with post-translational modifications by cytokine release. Seventeen T cell lines/clones (14 CD4+ and 3 CD8+) were found to be reactive with a broad range of islet-derived peptides. Of these CD4+ T cell lines, five were reactive with post-translationally modified islet peptides. All autoreactive T cell lines secreted pro-inflammatory cytokines. The remaining T cell lines/clones await determination of reactivity, including 20 CD8+ T cell lines from 5/9 of the islets isolated from donors with T1D and the separation and purification of 102 T cell lines that grew from islets of as mixtures of CD4+ and CD8+ T cells (from 6/9 donors with T1D). These studies are a major step forward in defining the repertoire of islet infiltrating, autoreactive T cells. These reactivities should impact induction of autoantigen-specific therapeutic efforts attempting to benefit subjects at-risk for, with recently diagnosed, and with established T1D in combination with islet replacement/regeneration.
CCL21 Expression in Beta Cells Induces Lymph Node Mimicry in the Pancreas and Prevents Autoimmune Diabetes

Maria Abreu¹, Mejdi Najjar², Allyson Bayer¹, Alberto Pugliese¹, Alice Tomei¹
¹University of Miami, Miami, FL, USA, ²University of Central Florida, Orlando, FL, USA

Currently there is no treatment to reestablish tolerance towards self-antigens, preventing islet autoimmunity and type 1 diabetes (T1D). In contrast, tumors can induce tolerance towards antigens they express. We have shown that tumor tolerance is dependent on the local expression of the chemokine CCL21 and on the formation of tolerogenic tertiary lymphoid organs (TLO) that we refer to as lymph node (LN) mimicry. CCL21 expression in b cells (TG+) prevents diabetes in non-obese diabetic (NOD) mice. Diabetes prevention in TG+ mice is not mediated by the blocking of islet-infiltration, but rather is associated with CCL21-induced LN mimicry in the pancreas. Islets of 4wk-old TG+ but not TG- NOD mice are surrounded by lymphoid stromal cells (LSCs) and immune infiltrates, which compartmentalize similar to that of LNs. LSCs found in LNs are known mediators of peripheral tolerance as they express and present self-antigens to autoreactive T cells leading to their deletion/inactivation in LNs. We find that splenocytes from TG+ mice are not able to transfer diabetes to NOD-scid mice and less b cell-autoreactive CD8 T cells are detected. Moreover, splenocytes from diabetic TG- NOD mice transfer diabetes to 25wk-old TG- but not TG+ mice, indicating that CCL21 transgenic expression in b cells suppresses the diabetogenic potential of TG- splenocytes after transfer into TG+ mice. Interestingly, TG+ islets transplanted into NOD-scid mice are protected from diabetes development via adoptive transfer of NOD splenocytes suggesting that CCL21 expression in b cells is sufficient to protect against autoimmunity.
T1D Cases show Epigenetic Marks Prior to T1D Diagnosis. The Diabetes Autoimmunity Study in the Young (DAISY)

Jill Norris¹, Fran Dong², Lauren Vanderlinden¹, Randi Johnson¹, Jennifer Seifert¹, Brigitte Frohnert², Kathleen Waugh², Katerina Kechris¹, Tasha Fingerlin³, Ivana Yang⁴, Marian Rewers²

¹Colorado School of Public Health, University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA, ²Barbara Davis Center, University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA, ³National Jewish Health, Denver, Colorado, USA, ⁴School of Medicine, University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA

Epigenetic tagging of genes, such as DNA methylation, may be involved in T1D etiology. We interrogated for DNA methylation signatures associated with the development of T1D in DAISY.

Peripheral blood DNA was obtained an average of 9 months prior to T1D diagnosis from 30 cases and 30 non-diabetic, islet autoantibody negative controls (frequency matched on age at autoantibody seroconversion and ethnicity). DNA methylation profiles were assessed using the Illumina Human Methylation 450K platform. After adjusting for age, sex and blood cell count, we selected the top 1000 differentially methylated positions and performed an enrichment analysis using PANTHER (http://pantherdb.org/) to identify biological function. Significantly differentially methylated positions represented genes in the cadherin signaling pathway (uncorrected p=1.1x10⁻⁵), and the Wnt signaling pathway (p=2.5x10⁻³). The most significant (false discovery rate(FDR)=0.032) and most differentially methylated site (change in beta=0.051) was located in the secretagogin gene. Cadherins mediate cell adhesion, thereby regulating morphology and function of many tissues as well as tissue trafficking of immune cells. In β-cells, cell-to-cell interactions, mediated by cadherins, promote glucose-stimulated insulin secretion and protection from apoptosis. The Wnt pathway promotes accumulation of β-catenin, which links cadherins to the cytoskeleton, representing an intersection of these two pathways. Secretagogin, expressed in β-cells and neuroendocrine cells, interacts with the actin cytoskeleton, influencing insulin secretion.

These results demonstrate different methylation patterns preceding T1D diagnosis. We are also examining whether differences between cases and controls exist at birth, in early childhood and the times surrounding islet autoantibody seroconversion in these children with HLA-conferred genetic risk.
Increased myeloid-derived suppressor cells correlate positively with Th17 and Th1 cells in patients with type 1 diabetes

Lenka Palová-Jelímková¹,², Klára Dáňová¹,², Pavla Strnadová¹, Zdeněk Šumník³, Lenka Petruželková³, Stanislava Koloušková³, Radek Špíšek¹,²
¹Sotio, a.c., Prague, Czech Republic, ²Department of Immunology, Charles University in Prague, Second Faculty of Medicine and University Hospital Motol, Prague, Czech Republic, ³Department of Pediatrics, Charles University in Prague, Second Faculty of Medicine and University Hospital Motol, Prague, Czech Republic

Myeloid-derived suppressor cells (MDSCs) represent a heterogeneous group of immature myeloid cells with immunoregulatory function. Expansion of MDSCs has been reported in some murine models and patients with autoimmune diseases, but the exact role of MDSCs in the pathogenesis of autoimmune diseases remains to be elucidated. This study sought to address the role of MDSC in the pathogenesis of type 1 diabetes (T1D). Compared to healthy donors, patients with active T1D as well as their first degree relatives with positive islet specific autoantibodies exhibited a profound expansion of CD14⁺CD11b⁺CD33⁺HLA-DR⁻/low monocytic MDSCs. Compared to healthy donors, the expansion of MDSCs in the peripheral blood of T1D patients was in strong positive correlation with increased Th17 as well as Th1 cells. Furthermore, circulating monocytic MDSCs were not associated with frequency of circulating CD4⁺CD25⁺FoxP3⁺CD127⁻low T regulatory cells. The mutual regulation of MDSCs and Th17 and Th1 cells may provide new insight into the pathogenesis of type 1 diabetes.
Modeling the consequence of reduced IL-2R signaling in NOD mice

Connor J. Dwyer, Allison L. Bayer, Carmen Fotino, Liping Yu, Cecilia Cabello-Kindelan, Kevin H. Toomer, Thomas R. Malek

1University of Miami Miller School of Medicine, Miami, FL, USA, 2University of Colorado Denver Anschutz Medical Campus, Aurora, CO, USA

Polymorphisms in IL2RA are a common genetic risk in type one diabetes (T1D). These SNPs lead to lower CD25 expression and reduced IL-2R signaling that affects both Tregs and T memory cells. Our lab developed a NOD mouse model in which all T lymphocytes expressed a mutant IL-2Rβ with three mutations in key tyrosine residues on its cytoplasmic tail (NOD-Y3). This model was designed to more closely model human T1D by targeting IL-2R signaling rather than IL-2 production. Consequently, NOD-Y3 Tregs exhibited a 2-fold reduction in IL-2 responsiveness measured by STAT5 phosphorylation. Male and female NOD-Y3 mice had increased autoantibody production, increased islet infiltration and accelerated diabetes onset. More antigen experienced CD4+ T effector cells and TNFα, IL-17 and IL-2 CD4+ T helper cells were found in the pancreas and pancreatic lymph node, respectively, of NOD-Y3 mice. For CD8+ T cells in NOD-Y3 mice, effector and effector memory cells were increased while central memory cells decreased. However, these effects are likely due to impaired IL-15 signaling. NOD-Y3 Tregs had reduced CD25 and Bcl-2 expression with increased Ki67, consistent with altered IL-2-dependent homeostasis. Mix bone marrow chimera experiments and adoptive transfers of central Tregs indicated that development of CD103+, Klrg1+ and ICOS+ effector Tregs were impaired in NOD-Y3 mice. These findings and current studies are consistent with accelerated diabetes onset in NOD-Y3 mice being primarily associated with defective Treg cells which promote increased autoreactive T cells.

(Supported by R01DK093866)
Low-dose IL-2 Therapy with a Novel IL-2-Based Fusion Protein Selectively Targets Tregs and Promotes Immune Tolerance

Natasha C. Ward¹, Liping Yu², Thomas R. Malek³

¹University of Miami Miller School of Medicine, Miami, FL, USA, ²University of Colorado Denver Anschutz Medical Campus, Aurora, CO, USA

Recent clinical trials suggest that low-dose IL-2 (~1 x 10⁶ IU) is an effective means to increase Tregs in patients with autoimmune diseases. In some studies, outward signs of clinical efficacy were observed. A clinical trial for T1D showed that low-dose IL-2 is safe and increases Tregs. Though promising, a limitation of low-dose IL-2 therapy is its short half-life that necessitates frequent administration. This led us to develop a new IL-2 biologic, an IL-2 and CD25 fusion protein (IL-2FP), to improve the expansion of Tregs. When evaluating IL-2-dependent pSTAT5 activation, Tregs were the main responsive population to IL-2FP, with minimal effects on conventional CD4⁺ and CD8⁺ T cells and NK cells. In comparison to IL-2, IL-2FP promoted longer duration of pSTAT5 activation, leading to greater Treg expansion and CD25 expression. These effects on Tregs are likely related to their increased expression of Ki67 and Bcl-2. A trend was also noted for increased numbers of KLRG1⁺ and CD103⁺ effector Tregs. In addition, these effects were much more notable in the pancreas than the pancreatic lymph node of NOD mice. IL-2FP, but not equivalent amount of IL-2, reduced insulin autoantibodies and prevented the onset of diabetes in NOD mice. In contrast to IL-2 with a half-life of 15-30 minutes, IL-2FP exhibits a half-life of 36-40 hours, which likely contributes to the improved stimulation of Tregs. Hence, IL-2FP shows promise as a new therapeutic to selectively target Tregs and promote immune tolerance in the context of autoimmune diseases.
Elucidating the Role of CD4 T Cells Reactive to Hybrid Insulin Peptides in the Pathogenesis of T1D

Rocky L Baker, Timothy A Wiles, Braxton L Jamison, Thomas Delong, Katherine Haskins
UC Denver - School of Medicine, Aurora, CO, USA

We previously reported that Chromogranin A (ChgA) and Islet Amyloid Polypeptide (IAPP) are target antigens for autoreactive CD4 T cells in the NOD mouse and that ChgA may be important to the initiation of disease since ChgA-deficient NOD mice do not develop diabetes. We recently established that hybrid insulin peptides (HIPs), formed spontaneously in islet beta cells by fusion of insulin C-peptide fragments to peptides of ChgA or IAPP, are ligands for diabetogenic CD4 T cell clones. To track HIP-reactive T cells in NOD mice, we used MHC class II tetramers loaded with HIPs. Both ChgA/HIP and IAPP/HIP tet+ cells were present in the islets of NOD mice starting at 6 weeks of age, and continued to accumulate throughout disease progression. A longitudinal analysis in the blood of NOD mice determined that T cells specific for a HIP containing a ChgA sequence are activated very early (before 8 weeks of age) whereas T cells specific for an IAPP/HIP are activated at a later time point. Upon comparing pre-diabetic with diabetic NOD mice we observed a shift of HIP-reactive T cells toward a Th1 phenotype and restricted V-beta usage by HIP-reactive T cells, suggesting a clonal expansion of T cells with an inflammatory phenotype. Using the tetramers, we isolated HIP-reactive T cell lines and demonstrated their diabetogenicity through adoptive transfer experiments. Our findings support the hypothesis that HIP-reactive T cells are critical players in the initiation and pathogenesis of T1D.
Identification of prediabetes biomarkers using single cell analysis  
Authors: Brian Abe¹, Marie Holt², Anne Costanzo², Louis Gioia¹, Andrew Su², Luc Teyton², ¹ Scripps Translational Science Institute, La Jolla, CA, ² The Scripps Research Institute, La Jolla, CA

Brian Abe, Marie Holt, Anne Costanzo, Louis Gioia, Andrew Su, Luc Teyton  
TSRI, La Jolla, California, USA

Purpose: The early prediction of autoimmunity in at risk patients for type 1 diabetes would extend the honeymoon period for therapeutic intervention. We hypothesized that CD4 T cell responses would precede autoimmune B cell responses.

Methods: We dissected the NOD mouse model to come to the conclusions that autoreactive T cells were in very small numbers and difficult to characterize at the population level. The conundrum is even more obvious when studying humans where only peripheral blood will be accessible. To overcome this limitation we developed single cell techniques combining pMHC tetramer isolation of CD4 T cells with gene expression profiling and TCR sequencing using microfluidic systems. Summary of results:
Expression profiling of 96 genes involved in T cell activation, tissue homing and residency, and cytokine signaling using real-time PCR, and using unbiased hierarchical clustering revealed that islet infiltrating T cells were unique and differentiable in their gene expression from lymph nodes and spleen. The validation of this expression pattern was confirmed by flow cytometry and allowed us to identify a similar CD4 T cell population in the peripheral blood of pre-diabetic mice. The paired TCR sequencing of these cells revealed unique islet-resident oligoclonal expansion.

Conclusions: These results raise the hopes of diagnosing pre-diabetes in patients at greatest risk of developing disease using peripheral blood and simple molecular techniques.
Single cell analysis of the anti-insulin CD4 T cell response in pre-diabetic NOD mice reveals the mechanism linking the MHCb57 polymorphism with type 1 diabetes. Authors: Luc Teyton, Anne Costanzo, Marie Holt, Lisa Kain, Brian Abe, Louis Gioia, Andrew Su. The Scripps Research Institute, La Jolla, California

Luc Teyton, Anne Costanzo, Marie Holt, Lisa Kain, Brian Abe, Louis Gioia, Andrew Su
TSRI, La Jolla, California, USA

Purpose: Test the relevance of the “P9 switch model” linking position b57 of MHC to the activation of autoreactive T cells to T1D.

I-A<sup>67</sup> tetramers specific for the insulin 12-20 and 13-21 peptides were produced and used to sort single cells from spleen, pancreatic lymph nodes (PLN), and islets. Expression analysis of 96 genes and TCR chain pairs sequencing were performed. Re-expression of TCR pairs in hybridoma T cells, recombinant protein expression and biophysical measurements, and retrogenic mice were carried out to test the hypothesis.

Results: At the early pre-diabetic phase of disease, selection, expansion, and islet infiltration were dominated by CD4 T cells specific of the insulin 12-20 peptide which retains no charge at the P9 position. This population presented the hallmarks of cytokine/type 1 interferon activation in PLNs, whereas signs of TCR-mediated activation were found only in islets. TCR sequencing revealed three unique features of anti-Ins12-20 T cells: diverse a and b chain usage in PLN, oligoclonal expansion in islets with an overwhelming usage of TRAV5D4A and TRVB1, presence of an acidic residue in the N-terminal portion of the TCR CDR3b in >90% of cells. The CDR3b acidic amino acid was shown to be critical for TCR recognition.

Conclusions: The anti-INS12-20 peptide recognition dominates the pre-diabetic phase and is governed by a single acidic residue in CDR3b. These results confirm that the “P9 switch model” is operational in vivo and constitutes the most likely mechanism supporting the association between T1D and position b57 of diabetogenic MHC molecules.
Antigen presentation in multiple lymphoid sites generates insulin autoantibodies targeting the islet of Langerhans

Xiaoxiao Wan¹, Hao Hu¹, James Thomas², Emil Unanue¹
¹Washington University School of Medicine, St Louis, MO, USA, ²Vanderbilt Medical School, Nashville, TN, USA

The presence of class-switched insulin autoantibodies (IAAs) is a consistent feature of patients with type 1 diabetes and NOD diabetic mice. The mechanisms underlying their production and pathogenic role are poorly understood. Production of class-switched IAAs is unexpected because insulin-reactive B cells are maintained presumably in a tolerant state. Furthermore, the source of T cell help is limited because central tolerance mechanisms, particularly mediated by insulin-expressing medullary thymic epithelial cells, are imposed upon thymocytes recognizing insulin. We find that a majority of the anti-insulin T cells in NOD mice recognize insulin peptides but not native insulin. A T cell receptor transgenic strain, 8F10, whose T cell reactivity is restricted to insulin peptides but not insulin protein is accompanied by increase titers of IAA, all directed to conformational epitopes. Furthermore, when an anti-insulin B cell receptor heavy chain transgene was introduced into the 8F10 mice, a marked increase in IAAs specific for native insulin was observed. Dynamic T-B cell interactions and spontaneous germinal center formation occurred in multiple lymphoid organs, indicating widespread insulin presentation. The T-B cell interactions took place because germinal center B cells, in contrast to naïve B cells, presented a broader repertoire of insulin epitopes, allowing the match of peptide-MHCII complexes with the 8F10 TCR. Finally, we demonstrate that IAAs localized into the islets of Langerhans, formed deposits on β-cells, and enhanced presentation of diabetogenic antigens. Thus, systemic recognition of a native circulating self-protein is sufficient for generation of autoantibodies that precipitate pathogenic outcomes in the target organ.
Immune Tolerance During Autoimmune Insulitis can be Restored by Inhibition of Hyaluronan Synthesis

Nadine Nagy1, Gernot Kaber1, Hedwich Kuipers1, Pamela Johnson2, Michel Gooden2, John Gebe2, Marika Bogdani2, Anthony Day3, Robert Vernon2, Daniel Campbell2, Thomas Wight2, Paul Bollyky1

1Stanford University School of Medicine, Stanford, CA, USA, 2Benaroya Research Institute, Seattle, WA, USA, 3University of Manchester, Manchester, UK

We recently reported that the extracellular matrix (ECM) polysaccharide hyaluronan (HA) accumulates at sites of autoimmune insulitis in human type 1 diabetes (T1D). However, the relevance of these HA deposits to T1D pathogenesis is unclear. We have evaluated the impact of 4-methylumbelliferone (4-MU), an oral inhibitor of HA synthesis, on disease progression in the DORmO (DO11.10xRIPmOVA) and Non Obese Diabetic (NOD) mouse models of T1D.

We find that islet HA deposits are also present in these animals and are temporally and anatomically associated with the development of insulitis, in patterns similar to those seen in human T1D. These HA deposits are characteristically low molecular weight HA fragments, known to promote inflammatory responses in other tissues. Moreover, treatment with 4-MU, halted progression to diabetes even after the onset of insulitis. In the NOD mouse model 1 week of treatment was sufficient to prevent subsequent diabetes. Mechanistically, 4-MU inhibits the activation of autoreactive T cells and prevents their polarization toward a Th1 phenotype. Instead, 4-MU promotes polarization toward a Th2 phenotype and induction of Foxp3(+) regulatory T cells in a CD44-dependent manner.

Together, these data suggest that HA synthesis is necessary for disease progression in T1D. We propose that 4-MU, already an approved drug used to treat biliary spasm, could be repurposed to prevent, and possibly treat, T1D in at-risk individuals.
Development of Hybrid Insulin Peptides (HIPs) as Biomarkers Among Type 1 Diabetic (T1D) Individuals

Janet Wenzlau, Thomas Delong, Anita Hohenstein, Kathryn Haskins
University of Colorado Anschutz Medical Campus, Aurora, CO, USA

Our lab has recently reported that HIPs are unique post-translationally modified antigens that may play a critical role in the immunopathology of T1D. Several diabetogenic T-cell clones from NOD mice show specific and robust responses to HIPs comprised of C-peptide fused to distinct granule proteins like C-peptide/WE14 and C-peptide/IAPP2. These and other HIPs derived from beta cell extracts have been confirmed via mass spectrometry.

Four HIPs have been identified as the cognate epitopes of human CD4 T cell lines/clones cultured from pancreatic islets from T1D organ donors. The C-peptide sequence in each HIP is identical, and like the mouse HIPs, each human HIP contains C-peptide fused to different natural granule protein cleavage products, IAPP1, IAPP2, NPY1, or insulin A chain. Together these data imply that HIPs play a salient role in human T1D. The existence of human HIP epitopes implies a new mechanism for autoantigen ligand formation and putative novel targets for autoantibodies.

We hypothesize that HIPs and/or autoantibodies targeting HIPs can be detected in the circulation of newly diagnosed T1D individuals. HIP biomarkers may serve directly or indirectly as early parameters for ensuing disease and as metrics for stratification of individuals at risk for T1D that would most benefit from (HIP-specific) immunotherapies. We are developing molecularly specific assays to optimize measurement of HIP-autoantibodies and other assays monitoring human HIPs directly to assess their utility as biomarkers. Beyond development of biomarker assays, these studies provide the prerequisites for eventual drug development using HIPs as the key players.
Partially exhausted CD8 T cells are associated with clinical response to teplizumab in new-onset type 1 diabetes

Alice Long¹, Jerill Thorpe¹, Hannah DeBerg¹, Vivian Gersuk¹, James Eddy¹, Kristina Harris², Mario Ehlers³, Kevan Herold⁴, Gerald Nepom¹, Peter Linsley¹
¹Benaroya Research Institute, Seattle, WA, USA, ²Immune Tolerance Network, Bethesda, MA, USA, ³Immune Tolerance Network, San Francisco, CA, USA, ⁴Yale University, New Haven, CT, USA

The inability of immunomodulatory agents to induce long-term benefit in type 1 diabetes (T1D) underscores the need to better understand immunologic mechanisms controlling disease progression. Typically, treatment results in transient stabilization of C-peptide levels in some patients, followed by progression at the same rate as in untreated control groups. Here, we compared immunological responses in subjects with the best response to the anti-CD3 monoclonal antibody teplizumab (responders) to subjects with limited response (non-responders) and untreated controls. Using an unbiased and integrated systems biology approach, we identified genes associated with C-peptide stabilization that correlated with CD8 T cells. These CD8 T cells expanded after treatment in responders and expressed high levels of the transcription factor EOMES, effector molecules, and multiple inhibitory receptors (IRs), including TIGIT and KLRG1, as measured by both transcriptional analyses and flow cytometry. We next isolated memory CD8 TIGIT+KLRG1+ T cells from responders to functionally define this population. TIGIT+KLRG1+ CD8 T cells had expanded TCR clonotypes, and transcriptionally resembled exhausted cells with increased expression of IR genes and decreased cell cycle genes following polyclonal stimulation. Importantly, further downregulation of T cell activation was achieved by triggering with a recombinant ligand for TIGIT, suggesting that the exhausted phenotype of CD8 T cells associated with favorable response to teplizumab was partial, not terminal. These findings identify and functionally characterize a cell type associated with response to teplizumab therapy and suggest that pathways regulating T cell exhaustion may play a role in successful immune interventions for T1D.
Erratic Blood Glucose Levels in a Diabetic Patient with Insulin Antibody

Josie Go, Jose Mejia
Nassau University Medical Center, East Meadow, NY, USA

Introduction:

In the era of insulin analogue, the development of antibodies to exogenous insulin is rare. Presence of these insulin antibodies can cause labile glycemic control.

Case Presentation:

A 56 year old African-American male with a history of insulin-dependent diabetes mellitus who presented with altered mental status and in hyperosmolar hyperglycemic state (HHS). HHS treated accordingly with resolution. Appropriate insulin therapy was restarted. Patient subsequently developed episodes of fasting hypoglycemia during hospital stay.

Laboratory results showed an elevated insulin antibody, normal serum insulin, low C-peptide, and elevated fructosamine. Glycosylated hemoglobin was 10.8%.

Diet modification with frequent small meals with complex carbohydrates was started. Hypoglycemic episodes persisted. Immunosuppressive therapy with prednisone 60mg daily was then added. With the current regimen, patient had better glycemic control. Amount of insulin required per day was also decreased.

Discussion:

Presence of insulin antibodies can present as a challenge in obtaining a good glycemic control. These antibodies will bind to insulin which can cause a marked increase in insulin resistance. This, in turn, reduces insulin action, thus, triggering hyperglycemia. Furthermore, insulin antibodies can also serve as a carrier, forming insulin antibody-insulin complex. When massive volume of these complexes dissociate, free insulin increases all at once, thus, leading to hypoglycemia.

Generally, the hypoglycemic attacks are relieved as insulin antibody titer decreased. Glucocorticoids, anti-CD20 antibody therapy or plasmapheresis could be another option for severe cases.

Conclusion:

In patients with recurrent episodes of hyperglycemia and hypoglycemia, the presence of antibodies to insulin should be considered.
Genetic susceptibility to type 1 diabetes leads to functional alterations in the gut 
microenvironment and perturbations of the microbiota

Jane Mullaney, Juliette Stephens, Saleh Alabbas, Cai Fong, Brooke Geeling, Emma Hamilton-Williams
The University of Queensland Diamantina Institute, Brisbane, Australia

It has recently been shown that alterations in the microbiota can be found in both individuals with or at 
risk of developing type 1 diabetes (T1D). These changes may be due to environmental drivers, genetic 
risk factors or both. We have used type 1 diabetes susceptible NOD mice to investigate whether T1D 
susceptibility genes drive alterations in the gut microbiota. We demonstrate that protective alleles of the 
Idd3, (IL2) Idd5 (CTLA4, Sic11a1 and Acadl) and MHC, regions, which all mediate profound protection 
from T1D, are associated with shifts in the microbiota. Through histological and gene expression studies 
of the intestine of NOD mice compared with protected strains, we found subclinical pathology in the NOD 
intestine including increased immune cell infiltrates, reduced goblet cell mucous production and reduced 
Paneth cell anti-microbial peptide production. Although NOD mice had a deficiency in IL-22 producing gut 
associated type 3 innate lymphoid cells, contributing to the loss of goblet cell homeostasis, we show that 
expression of protective Idd3 and Idd5 alleles in NOD mice resulted in increased goblet cell mucous 
production and immunotherapeutic administration of IL-2, which mimics the effects of the protective Idd3 
allele, reduced overall gut inflammation in NOD mice. Expression of the MHC alleles also results in 
functional improvement in Paneth cell produced anti-microbial peptides. These findings demonstrate for 
the first time that type 1 diabetes associated genetic variants that restore immune tolerance to islet 
antigens also result in functional changes in the gut immune system and resultant changes in the 
microbiota.
The mucosal immune system is an important barrier preventing pathogens from entering the body through tissues who are in continuous contact with the environment such as the oral cavity and the vagina. The immune cells who mediates immune responses are attracted to mucosal tissues through chemokines, such as CCL28, CXCL14, CCL25 and CXCL17. We recently found that the population that migrates in response to CXCL17 are a sub-population of macrophages that expressed the receptor CXCR8.

Additionally, we have found that under homeostatic conditions the CXCL17 expression is highly superior, compared with the other mucosal chemokines, in the superior GI system and vagina. Moreover under inflammatory conditions, CXCL17 increased its expression and begin to be expressed in another tissues such as caecum and colon. The surprising high expression of the CXCL17 in the stomach, and the predominant expression of its receptor, CXCR8 in the brain, make us propose that the chemokine could be participating in the food intake regulation. Therefore we evaluate the expression of the chemokine under starvation conditions, finding that the expression of the CXCL17 increases at a level comparable with ghrelin: the hunger hormone. We also evaluate the response of the Cxcl17−/− mice to a high fat diet. We found that Cxcl17−/− mice gain more weigh versus the wild type mice. Those results make us think that Cxcl17 is a chemokine that may influence the metabolism of the mice, in mechanism influenced by macrophages.
Gene expression differences between tolerogenic and inflammatory dendritic cells identify targets that neutralize T1D genetic risk and improve immune regulation

Tatjana Nikolic1, Nicky Woittiez1, Arno van der Slik1, Sandra Laban1, Antoinette Joosten1, Bobby Koeleman2, Bart Roep1,3, Laura Claessens1
1Leiden University Medical Center, Leiden, The Netherlands, 2Utrecht Medical Center, Utrecht, The Netherlands, 3City of Hope, Duarte, CA, USA

Tolerogenic dendritic cells (tolDCs) are a promising immunomodulatory adjuvant to regulate islet autoimmunity in patients suffering from Type 1 Diabetes (T1D). To understand the gene expression underlying the regulatory features, we compared the transcriptomes of tolDCs modulated with 1,25(OH)2vitamin D3 and dexamethasone with that of non-modulated mature inflammatory dendritic cells (mDCs).

For this aim, we performed deep RNA-seq analysis of mature VD3/Dex-modulated tolDCs and non-modulated mDC generated from monocytes of four healthy donors. Of 18,000 detected gene transcripts, 4500 genes differed at least 2-fold between tolDCs and mDCs and were further analysed.

In gene ontology terms, differentially expressed genes classified into response to extracellular stimulus and regulation of cell activation. Genes reduced in tolDC were involved in cytoskeleton organisation and amino-acid metabolism pathways. Genes increased in tolDCs compared to mDCs mainly contributed to energy homeostasis, endocytosis and antigen processing. Further, we analyzed 26 genes conferring genetic risk for developing T1D and expressed in DCs, of which 20 showed different expression between tolDCs and mDCs. Risk genes increased in tolDCs contribute to immune inhibition and MHC class II activity, whereas genes lower expressed in tolDC than in mDC classified as activators of immune responses.

In conclusion, modulation of monocytes into tolDCs alters gene transcription at several functional levels. Differentially expressed transcripts of candidate risk loci for T1D point to a role of these ‘risk genes’ in regulating immune responses and targeting immune modulation into ‘regulatory transcriptome’ may neutralize the contribution of these genes to risk for development of T1D.
Levels of Catecholamine Mediating Enzyme - Dopamine-Beta-Hydroxylase (DBH), Its Cofactors and Other Biochemical Parameters in Epileptic and Diabetic Neuropathy Patients of Bangladesh

Md Khalilur Rahman
Dhaka University, Dhaka, Bangladesh

The catecholamine neurotransmitter-mediating enzyme, Dopamine-Beta-Hydroxylase (DBH) is a Cu++ and ascorbic acid dependent enzyme that produces noradrenaline from dopamine in human and laboratory animals. In Bangladesh, many people are suffering from neurological diseases like epilepsy, diabetic and peripheral-neuropathies. The main aims and objectives of this study were to find out the levels of DBH activities in serum of the patients suffering from epilepsy and diabetic neuropathy diseases. The results were compared with those of healthy individual of similar age groups. In order to get a clear profile of DBH activity in these patients of Bangladesh, along with DBH activity, the co-factors of DBH (copper and ascorbic acid) and glucose, protein and other relevant parameters were also measured in the serum of patients and in healthy normal individuals. DBH activity was expressed as nmols/min/ml of serum (1 unit). DBH activity of juvenile epileptic patients were found to be 1.23±0.46 units in Group 1 (1-5 years), 2.73±0.43 units in Group 2 (6 - 10 years) and 4.25±0.69 units in Group 3 (11- 15 years) as compared to that in the age matched control Groups, these values were 5.83±2.02, 9.44±1.865 and 12.25±2.46 units, respectively. The results on the adult epileptic patients also showed the significant decreased activity of DBH activities as compared to the age matched control subjects. DBH activities were also drastically decreased in all the serum samples of diabetic-neuropathy and peripheral-neuropathy patients of Bangladesh.
Genetic risk for type 1 diabetes (T1D) is conferred primarily by the HLA class II region, with roughly 50 additional susceptibility loci contributing modest individual risk. Given the large number of risk loci, the combinations of these risk alleles may result in heterogeneous disease presentation and etiology. Our objective was to apply a combinatorial genetic risk score (GRS) composed of HLA imputing SNPs and putatively identified non-HLA risk SNPs to assess the combined genetic burden. METHODS: A cohort consisting of 298 controls, 543 first-degree relatives, 456 T1D, 24 other autoimmune, and 27 type 2 diabetes (T2D) subjects were genotyped on a custom SNP array. Subject HLA was imputed by DR3 and DR4 SNPs and combined with SNP typing of 29 additional T1D-risk loci. The GRS calculation was a log-additive model that incorporated the odds ratios and number of risk alleles carried for each locus. RESULTS: The GRS yielded a T1D vs control ROC-AUC of 0.844 (P<0.0001), and a GRS>50th T1D-centile was indicative of T1D with 92.9% specificity. Interestingly, the GRS negatively correlated with age at diagnosis (Pearson P=0.0002). CONCLUSIONS: Here, we report that a combined GRS aids in discriminating subject cohorts. Moreover, an elevated GRS negatively correlates with age at disease diagnosis. These results corroborate previous reports that a GRS can aid in cohort stratification, which will improve functional studies, biomarker identification, and subject selection for interventional and natural history trials.
Alteration of B cells subsets and receptor for B-cell activating factor BAFF in children with type 1 diabetes

Anna Sediva¹, Jana Kayserova¹, Zuzana Parackova¹, Michal Rataj¹, Irena Zentsova¹, Stanislava Kolouskova², Lenka Petruzelkova², Zdenek Sumnik²

¹Department of Immunology, Motol University Hospital and 2nd Faculty of Medicine, Charles University, Prague, Czech Republic, ²Department of Pediatrics, Motol University Hospital and 2nd Faculty of Medicine, Charles University, Prague, Czech Republic

Background:

T1D is considered to be a complex disorder initiated by an innate immunity and mediated by T cells. Lately, mounting evidence shows that B cells might also play a critical role in T1D development. Here we present alterations in B cells subsets including BAFF receptor (BAFFR) expression in cohorts of T1D patients and their relatives.

Patients:

Cohort of 304 patients with T1D was included in the study, 41 in onset, 263 with long-term disease and 96 their relatives. B cell panel included naive, transitional, MZ-like and switched memory B cells. We have also measured BAFF serum levels as well as BAFFR expression on both B and T cells.

Results:

We observe significant decrease of naive B cells in all tested cohorts, and decreased transitional B cells in long-term T1D patients in comparison to controls. While we do not see significant differences in MZ-like B cells among cohorts, we identified a subset of patients with an increase of this special B cell subpopulation. BAFFR expressing CD4 and CD8 T cells and B cells do not differ in frequency among T1D and controls, however, expression of BAFFR on those cells is significantly lower in T1D patients and also in their relatives in comparison to controls.

Conclusion:

Patients with T1D present with a decrease of populations of B cells in early stages of their development. Their B cells, as well as T cells are also characterized by a lower expression of BAFFR, suggesting further alterations in B cells activation upon BAFFR ligation.
Enrichment of antigen specific T cells in type 1 diabetes

Martin A Thelin^1, Stephan Kissler^1, Frederic Vigneault^3, Alexander L Watters^3, Des White^3, Sandeep T Koshy^2,3, Sarah A Vermillion^3, David J Mooney^2,3, Omar A Ali^3, Thomas Serwold^1

^1Joslin Diabetes Center, Boston, MA, USA, ^2School of Engineering and Applied Sciences, Boston, MA, USA, ^3Wyss Institute for Biologically Inspired Engineering at Harvard University, Boston, MA, USA

T cells are known to play an essential role in the development of type 1 diabetes (T1D) both in human and non-obese diabetic (NOD) mice. A major roadblock in the study of T1D is that the T cells that promote T1D, while abundant in the pancreas, are exceedingly rare in the blood. In order to enable the study of rare beta-cell specific T cells, we developed a method to enrich and harvest diabetogenic T cells in vivo using a biomaterial scaffold loaded with lysates from an insulin-producing beta-cell line. The scaffold is composed of a synthetic poly (lactic-co-glycolic acid) PGLA matrix and is implanted subcutaneously in NOD mice. T cells are recruited to the scaffolds in an antigen specific manner and these T cells induce diabetes after adoptive transfer into NOD.SCID mice. Additionally, we sequenced the T cell receptors (TCRs) and identified many expanded TCRs within the beta-cell scaffolds that were also expanded within the pancreases of NOD mice. Collectively, our data demonstrate the utility of biomaterial scaffolds loaded with beta-cell antigens to identify and study rare T cells in T1D.
Excess Body Mass Index in Childhood: A Modifiable Risk Factor for Type 1 Diabetes Development?

Christine Ferrara1, Susan Geyer2, Yuk-Fun Liu3, Carmella Evans-Molina4, Ingrid Libman5, Rachel Besser6, Dorothy Becker5, Henry Rodriguez4, Antoinette Moran7, Stephen Gitelman1, Maria Redondo8, TrialNet Type 1 Diabetes Study Group9

1University of California, San Francisco, San Francisco, CA, USA, 2University of South Florida, Tampa, FL, USA, 3Kings College London, London, UK, 4Indiana University School of Medicine, Indianapolis, IN, USA, 5Children’s Hospital of Pittsburgh of UPMC, Pittsburgh, PA, USA, 6Oxford University Hospitals NHS Foundation Trust, Oxford, UK, 7University of Minnesota, Minneapolis, MN, USA, 8Baylor College of Medicine, Houston, TX, USA, 9Type 1 Diabetes TrialNet Study Group, USA, USA

Objective: The rising incidence of type 1 diabetes parallels an increased prevalence of obesity, yet the causal association remains inconclusive. Analyses often examine BMI at a single time point without emphasis on duration of BMI elevation. We aimed to determine the cumulative effect of elevated BMI over time on the progression to type 1 diabetes in youth, and to study the impact of age and sex on this relationship.

Research Design and Methods: We studied 1,117 pediatric participants in the TrialNet Pathway to Prevention cohort, i.e. autoantibody-positive relatives of patients with type 1 diabetes. Longitudinally accumulated BMI above the 85th age- and sex-adjusted percentile was calculated to generate a cumulative excess BMI (ceBMI) for each subject. Recursive partitioning analysis and multivariate modeling yielded sex and age- specific thresholds for ceBMI that confer the greatest risk for type 1 diabetes progression.

Results: ceBMI ranged from -10 to +15.1 kg/m² (median -1.86), with 0 corresponding to the CDC definition of elevated BMI (>85th BMI percentile). Higher ceBMI corresponded to significantly greater risk of progressing to type 1 diabetes (p=0.0006). The increased risk of diabetes occurred at lower ceBMI values in children <12 years compared to older subjects, and in females versus males.

Conclusions: Elevated BMI is associated with increased risk of diabetes progression in pediatric autoantibody positive relatives, but the effect varies by sex and age. These data suggest that lifestyle modifications to lower BMI may delay the onset of type 1 diabetes and offers specific BMI thresholds for implementing these changes.
Measurement of autoantibodies to steroid 21-hydroxylase in patients with type 1 diabetes mellitus using a new ELISA

Shu Chen¹, Maria del Pilar Larosa¹, Hannah MacRae¹, Nora Steinmaus¹, Liang Guo¹, Artur Bossowski², Jadwiga Furmaniak¹, Corrado Betterle³, Bernard Rees Smith¹
¹FIRS Laboratories, RSR Ltd, Cardiff, UK, ²Department of Pediatrics, Endocrinology and Diabetes with a Cardiology Unit, Medical University in Bialystok, Bialystok, Poland, ³Unit of Endocrinology, Department of Medical and Surgical Sciences, University of Padua, Padua, Italy

Patients with type 1 diabetes mellitus (T1DM) are at risk of developing associated autoimmune conditions and approximately 33% of patients are positive for non-islet autoantibodies. Autoantibodies to steroid 21-hydroxylase (21-OHAb) are markers of adrenal autoimmunity and are helpful in prediction of Addison’s disease (AD). We have developed a new, sensitive and specific bridging ELISA for the measurement of 21-OHAbs.

The ELISA employs the ability of the autoantibodies in the test sample to form a bridge between the purified recombinant human 21-OH coated onto the plate well and biotinylated 21-OH in liquid phase. Bound 21-OH-biotin is detected by addition of streptavidin peroxidise and a colorogenic peroxidise substrate.

88/107 (82%) of samples from patients with autoimmune AD were positive in 21-OHAb ELISA compared to 85/107 (79%) in the immunoprecipitation assay (IPA) using ¹²⁵I-labelled 21-OH. 3/355 (1%) healthy adult blood donors were positive in the ELISA. None of the samples from adult patients with Graves’ disease (n=50), neuroimmunological diseases (n=7), systemic lupus erythematosus (n=9) and rheumatoid arthritis (n=16) were positive in the ELISA. However, 4/120 (3.3%) samples from children with T1DM, 2/54 (3.7%) samples from children with Graves’ disease and 6/73 (8.2%) samples from children with Hashimoto’s thyroiditis were positive in 21-OHAb ELISA.

The newly developed 21-OHAb ELISA is a convenient alternative to IPAs based on isotopic labels. The ELISA has the assay sensitivity and specificity at least as good as the IPAs and should be a useful tool for detection of 21-OHAb in the wide range of clinical applications.