1793-P

Enrichment Analysis Informs Rare Variant Association Tests of Type 2 Diabetes and Glycemic Traits in CHARGE Whole-Genome Sequence

SAMANTHA LENT, ALISA MANNING, JENNIFER WESSEL, JOSÉE DUPUIS, JAMES B. MEIGS, CHARGE DIABETES WORKING GROUP, *Boston, MA, Indianapolis, IN*

Whole genome sequence (WGS) data can give us a better understanding of the genomic architecture of type 2 diabetes (T2D) and related glycemic traits. Most WGS variants are rare (allele frequency <0.01) and in non-coding regions. We hypothesize that applying enrichment tests developed for common variants to our data will improve power by identifying biologically meaningful categories for rare variant tests and reducing multiple testing burden.

We analyzed WGS variants (average depth 7x) for association with T2D (N=3005), fasting glucose (FG; N=2825) and log-transformed fasting insulin (FI; N=2825) adjusted for age, sex and BMI (FI only), in European ancestry individuals from 3 cohorts in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium: Framingham Heart Study, Cardiovascular Heart Study, and Atherosclerosis Risk in Communities Study. We used LD score regression (LDSC) to test for enrichment in 120 different annotations, including LDSC baseline annotations and all tissue-specific functional region predictions from GenoSkyline Plus. We limited our analyses to common variants found in the 1000 Genomes Project (~1,070,000 variants) in order to use pre-calculated linkage disequilibrium statistics in LDSC.

We found 4 annotations nominally enriched (P<0.05) for FI associations-ovaries (P=0.006), promoters (P=0.010), muscle satellite cultured cells (P=0.016), and conserved regions (P=0.046). Repressed regions (P=0.023), coding regions (P=0.029), and the brain anterior caudate (P=0.045) were nominally enriched for FG associations. In the T2D case-control analysis, we found enrichment in enhancers (P=0.011), DNaseI Digital Genomic Footprinting regions (P=0.023), coding regions (P=0.032), and CD3+ T-cells (P=0.044).

We will use these annotations to group non-coding rare variants in known glycemic trait and T2D loci to evaluate if using prior knowledge gives biologically meaningful rare variant results.

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1794-P

Leptin Signaling Regulates Pluripotency of Stem Cells

MANOJ K. GUPTA, ANGE UWIMANA, ROHIT N. KULKARNI, *Boston, MA*

Leptin is a central regulator of metabolism. While leptin (Ob) and leptin receptors (ObR) are expressed in embryonic and induced pluripotent stem cells, the role of leptin in pluripotency and lineage development and their consequences of progression of metabolic diseases is poorly understood. Here we report molecular and functional characterization of induced pluripotent stem cells (iPSCs) reprogrammed from tail tip fibroblasts of leptin deficient ob/ob mice (N=3) and non-leptin deficient wild type (+/+) mice (N=3). Genomic DNA from control and ob/ob iPSCs was isolated and genotyped to validate the leptin mutation via polymerase chain reaction (PCR). Morphological and pluripotency analysis of iPSCs were performed using bright-field microscopy and alkaline phosphate staining respectively. The proliferation of iPSCs was determined by hematocytometer and MTT cell proliferation assay. Protein expression of pluripotency markers were decreased (Oct4 by 30%; Nanog by 60%) in ob/ob iPSCs confirmed by Western blotting and immunostaining. Oct4 and Nanog transcripts were also decreased by 30-50% as shown by quantitative real time PCR analysis. Interestingly, the phosphorylation of Stat3/Akt, key pluripotency signaling pathways, were attenuated (pStat3 decreased by 80% and pAkt decreased by 50%) in ob/ob iPSCs as compared to control iPSCs demonstrated by Western blotting. Finally, we differentiated the iPSCs into embryoid bodies (EBs) and observed relatively larger bodies at day 5 from ob/ob iPSCs. However, determination of differentiation potential of ob/ob iPSCs warrants further investigation. We report, for the first time, that a mutation in leptin impacts pluripotency of ob/ob iPSCs via the Stat3/Akt signaling axis and has implications for leptin in regulating pluripotency and differentiation of metabolic cells.

1795-P

Type 2 Diabetes Gene Bioinformatically Identified by Variants Mapping to Amino-Acid Changes in Three-Dimensional Protein Space

MARCIN VON GROTTHUSS, JOSE C. FLOREZ, JASON FLANNICK, *Cambridge, MA*

We hypothesized that three-dimensional (3-D) protein structure maps might enable identification of key type 2 diabetes genes due to more accurate groupings of variants for aggregate association tests. We developed a novel method to map all missense mutations to 3-D structures of proteins and use these maps in aggregate association analysis. We applied our methodology to analyze missense variants in TBC1D1 and TBC1D4 as identified from sequence data of 16,857 multi-ethnic T2D cases/controls; we chose these genes because TBC1D4 was recently identified to carry a Greenlandic variant of large effect on T2D risk and has an established role in insulin signaling, while TBC1D1 has a potentially (but unverified in humans) analogous role to TBC1D4 based on animal models. PTVs in TBC1D1 (OR=2.14, p=0.02), as well as combined PTVs and bioinformatically deleterious variants in TBC1D4 (OR=1.2, p=0.03), demonstrated nominally significant aggregate association with increased T2D risk in the heterozygous state. However, bioinformatically deleterious missense variants in TBC1D1 showed no association (OR=1.02, p=0.88). In contrast, our mapping algorithm identified a cluster of mutations localized on the surface of the TBC1D1 Rab-GAP binding site, which in aggregate demonstrated a nominal association with T2D risk (OR=2.7, p=0.038) on par with that observed for PTVs. Rab-GAP plays a known role in localization of the glucose transporter GLUT4 to the plasma membrane in insulin responsive tissues, with TBC1D1 and TBC1D4 binding necessary for Rab activation. Although functional and genetic follow-up is necessary, our preliminary results suggest (a) that disruption of TBC1D1 in humans increases risk for T2D, similar to disruption of TBC1D4; (b) that impaired Rab-GAP binding is responsible for this increase in risk; and (c) that analyzing variants in 3-D protein space can inform aggregate tests of missense variants when cellular assay data is unavailable.

IMMUNOLOGY

Moderated Poster Discussion: Cutting-Edge Studies in Inflammation and Diabetes *(Posters: 1796-P to 1801-P), see page 19.*

1796-P

Innate Immune Defects in Diabetes-Tuberculosis Comorbidity Are Corrected through Inhibition of IL-6 Signaling

JAMES DILISIO, ANDRES OBREGON-HENAO, DAVID ACKART, ALEXANDRA TODD, RANDALL J. BASARABA, BRENDAN K. PODELL, *Fort Collins, CO*

Guinea pigs with diet-induced insulin resistance and type 2 diabetes are highly susceptible to low-dose aerosol exposure of Mycobacterium tuberculosis. Similar to the mouse model of diabetes-TB comorbidity, we show that diabetic guinea pigs also manifest a delay in the onset of adaptive immunity. Since IL-6, a cytokine known to interfere with myeloid cell migration, is increased in lung of naive diabetic guinea pigs, we investigated the early innate response to M. tuberculosis and the impact of IL-6 on the interface of innate and adaptive immunity. At day 10 of infection, diabetic guinea pigs fail to develop pulmonary lymphangitis, which corresponds to a lack of detectable bacteria in the lung draining lymph nodes. Transwell assays using soluble lymph node homogenate as a chemoattractant indicate that myeloid phagocyte populations in bronchoalveolar lavage fluid have an impaired ability to migrate toward diabetic lymph node but not nondiabetic lymph node, indicating a chemotactic defect at this stage of infection. Further identification of myeloid subsets by flow cytometry indicates that neutrophils are not rapidly recruited to the lung of diabetics. In contrast, diabetic guinea pigs have high numbers of inflammatory macrophages in bronchoalveolar lavage fluid, which combined with high expression of CCL2 in lung, suggests an inappropriately mature innate response. Knockdown of IL-6 signaling by intrapulmonary administration of translation-blocking Vivo-Morpholinos targeting the IL-6 signal transducer, gp130, significantly improved pulmonary influx of neutrophils in diabetic guinea pigs, and restored migratory capacity of pulmonary phagocytes to lymph node chemoattractants. These data support the critical role for an early neutrophil response in the migration of antigen presenting cells to the lung-draining lymph node, which is impaired by preexisting diabetes-induced IL-6 in the lung.

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1797-P

Aid Deficiency Accelerated T1D by Promoting Lymphocyte Activation and Expansion in NOD Mice

QIYUAN TAN, NINGWEN TAI, YANGYANG LI, SEAN PENNETTI, F. SUSAN WONG, LI WEN, *New Haven, CT, Cardiff, United Kingdom*

Type 1 diabetes (T1DM) is an organ specific autoimmune disease mediated mainly by diabetogenic T cells. However, B cells also play an important role in the process of the disease. Activation-induced cytidine deaminase (AID) is an enzyme initiating somatic hypermutation (SHM) and class switch recom-

For author disclosure information, see page A751.

bination (CSR) in B cells. Increasing evidence has shown that both mice and patients with AID mutation present with many autoimmunity manifestations like lupus and autoimmune bowl disease. However, the role of AID in T1DM is unknown. To investigate the role of AID in T1DM, we generated AID$^{-/-}$ NOD mice. We found that AID$^{-/-}$ NOD mice developed accelerated T1DM with worse insulitis and increased anti-insulin autoantibody in the serum. Mechanistic studies revealed that in the absence of AID, both activation and proliferation of B cells and diabetogenic T cells (CD4+ and CD8+) were significantly increased as well with enhanced T-B cell interaction. Moreover, excessive lymphoid expansion in gut-associated lymph nodes was observed in AID$^{-/-}$ NOD mice. Thus, our study provides novel findings relating to the role of AID in T1DM. Our data also suggest that AID is a negative regulator of autoimmunity and ablation of AID can lead to exacerbated autoimmunity and development of autoimmune disease including T1DM.

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1798-P

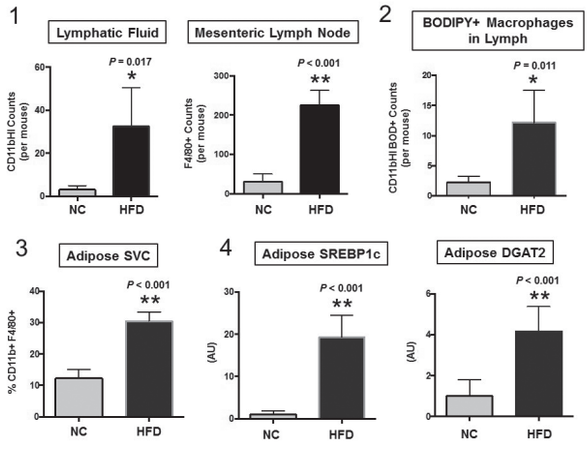
Lipid-Ingested CD11b+ Macrophages Are Increased in Mesenteric Lymphatic Duct after One Week of High-Fat Diet

HYE LIM NOH, RANDALL H. FRIEDLINE, HEE JOON KANG, SUCHAORN SAENGNIPANTHKUL, TAEKYOON KIM, JONG HUN KIM, SEZIN DAGDEVIREN, KUNIKAZU INASHIMA, XIAODI HU, KAREN KELLY, KI WON LEE, JASON K. KIM, *Worcester, MA, Seoul, Republic of Korea*

Lymphatic system plays an important role in the intestinal absorption of dietary fat and presents an environment of potential interaction between lipid and immune cells. We performed micro-surgery to collect lymph fluid from the mesenteric lymphatic duct of C57BL/6 mice fed a normal chow (NC) or high-fat diet (HFD) for 1 wk (n=6/group). Lymph samples were collected 1 hr after an oral gavage of Intralipid mixed with BODIPY-labeled free fatty acid, and flow cytometric analysis was conducted to characterize immune cell types. CD11b+ macrophage counts were increased by 16-fold in mesenteric lymphatic fluid after 1 wk of HFD (Figure 1; P<0.05). F4/80+ macrophage counts were also significantly elevated in mesenteric lymph nodes of HFD-fed mice (Figure 1). Interestingly, BODIPY+ macrophage numbers in the mesenteric lymph were significantly higher in HFD-fed mice, suggesting fatty acid uptake by lymphatic macrophages (Figure 2). These data were associated with a 3-fold increase in macrophage frequency in stromal vascular cells (SVC), and 20- and 4-fold increases in major lipogenic genes, SREBP1c and DGAT2, respectively, in adipose tissue after 1 wk of HFD (Figure 3 and 4).

In conclusion, we identify a physiological role of macrophages that act as an energy sensor of dietary fat intake in lymphatic system, and this process may regulate adipose tissue lipogenesis.

Figures.



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1799-P

Cell Surface Thiols Are Linked with T-Cell Activation and Diabetogenicity

HUBERT M. TSE, LINDSEY E. PADGETT, KATIE E. HEATH, *Birmingham, AL*

Free radicals contribute to type 1 diabetes (T1D) autoimmune responses. We recently demonstrated that superoxide-deficient CD4 T-cells exhibited increased effector responses and diabetogenicity. 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 autoreactive CD4 T-cell redox regulation is an increase in cell surface thiols, but how cell surface thiols contribute to T1D is unclear. We hypothesized that during T1D, activated CD4 T-cells will display an enrichment in cell surface reduced thiols and a concomitant increase in diabetogenic effector responses. To test this, alexa fluor 647-conjugated maleimide (ALM)-labeling of cell surface reduced thiols on diabetogenic mouse and human CD4 T-cells was performed. We observed an increase in ALM percentage and geometric mean fluorescence intensity (gMFI) from peripheral NOD mouse CD4 T-cells during progression to overt diabetes. Cognate autoantigen stimulation elicited a 2- and 20-fold increase in CD4 ALM T-cell percentage and gMFI, respectively. In addition to serving as a marker for T-cell activation, the gMFI of ALM reduced cell surface thiols on CD4 T-cells was enhanced (1.2-fold) with Th1 polarization and blunted (12.2-fold) following Treg polarization in comparison to Th0 conditions. Cell surface reduced thiols from human CD4 T-cells with recent-onset T1D exhibited elevated ALM percentage (1.6-fold, $p < 0.05$) and gMFI (1.7-fold, $p = 0.1503$) in comparison to healthy controls following polyclonal stimulation. Mirroring the increase in ALM with T1D individuals, PBMCs (n=12) also synthesized increased IFN-γ (1.2-fold, p=0.0031), TNF-α (1.3-fold, p=0.0022), and CXCL10 (1.2-fold, p=0.0145) in contrast to healthy controls (n=7). Our studies point to the exciting potential that oxidation of cell surface thiols on diabetogenic CD4 T-cells could abrogate effector responses and correlate with CD4 T-cell activation.

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1800-P

WITHDRAWN

1801-P

The Unexpected Neutrophil in the Inflammatory Microenvironment of Obese Human Adipose Tissue

ALECIA M. BLASZCZAK, WILLIAM LAFUSE, JOEY LIU, ANAHITA JALILVAND, ANDREW SUZO, SABRENA NORIA, BRADLEY NEEDLEMAN, VALERIE P. WRIGHT, DAVID BRADLEY, LARRY SCHLESINGER, WILLA A. HSUEH, *Columbus, OH*

Increasing evidence indicates that the inflammatory processes taking place within adipose tissue (AT) play a fundamental role in the development of chronic inflammation and obesity-related complications. Elevated IL-1β, IL-6, CRP, and CXCL8, as well as a corresponding increase in monocytes and neutrophils (PMNs), occur in peripheral blood of obese individuals. In mice fed high fat diet (HFD), there is an early transient increase in AT PMNs (up to 2% of the stromal vascular fraction, SVF) with a more permanent increase

in the abundance of pro-inflammatory T cells and macrophages. However, genetic loss of neutrophil elastase in mice improves HFD-induced insulin resistance, suggesting an important role for PMNs. Using flow analyses, we recently identified the presence of PMNs in human visceral adipose tissue (VAT). In obese subjects, they surprisingly account for as much as 30% of the SVF cells of VAT, but only up to 5% in lean. Their accumulation appears to result from increased VAT chemokine (IL8, CXCL2) expression and low rates of apoptosis. The PMNs are primarily located in VAT capillaries, but can also be found within the AT itself. Compared to peripheral blood PMNs, transcriptomic analysis suggests that AT PMNs are more activated than those in blood with an upregulation of genes involved in enhancing PMN function and inflammation including IL1β and IL1α with no alterations in IL6 or TNF genes, suggesting caspase 1 activation; and increased expression of genes involved in chemotaxis, PMN granule contents, ROS production, extracellular matrix proteins, and cytokines. Thus, VAT PMNs appear to contribute to chronic inflammation by altering the structure, function, and composition of AT to exacerbate the pro-inflammatory microenvironment of obesity. The cause of the marked increase in VAT PMNs will help identify the activating stimulus to inflammation in obesity and ultimately lead to the development of a treatment and prevention strategy for the inflammatory-driven complications of obesity.

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1802-P

Novel HLA Humanized NOD-Mouse Models for Type 1 Diabetes Therapy Development

JEREMY J. RACINE, HAROLD D. CHAPMAN, JENNIFER SCHLOSS, TERESA P. DILORENZO, DAVID V. SERREZE, *Bar Harbor, ME, New York, NY, Bronx, NY*

While contributing to our understanding of type 1 diabetes (T1D) pathogenesis, NOD mice have not yet enabled identification of clinically applicable therapies. This argues for a need to develop new better models for therapy development. Major histocompatibility complex (MHC in mice, HLA in humans) genes are the primary contributors to T1D susceptibility. Hence, the ongoing creation of HLA-"humanized" NOD mice represent improved models to develop possible interventions. In addition to previously characterized NOD.β2m$^{-/-}$.HHD (HLA-A2.1) mice, we recently generated a novel NOD.β2m$^{-/-}$.B39 (HLA-B39) model exhibiting aggressive insulitis with ~20% developing T1D. One problem with these strains is that to prevent expression of murine MHC I, they carry the β2m$^{-/-}$ mutation. Since β2m is a critical component of FcRn mediated IgG salvage, these mice have limited applicability for testing antibody-based therapies. Thus, to remove the need for the β2m$^{-/-}$ mutation, we used CRISPR/Cas9 to directly remove individual or all MHC class I (H2-K^{d}, H2-D^{b}) and class II (H2-A^{g7}) molecules expressed by NOD mice. While NOD.H2-K$^{d-/-}$ mice are currently being tested for T1D development, the NOD.H2-D$^{b-/-}$ stock is partially disease resistant and has reduced insulitis compared to fully MHC expressing controls. Hence, H2-D^{b} restricted antigenic responses contribute to T1D. An NOD stock deficient in both H2-K^{d} and H2-D^{b} expression (NOD.MHC1$^{-/-}$) recapitulates the T1D and insulitis-free status of NOD.β2m$^{-/-}$ mice. NOD.MHC1$^{-/-}$ mice provided a platform for production of a totally murine MHC deficient NOD stock (NOD.MHC1$^{-/-}$.H2-Ab1$^{-/-}$). NOD.MHC1$^{-/-}$ mice also enabled subsequent production of stocks completely lacking murine MHC class I molecules, but expressing in their place human HLA-B39 or HLA-A2.1 variants implicated as contributing to T1D development (designated NOD.MHC1$^{-/-}$.B39 and NOD.MHC1$^{-/-}$.HHD). These new NOD models will improve our ability to test HLA-targeted T1D therapies.

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1803-P

Serum Vitamin D and C-Reactive Protein as Predictors of Glucose Control in African Americans with Type 2 Diabetes

MARY L. GARVIN-SURPRIS, ERICA PRYOR, MARY A. HESS, MARTI RICE, FERNANDO OVALLE, SUSAN APPEL, *Atlanta, GA, Birmingham, AL, Tuscaloosa, AL*

Introduction: Evidence suggests that serum 25(OH)D (serum vitamin D), generally low in African-Americans, may play a role in glucose control. Elevated levels of chronic low-grade inflammation, measured by C-reactive protein (CRP), may also relate to glucose control. The purposes of this study were to determine whether serum 25(OH)D and CRP were independent predictors of glucose control in adult African-Americans with type 2 diabetes mellitus (T2DM), after controlling for other factors associated with glucose control, and to examine whether there are gender differences in these relationships.

Methods: A secondary data analysis was performed on de-identified clinical data obtained from electronic medical records from a university-affiliated health system in a large southeastern city. Fasting plasma glucose (FPG) and hemoglobin A1c (HbA1c) were the outcome measures of glucose control.

Results: In the CRP analysis sample dataset (n=260), no significant correlations were found between CRP and FPG (r=.115, p=.31) or between CRP and HbA1c (r=.21, p=.08). Significant inverse correlations were found between serum 25(OH)D and FPG (r=-.20, p=.003) and with HbA1C (r=-.22, p<.001) in the serum vitamin D analysis dataset (n=574). In separate regression models for each outcome, after controlling for selected covariates, serum 25(OH)D explained 2% of the variability in FPG (R2change=.02, p=.035) but only 1.1% of the variability in HbA1c (R2change =.011, p=.051). Gender was not a moderator of the relationship between serum vitamin D and either measure of glucose control. Males had lower mean vitamin D levels than females (t=-2.98, df=274.1, p=.003).

Conclusions: Serum vitamin D was an independent predictor for FPG but not HbA1c. There was no significant association between CRP and the two measures of glucose control. This study is one of the first to examine the relationships between serum vitamin D and CRP and measures of glucose control in African-American adults with T2DM.

1804-P

Chronic Exposure to IL-6 Induces Endoplasmic Reticulum Stress by Upregulating CHOP, BiP, and UPR in Monocytes

JENNIFER PASQUIER, BINITHA THOMAS, JESSICA HOARAU-VECHOT, ANNA HALAMA, SHAYMAA DIB, OMAR CHIDIAC, AMIN JAYYOUSI, JASSIM AL SUWAIDI, ARASH RAFII, CHARBEL ABI KHALIL, *Doha, Qatar*

Introduction: Chronic low-grade inflammation is the hallmark of diabetes complications. Among many cytokines involved in the inflammation process, IL-6 is known to be a strong mediator.

Objectives: Monocytes have been directly implicated in atherogenesis and other cardiovascular complications of diabetes. We sought to determine the effect of IL-6 on a monocytic cell line, independently of the presence of hyperglycaemia.

Methods: We treated THP-1 monocytes daily with IL-6 at 15 pg/ml—a concentration comparable to IL-6 levels in serum of T2D patients for 3 months. RNA sequencing was performed before and after treatment.

Results: Chronic exposure of THP-1 by IL-6 led to the expression of C/EBP homologous protein (CHOP) and binding immunoglobulin protein (BiP), the primary genes linked to Endoplasmic Reticulum (ER) stress. Further, disruption of the ER by IL-6 affected the physiological cellular activities, disturbed cell-type specific functions and viability, and induced apoptosis of THP-1 monocytes. Furthermore, we showed that the effect of IL-6 is similar to the N-linked glycosylation inhibitor, tunicamycin, on those cells. Finally, GRP94 (Glucose Regulated proteins) was also found to be up-regulated in our study. GRP94 plays a key role during the Unfolded Protein response (UPR) in the ER and is associated with degradation.

Conclusion: Our data suggest that IL-6 induces ER stress in monocytes and impairs the immune system by affecting the function of those cells, independently of the presence of hyperglycemia.

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1805-P

WITHDRAWN

For author disclosure information, see page A751.

Moderated Poster Discussion ADA-Supported Research

1806-P

A Long-Acting GLP-1R Agonist, CJC-1134-PC, Modulates Potent Inflammatory Effects on Diabetic Mice

XIPING LIU, SEAN HONG, SHIJUAN WU, ZHIWEN YAO, QIANG YAO, *Irvine, CA*

Growing evidence has shown that inflammation plays a role in the development of type 2 diabetes. However, the biological mechanisms linking the innate and adaptive immune responses with diabetes have not been fully understood. This study investigates the effect of CJC-1134-PC, a long acting GLP-1R agonist, on modulating inflammatory response beyond on controlling glucose homeostasis in-vivo. The diabetic mice (db/db) and the nondiabetic mice (C57BL6C) were used for the study. The diabetic mice were injected subcutaneously with 20nmol/kg/day CJC-1134-PC or vehicle for 4 weeks. Blood glucose levels were monitored daily at pre-dosing and hemoglobin A1c (HbA1c) levels were measured biweekly. After 4 weeks, the inflammatory cytokines from the mouse serum were assessed using Meso Scale Discovery (MSD) based assay. Compared with vehicle treated diabetic mice, blood glucose levels decreased significantly and then were maintained at steady-state persistently after the 3rd dosing, and HbA1c decreased by 1.34±0.47% after 4 weeks in CJC-1134-PC treated diabetic mice. Of the ten pro-inflammatory cytokines compared between diabetic and nondiabetic mice, IL-2 and IFN-γ were downregulated in diabetic mice. On the other hand, IL-6 and TNFα were upregulated in diabetic mice. Interestingly, after CJC-1134-PC treatment, IL-2 and IFN- γ levels increased (vehicle vs. CJC-1134-PC: IL-2: 0.31±0.09 vs. 0.53±0.23pg/mL, IFN- γ: 0.28±0.15 vs. 0.42±0.15pg/mL), and IL-6 and TNFα levels decreased (vehicle vs. CJC-1134-PC: IL-6: 25.35±11.10 vs. 14.39±7.13pg/mL, TNFα: 10.91±2.65 vs. 9.28±0.43pg/mL), making their cytokine profile similar to that found in nondiabetic mice. Overall, the data suggests that CJC-1134-PC is effective in glycemic control and also plays a role in modulating inflammation in diabetic mice.

1807-P

Reversal of Hyperglycemia by Microencapsulated Human Umbilical Cord Wharton Jelly-Derived Adult Mesenchymal Stem Cells (hUCMS) Graft (TX) in Spontaneously Nonobese Diabetic (NOD) Mice

PIA MONTANUCCI, TERESA PESCARA, MASSIMILIANO CAVALLO, SARA PARRETTINI, GIUSEPPE BASTA, RICCARDO CALAFIORE, *Perugia, Italy*

Reversal of Hyperglycemia by Microencapsulated Human Wharton Jelly-derived Adult Mesenchymal Stem Cells (hUCMS) Injection (IJ) in Overtly Diabetic NOD Mice.

We had shown that hUCMS, enveloped in clinical grade alginate microencapsules (cgaMC)/PBMC's from T1D patients, in vitro co-cultures, manifested strong immunoregulatory properties due to both, production of humoral factors (TGFβ, IDO, NO, IL6, PGE2, HGF, VEGF) and expression of HLA-E-F and G molecules (Clin Immun, 2015). We aimed to translate these hUCMS properties in vivo, by intraperitoneal IJ of hUCMS/cgaMC, into NOD's with overt T1D. 15 NOD's were divided into two groups: #1, n=5, with BG of 500 mg/dl; #2: n=10, with BG of 250 to 300 mg/dl and abnormal OGTT; 5 NOD's (with overt DM) served for controls. #1 showed poor response to IJ (BG range: 200-400 mg/dl); #2 showed BG decline to 150 mg/dl throughout 170 d of IJ, and near normal OGTT, still going. At 160 d of IJ, Tc immunophenotyping, from retrieved thymus and spleen, showed $CD4^+FoxP3^+CD25^{high}$ (Treg) levels that compared to normal, but not to overtly diabetic control NOD's (no Tregs detectable). Pancreatic sections of the long-term remitters were associated with minor insulitis in conjunction with detection of small/intact islet cells, unlike treated-failed or untreated diabetic controls, showing only atrophic endocrine tissue. CgaMC, retrieved from the remitter group, at 160 d of IJ, were freely floating in the peritoneal cavity and showed no fibrotic overgrowth. From the collected data, we can infer that there is a narrow window, upon the onset of overt DM in NOD's, well trackable by OGTT, when induction of acquired central tolerance towards autoreactive Tc clones, by administration of hUCMS in cgaMC, is possible. This would preserve the native pancreas from extensive B-cell destruction and foster initiation of pilot clinicals in patients with recent onset T1D to prevent exogenous insulin.

1808-P

Prostate-Specific Antigen, Prostate Cancer, and Diabetes Mellitus

CHEN MING, *Shanghai, China*

The risk of prostate cancer (PCa) in diabetes mellitus (DM) remains unclear. Here we studied the association of DM with PCa risk and grade among 456 patients who underwent prostate biopsy in our hospital from January 2010 to October 2016. Difference in serum prostate-specific antigen (PSA) levels between DM and non-DM men was compared. Risk evaluation on DM and PCa grade was conducted by multivariate-adjusted logistic regression analysis; PCa grade was classified according to the Gleason score (GS): low-grade (GS ≤6), intermediate-grade (GS 7), and high-grade (GS 8-10). Subgroups were divided according to age (Group A: 50~75 years; Group B: >75 years) and PSA (normal group: <4ng/ml; gray-zone group: 4~10ng/ml; abnormal group: >10ng/ml). The results showed: in 456 patients, 124 (27.2%) were diagnosed with PCa, including 60 diabetics. Serum PSA levels were lower in DM men compared with those in non-DM men (Ln transformation, 1.24±1.35 vs. 1.57±1.34, P=0.048); the PSA levels were negatively correlated with FPG (r=-0.113, P=0.015) and showed a trend of negative correlation with HbA1c (r=-0.090, P=0.053). In multivariate analysis, DM increased the risk of overall PCa (OR [95% CI] =1.910[1.058-3.450], P=0.032) and high-grade tumor (OR [95% CI]=3.067[1.237-7.603], P=0.016), but was not associated with low- or intermediate-grade tumor (P=0.216/0.899). In subgroup analysis, the risk of high-grade PCa was remarkably increased in Age Group B (OR=3.362, P=0.049) and displayed an increasing trend in PSA gray-zone group (OR=3.027, P=0.085). In the DM population, PCa risk was markedly increased accompanied by increase in BMI (OR=1.165, P=0.038); and there was no notable correlation of PCa risk with age, DM duration, FPG and HbA1c, irrespective of grade. It was suggested that low PSA levels in DM patients might delay and interfere with PCa detection, eventually leading to higher malignant degree of PCa when diagnosis. Hence, DM contributes to increasing the risk of high-grade but not low- or intermediate-grade PCa in China.

1809-P

Insulin Peptide Reactive T-Cells Detected in Ketosis-Prone Type 2 Diabetes

AKIRA SHIMADA, ATSUSHI SATOMURA, YUMI MIYASHITA, YOICHI OIKAWA, IKUO INOUE, MITSUHIKO NODA, *Saitama, Japan, Tokyo, Japan*

In recent years, an increasing number of ketoacidosis cases without precipitating cause have been reported in subjects with type 2 diabetes. These subjects are usually obese and have a strong family history of diabetes and a low prevalence of autoimmune markers, and recognized as ketosis-prone type 2 diabetes. Among them, when antibodies are absent (A-beta+ phenotype = autoantibodies absent, beta cell function present), multiple etiologies have been proposed, and it is unknown whether insulin reactive T-cell response exists in these subjects or not. Therefore, we examined insulin peptide T-cell response by using ELISPOT in the subjects with A-beta+ ketosis-prone type 2 diabetes (= KPD). Peripheral blood was obtained from enrolled subjects with informed consent, and stimulated with insulin peptides (insulin B9-23, B10-24, B11-25, B12-26) for 24 hours, then IFN-gamma spots were counted (duplicate). Maximum spots more than 2.5 were considered as positive based on the data from control subjects. This study was approved by institutional review board. Thirty seven point five percent (3 out of 8) of KPD (8 male, 0 female, mean age 39.0 years) was found to be positive, and this was similar level in type 1 diabetes (35.7% (25 out of 70), 37 male, 33 female, mean age 48.1 years). Only 1 out of 35 (0.03%) was positive in controls (26 male, 9 female, mean age 61.8 years). Spot level in KPD group was significantly higher as compared to that in controls ($p<0.05$). Actual mean spot number in KPD was 3.1, type 1 diabetes 2.0, and 1.0 in controls.

In conclusion, insulin peptide response was detected in ketosis-prone type 2 diabetes, therefore this group may have similar pathophysiology of type 1 diabetes.

1810-P

Cytotoxic CD8⁺ T-Cells Are Associated with Deterioration of Metabolic Parameters and Inflammation in Type 1 but Not in Type 2 Diabetes

MARIA APOSTOLOPOULOU, BARBARA MENART, RUTH RUETTER, BETTINA NOWOTNY, ULRICH GEHRMANN, JULIA SZENDROEDI, NANETTE C. SCHLOOT, MICHAEL RODEN, *Düsseldorf, Germany*

Infiltration of pancreatic islets with different lymphocyte subtypes may contribute to deterioration of glycemic control in T1D and T2D. Islet antigen-directed autoreactive CD8⁺ T-cells have been proposed to appear in patients with T1D only. We hypothesized that at 5 years of T1D duration cytotoxic CD8+ T-cells would associate with deterioration of β cell function, glycemic control and inflammation whereas CD4+ Th-cells would associate with these alterations among patients with T2D. A total of 76 patients with T1D (age: 36 ± 11 years, BMI: 25.5 ± 4.9 kg/m^2) and 130 with T2D (51 ± 11 years, 32.4 ± 6.9 kg/m^2) were studied within the first year of diabetes manifestation. At 5 years after diagnosis 31 different patients with T1D (43 ± 12 years, 25.9 ± 3.2 kg/m^2) and 73 with T2D (56 ± 9 years, 31.9 ± 6.2 kg/m^2) were included. Whole body insulin sensitivity (M-value) was assessed by hyperinsulinemic-euglycemic clamps, insulin secretion by glucagon-stimulation tests and white blood cells were analyzed by flow cytometry. At 5 years both diabetes groups had a mean HbA1c of 7.0% and were more insulin resistant than at diagnosis (M in mg*kg^{-1}*min^{-1}: T1D 6.3 vs. 8.6 and T2D 5.1 vs. 6.3, p<0.05). Total leukocytes, monocytes and neutrophiles did not differ at 5 years in both groups. Patients with T1D at 5 years had 15% lower percentages of CD8+ cells than those at diabetes manifestation, which correlated with fasting glycemia, total cholesterol and high-sensitive C-reactive protein (hs CRP) (all r>0.37, p<0.05) but not with insulin secretion. Patients with T2D had 7% higher percentages of CD4+ cells, which correlated positively with hsCRP (r=0.36, p<0.05), whereas CD8+ Tc-cells did not correlate with any metabolic parameter.

In conclusion, these data suggest a role of CD8+ cytotoxic T-cells in the deterioration of glycemia and in the progression of inflammation among patients with T1D but not T2D at 5 years after diagnosis.

1811-P

Natural IgM Prevents and Reverses T1D by Restoring Immune Homeostasis

CHRISTOPHER S. WILSON, PREETI CHHABRA, CALEIGH MORR, JOSHUA L. POSTOAK, BLAIR T. STOCKS, ANDREW F. MARSHALL, KENNETH L. BRAYMAN, DANIEL J. MOORE, *Nashville, TN, Charlottesville, VA*

The complex and diverse nature of the immune system required to protect mammalian health presents a high risk for autoimmune disease. This risk is mitigated by numerous endogenous regulatory mechanisms, which include both T lymphocyte and lesser-known B lymphocyte processes. It is attractive clinically to capture these endogenous processes as potential treatments for immune-mediated human disease. Naturally occurring IgM (nIgM) is secreted by several types of B lymphocyte and can be isolated by specialized methods to produce a potent preventive therapy for multiple autoimmune conditions. We extend these findings by demonstrating the potential of just two doses of nIgM immune therapy derived from non-autoimmune B6 mice to reverse spontaneous diabetes in the NOD mouse model of disease. This reversal is associated with reduction of autoreactive B cells and abrogation of detectable autoantibody production. Long-term protection from disease is further mediated by a B lymphocyte dependent 2-fold increase in thymic Treg development. nIgM derived from healthy human subjects given to NOD mice from 5 to 18 weeks of age was also disease protective in the mouse model for over 6 months of follow-up (8/10 ctrl NOD mice with diabetes vs. 0/15 nIgM treated, p<0.01) and led to expansion of human FoxP3+ Tregs in a humanized mouse model. Interestingly, nIgM derived from prediabetic NOD donors was less effective in enhancing FoxP3+ Treg production and modulating B lymphocytes, suggesting that failures in this regulatory mechanism may promote disease in some individuals with T1D. Overall, IgM immune therapy represents a newly identified safe and potent treatment for the prevention and reversal of human T1D by normalizing key cellular functions that promote immune stability and diminish autoreactivity.

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1812-P

Immunological Significance of CD4⁺ T-Cells in Acute-Onset Type 1 Diabetes Characterized by CD4⁺ T-Cells: Dominant Insulitis

YOICHI OIKAWA, KEN YAJIMA, AKINORI HASHIGUCHI, AKIRA SHIMADA, *Tokyo, Japan, Saitama, Japan*

Type 1 diabetes (T1D) is considered to be a Th1-type anti-islet autoimmune disease. In general, CD8⁺ T-cells are the predominant lymphocytes in the insulitis lesions, and CD4⁺ T-cell-dominant insulitis is very rare. We present a case with acute-onset T1D characterized by CD4⁺ T-cell-dominant insulitis. A 72-year-old woman presented with excessive thirst and a 3-month history of weight loss. She was in a state of ketosis, and her plasma glucose concentration and HbA1c value were elevated. Moreover, anti-islet autoantibodies were positive, thus acute-onset T1D was diagnosed. On this occasion, a tumour was detected in the pancreas; total pancreatectomy was performed 2 months later. The pathological diagnosis was intraductal papillary mucinous adenoma. Immunohistochemical staining of a sample of non-tumorous pancreatic tissue revealed 13 insulitis lesions infiltrated by both CD4⁺ and CD8⁺ T-cells, and interestingly there were more CD4⁺ T-cells than CD8⁺ T-cells in the lesions (84.7 ± 36.5 CD4⁺ T-cells/islet vs. 45.8 ± 27.2 CD8⁺ T-cells/islet). Although a Th1-type chemokine, CXCL10, was expressed in a part of the remaining beta cells in insulitis lesions, the frequency of infiltrating T-cells expressing CXCR3, a chemokine receptor for CXCL10, was relatively low (8.2 ± 10.5 cells/islet). Moreover, T-cells expressing FoxP3, a master gene for immunoregulatory T (Treg) cells, also infrequently infiltrated the lesions (13.0 ± 9.2 cells/islet). These findings suggested that the majority of infiltrating CD4⁺ T-cells might be neither CXCR3⁺ Th1 cells nor Treg cells. Meanwhile, the frequencies of infiltrating B cells and macrophages, which can act as antigen-presenting cells, were both positively correlated with CD4⁺ T-cell frequency (R^2 = 0.569 and R^2 = 0.670, respectively; P < 0.05), suggesting the immunological significance of CD4⁺ T-cells in the disease process of T1D in this case.

1813-P

WITHDRAWN

1814-P

The 70 kDa Heat Shock Protein DnaK Mediates the Macrophage Stimulatory Activity of the Autoantigenic Proinsulin B-Chain Peptide B11-23

ELIAS BLASIUS, ELKE GUELDEN, CHRISTIANE HABICH, VOLKER BURKART, *Düsseldorf, Germany*

Autoimmune reactivity against beta cell-derived peptide antigens, particularly insulin, is a dominant pathogenetic feature of type 1 diabetes. Increased immunogenicity is observed for peptides bound to heat shock proteins (Hsp). By chaperoning autologous peptides, Hsp may contribute to the development of autoimmunity. As innate immune cells play a prominent role in the initiation of beta cell-directed immunity, we hypothesized that Hsp70 and insulin peptides synergize in the activation of macrophages. Cells of the monocyte/macrophage lines J774A.1 and MM6 were exposed to various concentrations of the 70 kDa Hsp analogue DnaK, to proinsulin-

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derived peptides (13 amino acids long) or to combinations of DnaK and the 13mer peptides. The release of inflammatory mediators was quantified by ELISA and DnaK-peptide interactions were assessed by competition binding assays. Screening a set of overlapping 13mer peptides spanning the entire proinsulin sequence showed exceptionally high-affinity DnaK binding for the B-chain core peptide B11-23. Combinations of 10 µg/ml B11-23 and 1 µg/ml DnaK induced higher levels of tumor necrosis factor α (TNFα) and interleukin 6 (IL-6) from J774A.1 (446±143 pg/ml TNFα; 35±12 pg/ml IL-6; all p<0.05)) and MM6 (757±110 pg/ml TNFα; 572±42 pg/ml IL-6; all p<0.01)) than the individual reagents alone (<122 pg/ml TNFα; <10 pg/ml IL-6). Combinations of DnaK and B18-30 from the C-terminal region of the B-chain had no significant effects on cytokine release. Binding assays showed a 17.5 fold higher DnaK affinity of B11-23 than B18-30 (p<0.001). In a screening approach, macrophage activity remained unaffected by DnaK in the absence or presence of other 13mer peptides of proinsulin A- and B-chains or C-peptide. We conclude that the particular macrophage-stimulating potential of combinations of Hsp70 and B11-23 may contribute to the immunodominance of this insulin peptide in the development of beta cell-directed autoimmunity.

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1815-P

Distinct Phenotypes of GAD65-Specific CD4+ T Cells among the Three Subtypes of Type 1 Diabetes in Japan

DAISUKE CHUJO, AKITSU KAWABE, NOBUYUKI TAKAHASHI, TETSURO TSUJIMOTO, CHIHARU TSUTSUMI, FUMITAKA HASEDA, AKIHISA IMAGAWA, TOSHIAKI HANAFUSA, KUNIMASA YAGI, MASAYUKI SHIMODA, KOHJIRO UEKI, HIROSHI KAJIO, *Tokyo, Japan, Takatsuki, Japan, Kanazawa, Japan*

Type 1 diabetes (T1D) is classified into the following three subtypes in Japan based on disease onset and progression: acute onset (AT1D), slowly progressive (SP1D), and fulminant type 1 diabetes (FT1D). In T1D, while cellular immunity is considered to play a major role in pancreatic β cell destruction, phenotypes of autoreactive T cells, induced by islet antigens in each T1D subtype, remain largely unknown. Here, we analyzed the cytokine production profiles of GAD65-reactive CD4+ T cells in 17 AT1D patients, 15 SP1D patients, 15 FT1D patients, and 13 nondiabetic controls. Briefly, peripheral blood mononuclear cells, obtained from the participants, were stimulated for 48 hours with GAD65 peptide clusters, GAD65-C1 and GAD65-C2, containing 5 peptides per cluster to measure cytokine secretion. The peptide-reactive T cell population was further expanded by IL-2 for 5 days to analyze intracellular cytokine expression. While most AT1D patients, compared to SP1D patients and controls, displayed Th1 cells specific for GAD65-C1 (P<0.05 for IL-10-IL-13-IL-17-IFN-γ+CD4+ T cells), fewer AT1D patients, compared to controls, displayed Tr1 cells specific for GAD65-C1 and C2 (P<0.05 for IL-10+IL-13-IL-17-IFN-γ-CD4+ T cells). Interestingly, GAD65-C1-specific Th1 cells were more abundant in SP1D patients with HLA-DR9 than in SP1D patients without HLA-DR9 (P<0.05). Furthermore, SP1D patients, compared to controls and those with other T1D subtypes, dominantly displayed GAD65-C2-specific Th2 cells (P<0.05 for IL-10-IL-13+IL-17-IFN-γ-CD4+ T cells). However, significantly lesser GAD65-C1-specific Th2 and GAD65-C2-specific Tr1 cells were displayed by FT1D patients than that by controls and patients with other T1D subtypes.

In conclusion, our study demonstrated that phenotypes of islet antigen-specific CD4+ T cells differ among the three T1D subtypes. These distinct phenotypes of pathogenic T cells may be associated with the manner of progression of pancreatic β cell destruction.

1816-P

Permanent Epigenetic Changes in Treg Signature Genes of Type 1 Diabetic Subjects after In Vivo BCG Vaccinations

WILLEM KUHTREIBER, SARA PLAGER, TAESOO KIM, HUI ZHENG, DENISE L. FAUSTMAN, *Charlestown, MA*

Increasing the potency or numbers of active regulatory T cells (Tregs) is a goal of many clinical trials seeking to halt autoimmunity. In a published study, BCG-treated subjects with type 1 diabetes (T1D) had statistically significant increases in Treg numbers for 4-6 weeks after repeat BCG vaccination. A longer-lasting effect would be optimal and may be able to stably reverse diabetic autoreactivity. Chronic Mycobacteria infections evade host recognition on a cellular level via measurable Treg increases and induction of host Tregs. For BCG's virulent counterpart, tuberculosis, epigenetic imprinting of host genes is a mechanism for host Treg production and infection chronicity. We therefore investigated if the effect of repeat BCG vaccinations on Tregs could be permanent and driven by host epigenetic modifications to Treg signature genes. BCG's impact on methylation was studied at methylation sites on 6 Treg signature genes (FoxP3, TNFRSF18, IL2RA, IKZF2, IKZF4, CTLA4) by profiling transcriptional start site (TSS) clusters located within the Treg-specific demethylation region in T1D subjects before and 8-weeks after in vivo BCG dosing. BCG induced demethylation at almost all TSS in all 6 signature genes. In vivo documented epigenetic changes correlated with increased mRNA expression of all 6 Treg signature genes in isolated CD4 T cells. Overall, repeat BCG vaccinations reset the immune system by consistent and rapid demethylation of all 6 key Treg genes for enhanced mRNA expression, as monitored via CD4 T cells in blood from BCG-treated T1D subjects. This suggests that not only are Treg cell numbers transiently elevated after BCG vaccination, but also that permanent epigenetic expression of Treg genes that control Treg potency is stably re-established by BCG treatment. BCG vaccination, like tuberculosis, powerfully modulates Treg induction. Studies are underway to follow vaccinated T1D subjects for long-term beneficial clinical effects of Treg upregulation by BCG.

1817-P

The Impact of Diabetes on BCG Outcomes in Patients with Nonmuscle Invasive Bladder Cancer

WAYNE G. BRISBANE, SARAH K. HOLT, JOHN L. GORE, JONATHAN L. WRIGHT, EVAN Y. YU, JORGE D. RAMOS, GEORGE R. SCHADE, *Seattle, WA*

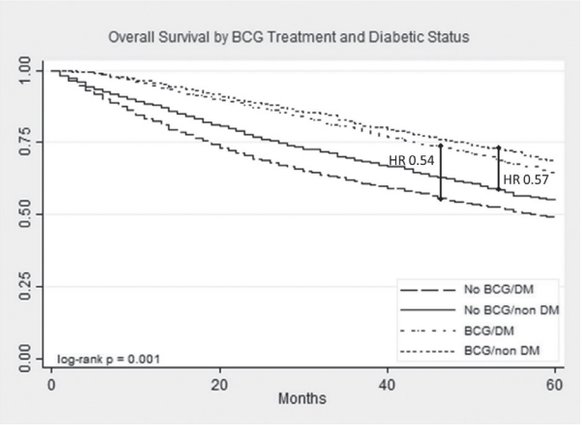
Introduction: Intravesical bacille Calmette-Guerin (BCG) is the standard therapy for patients with high grade non-muscle invasive bladder cancer (NMIBC). The efficacy of BCG is dependent on cell mediated cytotoxic killing of bladder cancer cells. Diabetes mellitus (DM) results in immune aberrations. As a result, we evaluated the impact of DM on BCG outcomes among patients with high-risk NMIBC.

Methods: Utilizing the Surveillance, Epidemiology and End Results (SEER)-Medicare database we identified 10,972 patients with high grade NMIBC between 2002 and 2011. We stratified patients on the basis of DM controlling for severity and compared outcomes of BCG treatment utilizing a Cox proportional hazard ratio.

Results: DM patients had significantly increased risk of all-cause mortality (HR 1.18, p<0.01). As seen in the Figure, intravesical BCG was associated with decreased mortality in patients with and without DM. (HR 0.54 p<0.01, HR 0.57 p<0.01). Yet, diabetic patients have an increased mortality compared to nondiabetic patients, when both are treated with BCG (HR 1.17 p=0.02)

Conclusion: Patients with diabetes significantly benefit from BCG. While there is evidence of deleterious effects of diabetes on the immune system, this does not translate into lack of benefit from immune therapy with BCG.

Figure.



1818-P

Clinical Spectrum of Autoimmune Brain Disease in Patients with Type 1 Diabetes

VINOD METTA, RAJITHA CHICHILI, SABITHA CHICHILI, ANIL KUMAR REDDY ISKA, *London, United Kingdom, Hyderabad, India, Nellore, India*

Background: Autoimmune brain disease (AIBD) is a syndrome of central nervous system caused by antibodies that attacks brain, characterised by broad clinical spectrum of CNS disorders. It often associated with autoimmune conditions like type 1 diabetes independent of duration and age of diagnosis.

Description: We report 4 patients with no other associated autoimmune conditions or neurological or significant family history presented with neuro behavioural symptoms, facio brachio dystonic seizures and found to have

super high titres of dual antibodies anti Glutamic acid decarboxylase 65 (Anti-GAD65) and Voltage gated potassium channel antibodies (VGKC). Anti GAD has been linked with type 1 diabetes but not VGKC. Incidence of dual autoantibodies in patients with type 1 diabetes is unknown and under reported.

Results: All our patients underwent thorough autoimmune, paraneoplastic work up, CSF analysis, EEG, MR brain and PET imaging. Mean age of diagnosis of type 1 diabetes (23.25, range 10-39) mean onset of AIDB symptoms (29.25, range 16-43) mean serum GAD levels (16,000 pmoles/L, range 11000-21000) mean CSF GAD levels (14,750, range 11000-190000) mean serum VGKC (250 Pmoles/L, range 260-370), mean CSF VGKC levels (310, range 290-340) no cancers were detected, subsequently treated with Immunoglobulins, steroid sparing agents, 2 out of 4 were treated with plasma exchange resulted in full functional recovery with in a span of 3-6 months. Disappearance of clinical findings and seroreversion after immunotherapy suggest dual antibody process might be involved in pathogenesis.

Conclusion: We highlight the importance of recognizing spectrum of autoimmune brain disease ranging neuropsychiatric, neuro-behavioral, and other treatment resistant epileptic disorders in patients with pre-existing autoimmune diseases such as type 1 diabetes, as prompt diagnosis and treatment can have major impact on patient outcome and quality of life.

1819-P

Profound Hypoglycemia in an Atypical Case of Type B Insulin Resistance Confirmed by a Novel Immunoassay

SCHAFER BOEDER, PINKY KURANI, MICHELLE LEVIN, LAURA BARBA, NAI WEN CHI, DANIAL MIR, CHRISTIAN SCHWIEBERT, WALDEMAR MINICH, LUTZ SCHOMBURG, TRICIA SANTOS CAVAIOLA, *San Diego, CA, Berlin, Germany*

Type B insulin resistance (TBIR), a rare disease caused by autoantibodies against the insulin receptor, predominately affects African-American females with multiple autoimmune diseases like SLE. Affected individuals usually present with hyperinsulinemia (due to delayed clearance of insulin by its receptor) and hyperglycemia. The latter can evolve into hypoglycemia as antibody titers wane. We report a case of a 64-year old Hispanic male with ESRD, hypothyroidism, acanthosis, and DM2 on oral agents. He developed recurrent fasting hypoglycemia that persisted after stopping antidiabetic agents. Based on an insulin level of 43 uIU/ml with a glucose of 47 mg/dL, he underwent an extensive search for insulinoma at another hospital. After negative EUS, octreotide scan, and intra-arterial calcium stimulation test, he was diagnosed with ectopic insulinoma and treated with depot Octreotide. However, the frequency and severity of hypoglycemia progressed despite frequent feeding. He suffered hypoglycemic coma and was brought to our institution. We confirmed the hypoglycemia was mediated by the insulin receptor based on glucagon responsiveness and undetectable beta-hydroxybutyrate. Insulinoma was unlikely given a plasma insulin level of <1 mIU/ml with a glucose of 25 mg/dL. IGF-2-oma was excluded based on serum IGF-2, ratio to IGF-1, and extensive imaging. To diagnose TBIR we developed a luminescence assay for anti-insulin receptor antibody (details to be presented) that confirmed an elevated titer of 195.8 (reference <3.0). The patient was given prednisone 40 mg/d. Within weeks his autoantibody titer decreased, his hypoglycemia largely resolved, and he developed postprandial hyperglycemia (CGM data to be presented), which prompted a steroid taper. Our patient highlights several unusual aspects of TBIR: fasting hypoglycemia can be the dominant feature, hypothyroidism can be the only other autoimmune disease, and hyperinsulinemia can lead to the false diagnosis of insulinoma.

1820-P

The Depletion of Peripheral NK Cells and NK T Lymphocytes in a Spontaneously Diabetic Nonhuman Primate (NHP) Model

GAO SUN, XIAOLI WANG, WEI QIAO, YONGQIANG LIU, BINGDI WANG, WEIWEI YE, JIQIU QIAO, XIAOYU ZHANG, XIAOYU AN, YONG FU XIAO, YI XIN (JIM) WANG, *Taicang, China*

Peripheral immune cells are associated with type 2 diabetes (T2D) in human and rodents. Spontaneously diabetic NHP is a highly translatable animal model for metabolic researches as it mimics many characters of T2D patients. The aim of the study is to investigate the dynamics of peripheral immune cells in NHPs.

Plasma from male diabetic (DM) (n=6) and normal (NM) (n=7) cynomolgus was collected for FACS analysis. Different cell types were identified by gating cells with combinations of antibodies against immune cell surface markers.

NK and NK T cells from DMs were 50% less than NMs, while dendritic and T cells were similar between groups. The changes in NK cells in DMs were consistent to those observed in T2D patients. Further analysis revealed negative correlations between NK cells and fasting blood glucose levels in DMs.

The results provide an another good evidence that spontaneous diabetic cynomolgus is an excellent tool for diabetic or related researches with the possibility of its extended usage in the development of drug candidates that might modulate specific immune cell populations.

1821-P

WITHDRAWN

TRANSPLANTATION

Moderated Poster Discussion: Novel Approaches in Islet Transplantation *(Posters: 1822-P to 1827-P), see page 19.*

1822-P

GCSF in Allogeneic Clinical Islet Transplantation Is Not Associated with Improved Long-Term Graft Survival

ANNA LAM, SHARLEEN IMES, ANDREW J. MALCOLM, JAMES SHAPIRO, PETER A. SENIOR, *Edmonton, AB, Canada*

Granulocyte colony stimulating factor (GCSF) is routinely used to treat neutropenia in clinical islet transplant (CIT) recipients. Recent recognition of GCSF as a regulator of T cell responses has led to interest in the potential of GCSF to improve β cell survival. Indeed, retrospective studies in CIT suggest improved islet graft survival in subjects receiving GCSF and exenatide. Additionally, the combination of anti-thymocyte globulin (ATG), a potent lymphodepleting (LD) agent, and GCSF has preserved β cell function in new onset type 1 diabetes (T1D).

This study was performed to determine the effect of GCSF alone on islet graft survival and whether this effect is influenced by the use of LD agents for induction. We retrospectively analyzed data from 164 patients with T1D who underwent CIT at our institution between 1999 and 2015. Immunosuppression induction included LD agent (ATG or alemtuzumab, n=84) or non-LD agent (daclizumab, n=80). Graft survival (C-peptide > 0.1 nmol/L) from first CIT was 6.6 ± 0.4 years (mean ± standard error). 47 patients were treated for neutropenia with GCSF for a median total dose of 900 mcg (300-9600 mcg). GCSF treatment did not significantly affect graft survival (hazard ratio (HR) 0.70; 95% confidence interval (CI) 0.28-1.73, p=0.41). Furthermore, graft survival was not different with GCSF treatment when an LD agent (HR 0.98,

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95% confidence interval (CI) 0.23-4.10, p=0.97) or a non-LD agent (HR 0.61, 95% CI 0.18-2.10, p=0.44) was used for induction.

The data presented here represents the largest reported cohort of CIT patient who have received GCSF and our retrospective analysis shows that GCSF treatment alone (without exenatide) is not associated with improved long term graft survival. Our results do not preclude further prospective studies of GCSF in CIT, but suggest that patient selection, timing and dose of GCSF be carefully considered in the study design.

1823-P

Falcarinol in Devil's Club Extract Protects Pancreatic Islets by Alleviating ER Stress

SUSAN S.C. CHEUNG, DJAMEL KHELIFI, JIANQIANG HAO, JOSEPH TAI, DAVID HASMAN, MICHAEL KUO, HONGJIAO WAN, ZILIANG AO, GARTH WARNOCK, *Vancouver, BC, Canada, Burnaby, BC, Canada*

We previously showed that 70% ethanol root bark extract from Devil's Club (DC), Oplopanax horridus, a native medicinal plant of North America, alleviate endoplasmic reticulum (ER) stress in freshly isolated human islets. Falcarinol (FC) is one of seven natural polyacetylene molecules in DC; it is also a bioactive component in ginseng and carrot. Here, we investigate whether FC can improve islet function and enhance islet quality. Groups of 200 islets isolated from 24-week-old C57BL/6 mice were treated for 20 h with DC, FC or thapsigargin (Tg, an ER inducer). Islet function was assessed by glucose stimulated insulin secretion (GSIS) and expressed as Stimulation Index (S.I.). Insulin and transcription factor X-box binding protein 1 (Xbp1, a mediator in ER stress signaling) mRNA transcript expression were determined by quantitative RT-PCR. Islets treated with DC (1/2000 dilution), FC (500 ng/mL) or Tg (1 μM) show increased S.I., respectively at 1.43±0.17 (N=8), 1.65±0.32 (N=10), and 1.6±0.44 (N=10) compared with untreated control at 1.05±0.1 (N=9), combined 500 ng/mL FC and 1 μM Tg treatment (FC+Tg) significantly increased S.I. to 3.23±0.26 (N=5, $p<0.001$ vs. control). Insulin mRNA transcript, expressed as Log 2 [-ΔΔCt (test - control)], in islets treated with DC, FC, Tg or FC+Tg were, respectively, 0.56±0.14 (N=4), 1.6±0.34 (N=14), 0.56±0.15 (N=9), 3.48±0.03 (N=4, $p<0.001$) compare with control at 1.13±0.59 (N=14). Xbp1 mRNA expression in islets treated with FC+Tg was significantly lower at 1.28±0.12 (N=4, p=0.001 vs. Tg alone) than those treated with Tg alone at 4.68±1.14 (N=8), while islets treated with FC alone was lower at 0.64±0.11 (N=17) than control at 1.14±0.3 (N=7). The data suggest FC can alleviate Tg induced ER stress in mouse islets, FC can improve islet function with increased S.I. in GSIS assay and insulin mRNA expression. FC has the potential application for protecting islets from ER stress.

Supported By: Lotte and John Hecht Memorial Foundation

1824-P

Long-Term Effect of Human Placental Lactogen Isoform A on Glucose Homeostasis in Mice with Pancreatic Islets Transplantation

GIULIA DONADEL, ROBERTO ARRIGA, BARBARA CAPUANI, VALENTINA MARCHETTI, FRANCESCA PACIFICI, ANDREA COPPOLA, DONATELLA PASTORE, DAVID DELLA MORTE, DAVIDE LAURO, *Rome, Italy, Vancouver, BC, Canada*

Background: Type 1 diabetes (T1D) is characterized by the progressive destruction of pancreatic beta cells with the subsequent lack in insulin release. Besides classical insulin administration therapy, transplantation of pancreatic islets and the search for factors promoting beta cells survival and proliferation secreting insulin are emerging therapy for T1D. Among tested growth factors the human placental lactogen (hPL) shows stronger activity in promoting islets proliferation and survival. Therefore, the aim of our study was to investigate the biological activity of hPL isoform A (hPL-A) in a model of diabetic murine engrafted islets.

Research Design and Methods: A single intraperitoneal dose of streptozotocin was used to induce type 1 diabetes mellitus in mice. We performed a collagenase p perfusion directly into the common bile duct and a Histopaque 1110 gradient solution to isolate pancreatic islets. For transplantation, the anterior chamber of the eye was used to inject 200 isolated islets. Iris dissections of the right and left eye were analyzed by confocal microscopy.

Results: First, we evaluated the engraftment of freshly isolated pancreatic islets into the anterior chamber of the eye of C57BL6/J mice. Following a 15 days post pancreatic islet transplantation, iris dissections still showed a positive staining for insulin and glucagon. Next, we evaluated the effect of islets treated with hPL-A on glucose homeostasis in streptozotocin mice. Starting from 1 months after transplantation, all mice injected with islets stimulated with hPL-A (500ng/ml) showed an improvement in non-fasting glycaemia ($p<0.05$) compare to mice transplanted with islets alone and mice untreated.

Conclusion: Taken together our data suggest that hPL-A could have a long-term effect for islet graft function and survival and can be considered as potential therapy against type 1 diabetes mellitus.

Supported By: Fondazione Roma

1825-P

Deriving Insulin-Producing Cells from Trans-differentiating Adipose-Derived Mesenchymal Stem Cells

CHUI SUN YAP, MIAN MIAN TONG, XIUBO FAN, YONG MONG BEE, GUO DONG LI, *Singapore, Singapore*

Diabetes is a metabolic disease characterized by a deficiency of insulin relative to metabolic needs, leading to poor blood glucose regulation. Although the symptoms can be managed with administration of insulin/drugs, transplantation remains the only cure. Limited by the scarcity of donors and tissues, stem cell-derived insulin-producing cells (IPCs) are regarded as a viable option. With the aim to establish a mouse IPC transplant model, we chose to reprogram adipose-derived stem cells (ADSCs) isolated from Mouse Insulin Promoter (MIP)-GFP mice. To obtain IPCs with high levels of insulin expression, we are screening transcription factors involved in the development of beta-cells (e.g., PDX1, NGN3 and FoxA2), activators/inhibitors of various developmental/signaling pathways (e.g., retinoic acid, activin A and Wnt agonist), reagents known to induce insulin expression (e.g., exendin 4 and nicotinamide), epigenetic modifiers (e.g., trichostatin A and procaine), 3D matrices (e.g., matrigel and fibrin-gel), and components of extracellular matrices (e.g., laminins and vitronectin). PDX1 expression proved to be a critical factor in reprogramming ADSCs into IPCs. Co-expressions of FoxA2 and/or NGN3 with PDX1 did not significantly increase insulin expression levels. However, they induced the expression of glucokinase, which is crucial for glucose sensing. We identified 13 factors in our successful protocols, which are able to induce the expression of pancreatic hormones (insulin and glucagon) in $ADSC^{PDX1}$ cells. The 13 factors were tested in various combinations in a series of fractional factorial design experiments. In 2 rounds of experiments, foetal bovine serum, retinoic acid and nicotinamide were found to be significant contributors to the reprogramming of ADSCs and/or induction of insulin expression, while activin A's contribution was marginal. These factors will be included in further screens to obtain IPCs with robust insulin expression and other beta-cell functions.

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1826-P

M2-Like Macrophages and Islet Allograft Immune Tolerance

LUIS HERNANDEZ, OLIVER UMLAND, PER-OLOF BERGGREN, MIDHAT H. ABDULREDA, *Miami, FL, Stockholm, Sweden*

Pancreatic islet transplantation confers significant improvement in glycemic control and prevents life-threatening severe hypoglycemia in type 1 diabetes (T1D) patients. However, chronic immunosuppression which is required to avoid rejection of the transplanted islets, is associated with severe complications (e.g., protracted infections and increased risk of cancer). Moreover, islet graft rejection and/or recurrent anti-islet autoimmunity still occur despite immunosuppression. Thus, there is a significant need for new approaches for inducing transplant immune tolerance to ensure durable islet graft acceptance without immunosuppression or its complications. We show here that transient immune intervention during transplantation of allogeneic islets into the immune privileged anterior chamber of the eye results in transplant immune tolerance and sustained survival of intraocular islet allografts (DBA/2) long after stopping immunosuppression in recipient mice with (NOD) and without (B6) autoimmune diabetes. We also demonstrate that long-term survival of islet allografts can be achieved by different transient peri-transplant immune interventions administered either systemically or locally/topically via eye drops. Our new data further show increased intra-graft M2-like macrophages in association with elevated Th2 cytokines within the local environment of tolerated islet allografts. Thus, our findings using mouse models of islet allotransplantation is recipients with and without recurrent autoimmune diabetes: 1) demonstrate long-term survival of allogeneic islets following transplantation with transient systemic or local immune intervention; and 2) provide novel mechanistic insight into an important local role of Th2-induced intra-graft M2-like macrophages in the induction and maintenance of islet allograft immune tolerance.

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1827-P

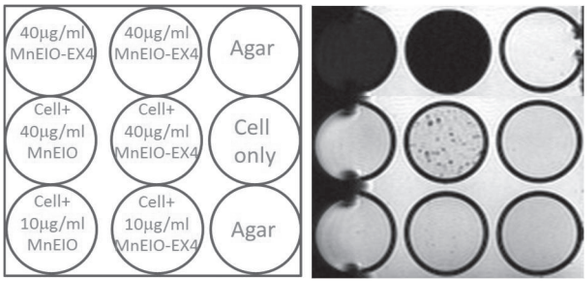
Magnetic Resonance Imaging of β-Cells Using Manganese Magnetism-Engineered Iron Oxide Nanoparticles Conjugated with Exendin-4

JYUHN HUARNG JUANG, CHIA RUI SHEN, SHU TING WU, JIUN JIE WANG, ZEI TSAN TSAI, YUN MING WANG, *Taoyuan, Taiwan, Hsinchu, Taiwan*

To detect and trace endogenous pancreatic β-cells as well as transplanted β-cells by magnetic resonance imaging (MRI), we conjugated manganese magnetism-engineered iron oxide (MnMEIO) nanoparticles with exendin-4 (MnMEIO-Ex4) which can specifically bind glucagon-like peptide-1 receptors on β-cells. The fluorescence of propidium iodide was used to determine MIN6 cell death. The iron in MIN6 was stained by Prussian blue. The insulin secretory response of MIN6 was determined by static incubation at low and high glucose. Proinsulin mRNA expression in MIN6 was determined by qualitative PCR. The distribution of the nanoparticles in MIN6 was examined using a transmission electron microscope (TEM). In vitro MR imaging was performed on a 7.0 T MRI system. MnMEIO with iron content 5-40 μg/mL had no toxic effect to MIN6. Positive iron staining was found in the MIN6 loaded with MnMEIO-Ex4 but not with MnMEIO. TEM confirmed the intracellular location of iron particles. In vitro MR image showed loss of intensity in MIN6 loaded with MnMEIO-Ex4 but not with MnMEIO (Figure). With the addition of poly-L-lysine, only MIN6 incubated with MnMEIO-Ex4 but not with MnMEIO could stimulate proinsulin mRNA expression and insulin secretion.

In conclusion, our in vitro results indicate MnMEIO-Ex4 nanoparticles are safe and effective for β-cell detection by MRI.

Figure.



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1828-P

Reversing Type 1 Diabetes without Insulin: Using Adult Brown Adipose Tissue Transplants and IGF-1

SUBHADRA C. GUNAWARDANA, DAVID W. PISTON, *St. Louis, MO*

We previously demonstrated the feasibility of reversing type 1 diabetes (T1D) without insulin in mouse models, through subcutaneous transplantation of embryonic brown adipose tissue (BAT). Euglycemia following BAT transplants is rapid and long-lasting, accompanied by decreased inflammation and regenerated healthy white adipose tissue (WAT). Translating this approach to human therapy requires practical alternatives for embryonic tissue. BAT-derived stem cell lines or adult BAT transplants alone fail to reverse T1D, perhaps due to lack of growth factors abundant in embryonic tissue. Adding growth factors may enable transplants to survive and vascularize in the recipients' subcutaneous space as well as stimulate adipogenesis and decrease inflammation in the surrounding host tissue. Preliminary data point to insulin-like growth factor-1 (IGF-1) as a possible candidate. IGF-1 is expressed more abundantly in donor embryonic BAT and in newly-formed WAT in transplant recipients than it is in the WAT of diabetic or normal controls. Plasma IGF-1 levels increase soon after transplant placement, and continue to increase in negative correlation to pro-inflammatory cytokines.

Here we tested the ability of adult BAT transplants to correct T1D, aided by temporary administration of exogenous IGF-1. Fresh BAT from healthy adult CB7BL/6 donors were transplanted in the subcutaneous space of NOD recipients. Exogenous IGF-1 was administered daily for a week following transplant, at 100 μg/Kg SC. Adult BAT transplants with IGF-1 supplementation resulted in long-lasting reversal of T1D at a 50% success rate (4 out of 8 recipients showed a lasting decrease of non-fasting blood glucose from 356.3±86.2 to 144.5±24.9 mg/dl within a week of treatment), in contrast with no recovery in the control groups who received adult BAT alone, IGF-1 alone, or no treatment. These data show the importance of IGF-1 in BAT transplant function, and provide a possible route to translate this T1D treatment to human patients.

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1829-P

Abnormal Activation of Bone Marrow-Derived Ly6C^{high} Monocyte and Chronic Inflammation at Multiple Organs in Diabetes

YOSUKE IKEDA, BATTSETSEG BACHULUUN, CHIHIRO IWAYA, YUKA KIMURA, SHINICHIRO KIMURA, YASUTAKA MAEDA, NORIYUKI SONODA, RYOICHI TAKAYANAGI, YOSHIHIRO OGAWA, TOYOSHI INOGUCHI, *Fukuoka, Japan*

Accumulating evidence has indicated that diabetes and obesity are associated with chronic low grade inflammation in multiple organ failure, but the detailed pathogenic mechanism is not fully understood. Macrophage is a key character of chronic inflammation. Especially, adipose tissue inflammation is initiated by the accumulation and activation of inflammatory M1 macrophages and caused hyperglycemic condition containing insulin resistance. Here, we focused on monocyte which is a precursor of macrophage, and investigated the abnormal activation of bone marrow-derived pro-inflammatory Ly6C^{high} monocytes. Ly6C^{high} monocytes were harvested from bone marrow of male streptozotocin (STZ)-induced diabetic mice, type 2 diabetes model db/db mice, high fat diet-induced obese mice, and each control mice using a BD FACS Aria™ II flow cytometer. The expression of oxidative stress and inflammation related genes was increased in Ly6C^{high} monocytes from STZ-induced mice and especially from db/db mice, comparing to each control mice by qRT-PCR. In addition, we harvested bone marrow-derived Ly6C^{high} monocytes from db/db or db/+ mice, labeled them with PKH26 fluorescent dye (red), and then injected them into other db/db recipient mice. After 3 weeks of observation, mice treated with db/db-derived monocytes exhibited significantly increased levels of both serum glucose and fructosamine, and worsening of glucose tolerance, comparing to those with db/+-derived monocytes. In histological examination, mice treated with db/db-derived monocytes exhibited upregulated infiltration to liver, kidney, and AT.

In summary, abnormal activation of Ly6C^{high} monocytes occurred at bone marrow level of STZ-induced mice and especially db/db mice, and this new observation may at least in part contribute to chronic low grade inflammation at multiple organs.

1830-P

Engineering, Expression, and Purification of a Single-Chain Fragment for Beta-Cell Targeting

JYUHN HUARNG JUANG, WOLFGANG SCHECHINGER, SHU TING WU, CHIA RUI SHEN, *Taoyuan, Taiwan*

In order to trace endogenous and transplanted pancreatic beta cells, developing probes which specifically recognize these cells could be a valuable tool for in vitro and in vivo detection and monitoring of beta cells. Towards this goal, a recombinant beta cell specific single-chain fragment (ScFv) was engineered, expressed, purified and characterized. We optimized a previously obtained hit from a phage display library screening experiment of ScFvs for secreted eukaryotic expression and engineered a 3T3 mouse fibroblast cell line which is stably expressing the codon-optimized cDNA. The ScFv was purified from conditioned media by immobilized ion affinity chromatography (IMAC) and size exclusion chromatography (SEC). Its purity was analyzed by SDS-PAGE/Western Blot and SEC, and the specific targeting of beta cells was assessed by immunofluorescence analysis on the formaldehyde-fixed beta-cell lines, beta TC1 and MIN6, and pancreatic sections from C57BL/6 mice. In SDS-PAGE and Western Blot analysis, the ScFv was running as 2 bands with relative molecular weights of 35 and 70kD after the initial purification process. The re-injection of the 35kD band on the SEC column resulted in two bands of 35 and 70kD again. In immunofluorescence experiments of beta-cell lines and pancreatic sections, the ScFv in beta-cell cytoplasm did not colocalized with insulin. The resulting 2 protein bands/fractions strongly suggest that our ScFv exists in 2 forms, putatively as monomer and as dimer which might be in an equilibrium. The dimer seemed to be very stable, as it was resistant to reduction with beta-mercaptoethanol (BME) and tris (2-carboxyethyl) phosphine (TCEP) and to denaturation with SDS.

In conclusion, we have developed, expressed and purified the functional beta-cell specific ScFv which has potential application in the detection and monitoring of beta cells.

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For author disclosure information, see page A751.

1831-P

Islet Cell Autotransplantation (IAT): A Successful Endocrine Procedure after Total Pancreatectomy in Children with Chronic Pancreatitis

MOHAMED SALEH, ABHINAV HUMAR, NURSEN GURTUNCA, MARK LOWE, RITA BOTTINO, MARTIN WIJKSTROM, ARMANDO GANOZA, MASSIMO TRUCCO, LUIGI GARIBALDI, *Pittsburgh, PA*

Introduction: Total pancreatectomy (TP) markedly improves quality of life in children with chronic pancreatitis (CP), but results in brittle, insulinopenic diabetes. TP with IAT (TPIAT) may preserve insulin secretion. Minimal data are available regarding TPIAT in pediatric patients (Pts).

Methods: Between 2009-2016, TPIAT was performed in 13 Pts (7 boys) with median age 10.3 yrs (range 7-17 yrs). Six (46%) had a PRSS1 (protease, serine, 1) mutation, 2 (15.4%) had a CFTR (Cystic fibrosis transmembrane conductance regulator) mutation, 2 (15.4%) had combined CFTR/SPINK1 (serine protease inhibitor, Kazal-type, 1) mutations. All were euglycemic prior to TPIAT (normal fasting glucose and HbA1c; normal response to mixed meal tolerance test in a subset). Islet cells, isolated after TP, were infused in the portal vein. Pts were kept on an insulin infusion for (average) 6.8 days, then switched to MDI, with tight glycemic control.

Results: Six months after TPIAT, 5 Pts (38%) did not require insulin (HbA1c 5.5-6.1%), 3 Pts (23%) were on basal insulin only (0.03-0.35 U/Kg/day), with HbA1c of 5.5-6.7%, and 5 Pts (39%) required basal/bolus insulin therapy (0.5-1 U/Kg/day, median 0.6) with a HbA1c of 6.8-10.2%, median 7.6. Insulin requirements did not correlate with BMI-SDS (r=0.18) or number of islets/kg infused (r=-0.21). Pts with PRSS1 mutation had borderline lower (p=0.05, t-test) insulin requirements (0.1 U/kg/day) than Pts with CFTR mutation (0.46 U/kg/day). Ten Pts (77%) discontinued pain medication with complete pain resolution within 3 months. Complications including pyloric stenosis, intra-abdominal adhesions, and gastroparesis occurred in 3 Pts; Spontaneous/exercise-induced hypoglycemia in 2 Pts.

Conclusions: TPIAT is an effective treatment for CP in children and adolescents. Within 6 months, it provided pain resolution in 77% and allowed good glycemic control with no insulin or low dose basal insulin in 61% of patients in this series.

1832-P

The Role and Mechanism of Exosomes in Regulation of β-Cell Function

CHEN WANG, YUN SUN, YU ZHAO, WEIPING JIA, *Shanghai, China*

Objective: In this study, we used the exosomes derived from the pancreatic islet β cells and transplant the exosomes into the diabetic mice model to observe their effects on β cell function in vivo and explore the underline mechanisms.

Methods: Beta-cell lines were cultured in exosome-free medium for 48 hours. Ultracentrifugation method was used to collect the exosomes derived from the cells. Transmission Electron Microscopy (TEM), Tunable Resistive Pulse Sensing (TRPS) technique and Western blot were used to identify the exosomes. Streptozotozin was used to establish diabetic mouse model.

Results: With ultracentrifugation method, the exosomes we collected from beta-cell culture medium. TRPS exhibited the mean or median diameters of the exosome were 77nm or 101nm with a cup or round-shaped morphology. The Western blot showed that CD9 was highly expressed in the exosomes. Transplantation of the exosomes into diabetic mice resulted in a longer median survival time compared with control mice ($P < 0.01$). In mice with abnormal glucose tolerance, transplantation of the exosomes could not only improve the glucose tolerance, but increase the insulin contents in pancreas as well. Moreover, expression of CD31, a marker of endothelial cells, increased significantly in pancreas after the exosome transplantation.

Conclusion: Transplantation of the exosomes derived from beta-cells improved glucose metabolism in mice treated with STZ. Such protective effect was associated with increased CD31 expression in pancreas.

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INSULIN ACTION—ADIPOCYTE BIOLOGY

Moderated Poster Discussion: Signaling in Adipose Tissue

(Posters: 1833-P to 1838-P), see page 16.

1833-P

Characterizing the Wilms Tumor 1 (Wt1) Adipose Subpopulation

QUYEN V. LUONG, RITA SHARMA, KEVIN Y. LEE, *Athens, OH*

Although white adipose tissue was once thought to serve only as an energy reservoir, it is now known to play an active role in energy homeostasis and insulin sensitivity. Excess visceral fat has been strongly associated with an increased risk of metabolic and cardiovascular diseases. Wilms Tumor 1 (Wt1) has been shown to be expressed in mesothelial cells and a major regulator of developing kidney and heart. Notably, a subset of visceral adipocytes has been shown to be derived from Wt1 positive mesothelial cells, and adipocyte-specific ablation of Wt1 has been shown to lead to altered thermogenic and inflammatory pathways in adipose tissue. To further characterize the adipocyte population derived from the Wt1 lineage, we labelled these adipocytes by crossing the tamoxifen-induced Wt1-CreERT2 mice to the cell membrane-targeted ROSA26^{mTmG} fluorescent Cre reporter mice. Recombination was induced during embryogenesis by a single administration of tamoxifen (8mg/40g body weight) at e14.5. At 6 months, adipose tissues from recombined mice were studied using confocal microscopy and fluorescence-activated cell sorting (FACS) analysis. Confocal microscopy revealed the presence of adipocytes from the Wt1 lineage in visceral depots, including the pericardical fat (22.9 +/- 8.8%), perigonadal fat (30.7 +/- 4.2%), and perirenal fat (18.0 +/- 2.9%). On the other hand, no adipocytes from the Wt1 lineage were observed in either subcutaneous depots examined, including the inguinal subcutaneous and scapular white fat. Moreover, a lineage-dependent effect on adipocyte size was not observed. FACS analysis showed that pre-adipocytes from the Wt1 lineage made up 10.0 +/- 3.7% and 6.6 +/- 4.1% of total adipocytes in perigonadal and perirenal fat, respectively, while pre-adipocytes from the Wt1 lineage were undetectable in the subcutaneous inguinal and scapular white fat. Future work will demonstrate the effects of high-fat diet induced obesity on Wt1 derived adipocytes and their roles in regulating adipocyte function and inflammation.

1834-P

Inverse Association between Fasting Insulin Levels and Postprandial Changes of Plasma Asprosin Concentration in Patients with Type 2 Diabetes

SHINSUKE TOKUMOTO, SACHIKO HONJO, EMI OKAMURA, MEGUMI ABE, YOSHIHARU WADA, AKIHIRO HAMASAKI, *Osaka, Japan*

Background and Aims: Asprosin, a fasting-induced protein secreted by adipose tissue, has recently been discovered as a glucogenic hormone that promotes hepatic glucose release in mice and humans. While circulating Asprosin levels are reported to physiologically decrease after feeding, the pathophysiological role of Asprosin in patients with glucose intolerance remains poorly understood. The aim of this study was to evaluate the relationship between Asprosin postprandial concentration kinetics and other biomarkers.

Materials and Methods: 11 healthy subjects and 23 type 2 diabetes mellitus (T2DM) patients underwent a 2-h meal tolerance test in the morning after an overnight fast; the meal consisted of 460 kcal of total caloric load with 56.5 g of carbohydrates, 18 g of protein and 18 g of fat. Blood samples were collected immediately before and 2 hrs after meals. HbA1c, fasting plasma glucose (FPG), total cholesterol and other biomarkers were measured in all subjects. Plasma Asprosin levels was determined by enzyme-linked immunosorbent assay according to the manufacturer's protocol (Eiaab, Catalogue No. E15190h). Correlations were evaluated by Spearman's rank test. P values <0.05 were considered statistically significant.

Results: Fasting Asprosin levels in healthy subjects were significantly less than those in T2DM patients ($p < 0.05$). In T2DM patients, postprandial reduction of Asprosin levels showed a significant negative association with fasting insulin levels ($r = -0.41$ $p < 0.05$). No significant correlation was found between Asprosin levels and other biomarkers including FPG.

Conclusion: To our knowledge, this is the first report of a negative correlation of fasting insulin levels with postprandial reduction in Asprosin levels and higher fasting Asprosin levels in T2DM patients than healthy controls. Our results suggest that insulin resistance could be associated with the regulation of circulating Asprosin levels.