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Submitted Abstracts that will not be Presented at the Meeting
Georgia Fousteri (Ospedale San Raffaele)

Abstract Title

Genetic determinants of type 1 diabetes in individuals with weak evidence of islet autoimmunity at disease onset

Authors

Paola Carrera, Ilaria Marzinotto, Ricardo Bonfati, Luca Massimino, Tatiana Jofra, Valeria de Giglio, Clara Bonura, Angela Stabilini, Valeria Favalli, Simone Bondesan, Maria Pia Cicalese, Andrea Laurenzi, Amelia Caretto, Giulio Frontino, Andrea Rigamonti, Chiara Molinari, Marina Scavini, Federica Sandullo, Ettore Zapparoli, Caridi Nicoletta, Silvia Bonfiglio, Valeria Castorani, Federica Ungaro, Alessandra Petrelli, Graziano Barera, Alessandro Aiuti, Emanuele Bosi, Manuela Battaglia, Lorenzo Piemonti, Vito Lampasona, Georgia Fousteri

Purpose

Islet autoantibodies (AAbs) are detected in >90% of individuals with clinically suspected type 1 diabetes (T1D) at disease onset. A single AAb and sometimes at low titer is often detected in some individuals making their diagnosis uncertain. The T1D genetic risk score (GRS) is a useful tool for discriminating polygenic autoimmune T1D from other types of diabetes and particularly the monogenic forms, but testing is not routinely performed in the clinic. Here, we leveraged the T1D GRS to screen for monogenic diabetes in individuals with weak evidence of autoimmunity, i.e., with a single AAb at disease onset.

Methods

In a pilot study, we genetically screened 142 individuals with suspected T1D, 42 of whom were AAb-negative, 27 single AAb-positive and 73 multiple AAb-positive at disease onset. Next generation sequencing (NGS) was performed in 41 AAb-negative, 26 single AAb+, and 60 multiple AAb-positive patients using an analysis pipeline of >200 diabetes-associated genes.

Summary of Results

The T1D GRS was significantly lower in AAb-negative individuals than in those with a single and multiple AAbs. Pathogenetic class 4/5 variants in maturity-onset diabetes of the young (MODY) or monogenic diabetes genes were identified in 15/41 (31.7%%) AAb-negative individuals while class 3 variants of unknown significance were identified in 17/41 (41.5%). Residual C-peptide levels at diagnosis were higher in mutated patients compared to those without pathogenetic variants. Class 3 variants of unknown significance were found in 11/26 (42.3%) of single AAb-positive individuals while pathogenetic class 4/5 variants were present in 2/26 (7.7%) individuals. While no pathogenetic class 4/5 variants were identified in multiple AAb-positive individuals, class 3 variants of unknown significance were identified in 19/60 (31.7%) patients. Several patients across the three groups had more than one class 3 variants.

Conclusions

These findings provide insights into the genetic makeup of patients with weak evidence of autoimmunity at disease onset. Absence of islet AAbs or presence of a single AAb together with a low T1D GRS can be indicative of a monogenic form of diabetes and NGS can improve diagnosis.

Research Category

Type 1 Diabetes Etiology & Environment

Zachary Morse (University of British Columbia)

Abstract Title

Enterovirus-induced intestinal dysbiosis promotes type 1 diabetes development

Authors

Zachary Morse, Rachel Simister, Sean Crowe, Lisa Osborne, Marc Horwitz

Purpose

Environmental factors including enterovirus infection and microbiome dysbiosis have been independently linked to type 1 diabetes in both humans and in mouse models, thus we aimed to determine how these risk factors can communicate to influence predisposition to diabetes development by skewing host physiology and immune homeostasis.

Methods

Non-obese diabetic (NOD) mice were infected with coxsackievirus B4 (CVB4) and microbial community profiling using 16S rRNA sequencing and targeted metabolomics was used to determine how infection alters the intestinal microbiome profile and functional capacity. The changes in intestinal physiology and host antibody responses to commensal bacterial antigens were measured using histology, immunoassays, and flow cytometry. Lastly, the infection-induced dysbiotic microbiome was given via fecal microbiome transfer (FMT) to naïve antibiotic-depleted recipient mice which were monitored for diabetes development and changes to intestinal immunity.

Summary of Results

Infection of NOD mice with CVB4 induced onset of diabetes along with reorganisation of the intestinal microbiota community composition. The dysbiosis caused by infection resembled that of mice which spontaneously developed diabetes and results in reduced production of short chain fatty acid metabolites. Accompanying this dysbiosis, colonic mucosal barriers and gene expression of tight junction proteins resulted in increased intestinal permeability and bacterial translocation to the pancreatic lymph nodes. Furthermore, host antibody IgG and IgA responses to commensal bacterial antigens were increased both systemically and within the gut of infected mice. Finally, FMT of the CVB4-induced "diabetogenic" microbiome was sufficient to promote diabetes onset in recipient mice in the absence of virus infection by reducing regulatory immune responses in the intestine.

Conclusions

Virus infection can drive dysbiosis and disrupts intestinal homeostasis in a way that contributes to diabetes autoimmunity in NOD mice. These findings signify cross communication between virus infection, commensal microbes, and the host to further our understanding of the complex dynamics for the environmental contribution to type 1 diabetes development.

Research Category

Type 1 Diabetes Etiology & Environment

David Leslie (Blizard Institute, University of London)

Abstract Title

Exocrine proteins including Trypsin(ogen) as a key biomarker in type 1 diabetes

Authors

David Leslie, Lilianna Bakinowska, Tanwi Vartak, Sam Jerram, Kathleen Gillespie

Purpose

Proteomic profiling can identify useful biomarkers. Monozygotic(MZ) twins, discordant for a condition represent an ideal test population. We aimed to investigate and validate proteomic profiling in twins with type 1 diabetes and in other well characterised cohorts.

Methods

A broad, multiplex analysis of 4068 proteins in sera from MZ twins concordant (n=43) and discordant for type 1 diabetes (n=27) identified major differences which were subsequently validated by a trypsin(ogen) assay in MZ pairs concordant (n=39) and discordant (n=42) for type 1 diabetes, individuals at-risk (n=195) and with type 1 diabetes (n=990), as well as with non-insulin requiring adult-onset diabetes diagnosed as either autoimmune (n=96) or type 2 (n=291).

Summary of Results

Proteomic analysis identified major differences between exocrine enzyme levels in discordant MZ twin pairs despite strong correlation between twins, whether concordant or discordant for type 1 diabetes ($p<0.01$ for both). In validation experiments, trypsin(ogen) levels were lower in twins with diabetes compared with non-diabetic co-twins ($p<0.0001$) and healthy controls ($p<0.0001$). In recently-diagnosed cases, trypsin(ogen) levels were lower than in controls across a broad age range. In at-risk relatives, levels <15 ng/ml were associated with increased risk of progression (uncorr. $p=0.009$). Multiple linear regression in recently-diagnosed cases showed that trypsin(ogen) levels were associated with insulin dose and diabetic ketoacidosis while age and BMI were confounders.

Conclusions

Type 1 diabetes is associated with altered exocrine function, even before onset. Twin data suggest roles for genetic and non-genetically determined factors. Exocrine/endocrine interactions are important under-investigated factors in type 1 diabetes.

Research Category

Type 1 Diabetes Etiology & Environment

Jodi Ye (Albert Einstein College of Medicine)

Abstract Title

PGM1, a Type 1 Diabetes susceptibility gene, rewires islet glucose metabolism and stress responses following viral infection

Authors

Jody Ye, Yunping Qiu, Shuibing Chen, Irwin Kurland, Yaron Tomer.

Purpose

Phosphoglucomutase 1, encoded by PGM1, is a risk gene associated with T1D from GWAS studies. Phosphoglucomutase 1 mediates the bi-directional transfer of a phosphate group between the 1&6 positions of glucose, which in turn regulates glucose metabolism, protein glycosylation, and mitochondrial respiration. Previous evidence indicated that PGM1 expression in beta cells was suppressed by viral infection. We hypothesized that PGM1 deficiency dysregulates glucose metabolism, elevates beta cell stress and immunogenicity in T1D.

Methods

PGM1 mRNA and protein levels were assessed in human islets following an intracellular Poly I:C treatment, as well as a pro-inflammatory cytokine cocktail IL-1b, TNF-a, and IFN-g treatment. For the downstream effects of PGM1 suppression, PGM1 was knocked down in human islets infected with lentiviral particle carrying the PGM1 shRNA. Markers of ER stress were assessed using qRT-PCR; mitochondrial Reactive Oxygen Species (ROS) was assessed using MitoSOX dye staining; glucose metabolism was examined using the Seahorse assay and the 13C tracer analysis; glucose stimulated insulin secretion was examined using ELISA. To examine the effect of PGM1 on beta cell development, PGM1 was knocked out using CRISPR-Cas9 in human embryonic stem cells (hESCs). Flow cytometry was used to characterize markers of beta cell progenitors during in vitro beta cell differentiation.

Summary of Results

In human islets, PGM1 mRNA and protein expression was suppressed after intracellular Poly I:C treatment but not after pro-inflammatory cytokine cocktail IL-1b, TNF-a, and IF-g treatment. Upon PGM1 shRNA lentiviral infection, mRNA expression of ER stress markers such as BIP, ATF3, CHOP, but not spliced XBP1 was significantly elevated. There was an approximately two-fold increase in mitochondrial ROS, as reflected by mitoSOX staining. For glucose metabolism, PGM1 suppression lowered the glucose flux via glycolysis as quantified by the Seahorse assay. 13C tracer analysis showed that glucose flux was directed to the TCA cycle more via pyruvate dehydrogenase than pyruvate carboxylase, supporting cell energetics. PGM1 suppression did not appear to affect beta cells' response to release insulin after glucose stimulation. Apart from stress responses and metabolic regulation, PGM1 is also required for beta cell development. A PGM1 deficient hESC clone yielded significantly less pancreatic progenitors (~6% PDX1+NKX6.1+) compared to a WT hESC clone (~30%).

Conclusions

Phosphoglucomutase 1 could be a critical metabolic and stress regulator in pancreatic beta cells. Its relevance to T1D is highlighted by the downregulation of PGM1 after viral infection. The consequence of PGM1 deficiency, including the suppression of glycolysis, elevated ER stress and oxidative stress potentially promotes beta cell immunogenicity. Further studies are required to test whether activating phosphoglucomutase 1 exerts therapeutic advantage of lowering beta cell stress in T1D.

Research Category

Beta Cell Physiology and Dysfunction

Giuseppe Matarese (Università degli Studi di Napoli "Federico II")

Abstract Title

Unveiling the role of metabolic pressure in the break of self-immune tolerance in type 1 diabetes

Authors

Claudio Procaccini, Alessandra Colamatteo, Claudia Russo, Fortunata Carbone, Maria Teresa Lepore, Clorinda Fusco, Giusy De Rosa, Giuseppe Matarese

Purpose

Type 1 Diabetes (T1D) is characterized by a T cell-mediated destruction of pancreatic beta-cells. It has been demonstrated that regulatory T (Treg) cells are essential for the maintenance of tolerance against self-tissues and qualitative/quantitative alterations of Treg cells have been correlated with break of immune tolerance. Our group has shown that the adipocyte-derived hormone leptin can play a significant role in the pathogenesis of several autoimmune diseases among which T1D. Indeed, leptin sustains pro-inflammatory T cell proliferation and cytokine secretion, while it exerts opposite effect on Treg cells, inhibiting their own proliferation. Since an inverse relationship between leptin secretion and the frequency of Treg cells has been described in autoimmune diseases and leptin neutralization has been shown to induce a robust expansion of human Treg cells, our objective is to investigate the precise relationship between leptin and Treg cells in the context of T1D.

Methods

In human cryopreserved spleen cells from the nPOD repository, from 3 groups of subjects (auto-Ab-positive, T1D and no-diabetes), we analyzed through polychromatic FACS analysis the expression of Foxp3, its splicing variants containing the exon 2 (Foxp3E2), Treg cell lineage-specific markers (PD1, CTLA4, CD69, CCR7, CD31, CD71, Ki67) and S6 phosphorylation, as readout of mammalian target of rapamycin (mTOR) activation. In parallel, spleen cells were also in vitro activated either polyclonally with anti-CD3/CD28 beads, or with recall-antigen specific stimulation (purified protein derivative, PPD), or with a T1D autoantigen-specific stimulation (recombinant human GAD65). These stimulations were analyzed for their capacity to induce T cell activation (evaluated as CD25 and CD69 expression), nutrient energy sensing mTOR pathway and Foxp3 induction/expression. Finally, we measured the capacity of these in vitro activated cells to express and produce leptin receptor and leptin (LepR/Lep).

Summary of Results

Flow-cytometric analysis of freshly isolated spleen cells from T1D, auto-Ab-positive and no-diabetes subjects (n=4/group) revealed increased frequency of CD4+Foxp3all+ and CD4+FoxP3E2+ cells in T1D subjects, as compared to auto-Ab-positive and no-diabetes subjects, but associated with a reduced expression of Treg-associated suppressive and proliferative markers (CCR7, CTLA4, CD31, CD69 and Ki67, respectively).
Moreover, recombinant human GAD65 stimulation revealed that CD4+ T cells from T1D subjects displayed an overall increased activation state (as testified by enhanced fraction of CD25+ and CD69+ cells) and mTOR over-activation (increased S6-phosphorylation). These events associated with an altered leptin/lepR expression both in CD4+ and Treg cell compartment of the T1D subjects, while no difference in the expression of CD4+ activation and metabolic markers among the three experimental groups was observed neither upon polyclonal (anti-CD3/CD28) or with recall-antigen in vitro stimulation

Conclusions

Taken together, these data show that ex-vivo peripheral Treg cells from T1D patients are increased in percentage as compared to auto-Ab-positive and no-diabetes subjects, but they display a lower expression of Treg cell lineage-specific markers, suggesting an impaired immunoregulatory function. Differently from the other experimental groups, T1D patients displayed an increased CD4+ T cell activation upon GAD-specific stimulation, associated with an altered mTOR/leptin/lepR axis, suggesting that these cells are subjected to a higher "metabolic pressure", as compared to CD4+ T cells from auto-Ab-positive and no-diabetes subjects. Our results lay the foundation for further investigations on how "metabolic pressure" could represent a novel pathogenic event leading to the breach of immunological self-tolerance in T1D.

Research Category

Immunology

Sofia Thomaidou (Leiden University Medical Center)

Abstract Title

IFN gamma but not IFN alpha increases recognition of insulin neoantigen to amplify islet autoimmunity

Authors

Sofia Thoumaidou, Amadeo Munoz Garcia, Jin Gan, Arno van der Silk, Rob C. Hoeben, Bart O. Roep, Françoise Carlotti, Arnaud Zaldumbide

Purpose

Increasing evidence suggests that the beta cell environment triggers expression and accumulation of neoantigens to which central tolerance is lacking, driving islet autoimmunity. We have recently demonstrated that the inflammatory milieu characteristic of insulitis affects translation fidelity and generate defective ribosomal products (DRiPs) that participate to autoimmune beta-cell destruction. Alike translation, protein degradation is a dynamic process that can be regulated in response to extracellular signals to ensure homeostasis, survival or apoptosis. In this process, the proteasome is playing a key role regulating protein turn-over and shaping peptides presented at the cell surface. In tumors, several specific epitopes were shown to be derived from the immunoproteasome rather than by the constitutive proteasome.

Methods

We evaluated the proteasomal composition of beta cells upon IFNα or IFNγ treatment, representing innate and adaptive immunity, using single cell transcriptomics. We specifically silenced catalytic subunits of the immunoproteasome in EndoC-βH1 cells and evaluated consequences on the crosstalk with diabetogenic insulin DRiP specific CD8+ T cells.

Summary of Results

Expression of the PSMB10 catalytic subunit during IFNγ, but not IFNα, stimulation discriminated beta cells from other endocrine cells and enhanced the presentation of insulin DRiP derived peptide to specific CD8+ T cells. In addition, PSMB10 was upregulated in T1D patients compared to auto-antibody positive and healthy controls, solely in a subpopulation of beta cells prone to the expression of immune related pathways.

Conclusions

Our data reinforce the participation of the degradation machinery in beta cell immunogenicity and position the immunoproteasome catalytic subunits as potential therapeutic targets to limit neoantigens processing and beta cell destruction.

Research Category

Immunology

Maria Pilar Toledo (Florida State University)

Abstract Title

A high throughput system to generate recombinant adenoviruses for CRISPR-Cas9 mediated gene knockouts

Authors

Julie Yue Wang, Maria Pilar Toledo, Pamela Sandoval Sanchez

Purpose

Adenoviruses have a very high transduction efficiency in primary mammalian cells. However, the big vector size precludes the conventionally cut-and-paste cloning method. The current most popular adenoviral cloning system (pAdEasy) relies on bacterial cell homologous recombination that is laborious and low efficiency. Our goal is to establish a facile all-in-one adenoviral backbone that is amenable for high throughput in vitro gRNA cloning.

Methods

The gateway cloning method achieves high efficiency in vitro homologous recombination and offers a reliable negative selection. Toward this, we designed the pAdEasy all-in-one vector that contains the Cas9 expression cassette, the gRNA expression cassette, and the GFP fluorescent marker. We also incorporated the attR sites on the vector to make it compatible with gateway cloning. gRNAs will be introduced via a two-step procedure: (1) gRNA oligos are cloned in to the pENTR vector to create pENTR gRNA entry vector by the high-efficiency golden gate assembly; and (2) the pENTR gRNA entry vector is recombined with the pAdEasy destination vector via LR reaction to create the final pAdEasy all-in-one expression vector.

Summary of Results

With our newly designed system, all the cloning steps can be achieved in vitro with highly optimized reaction conditions: both cloning steps have an efficiency of more than 2000 colonies per 2 μl of reaction. Restriction digestion confirmed that more than 92% of the colonies contained the correct product.

Conclusions

This study shows that an all-in-one adenoviral vector can be made relatively easily with sufficient efficiency to test different gRNAs and has the capability to test whole genome screening. Further, with the novel use of gateway assembly and ccdb, we dramatically reduced the background vectors leading to cleaner results. CRISPR genetic perturbation research is of immense importance for the further health of our species. Adenoviruses are optimal for transducing hard-to-transfect cells including the primary human islets. By creating the all-in-one adenoviral cloning system, our work will facilitate functional genomic research in various biotechnology fields.

Research Category

Novel Technologies

Alexandra Title (inSphero AG)

Abstract Title

Liraglutide protects β-cells in novel human islet microtissue models of type 1 diabetes

Authors

Burcak Yesildag, Joan Mir-Coll, Aparna Neelakandhan, Claire Gibson, Nikole Perdue, Chantal Rufer, Maria Karsai, Adelinn Biernath, Felix Forschler, Patricia Wu Jin, Patrick Misun, Alexandra Title, Andreas Hielermann, Frederick Kreiner, Johnna Wesley, Matthias von Herrath

Purpose

Type 1 diabetes (T1D) is an autoimmune disease in which insulin-producing β-cells are destroyed by auto-reactive T-cells, leading to a severe decline in β-cell function and mass. Robust in vitro human models that enable high-throughput and longer-term studies of islet-immune interactions are lacking. The use of native human islets in experimental research is challenging due the high degree of heterogeneity between islets as well as their limited viability in culture. Previously, we developed a human islet microtissue (hIsMT) model from primary human islets, that display homogeneous size, tissue architecture and cellular composition, as well as robust and stable functionality and viability in culture. Here, we utilize hIsMTs to establish three T1D-relevant islet-immune injury models by culturing them with (1) proinflammatory cytokines, (2) activated peripheral blood mononuclear cells (PBMC), or (3) HLA-A2-restricted preproinsulin-specific cytotoxic T lymphocytes (CTL). We characterize these models and use them to evaluate the protective role of GLP-1 receptor agonist liraglutide in T1D-related stress.

Methods

Native human islets were dispersed and reaggregated using a hanging drop system. The resulting hIsMTs were cultured with a cocktail of T1D-relevant cytokines for 7 days, or co-cultured with CD3/CD28 pre-activated PBMCs or preproinsulin-specific CTLs for 3 days. The role of liraglutide was evaluated by exposing hIsMTs to the compound in parallel to the islet-immune stress. β-cell function was evaluated by glucose-stimulated insulin secretion assay (GSIS), and islet MT viability was evaluated by measuring total ATP and Caspase 3/7 activity. Further evaluation of the models was performed via microscopic analysis of hormone expression, β-cell count, T-cell infiltration, and HLA expression, and through the culture supernatant cytokine measurements.

Summary of Results

In all T1D models, β-cell function declined, as manifested by increased basal and decreased GSIS and decreased intracellular insulin content. Additional hallmarks of T1D progression such as loss of the first-phase insulin response (FFIR), increased proinsulin-to-insulin ratios, HLA-class I expression, and inflammatory cytokine release were also observed. Liraglutide was found to prevent loss of GSIS under T1D-relevant stress, by preserving the FFIR and decreasing immune cell infiltration and cytokine secretion.

Conclusions

Our results suggest that liraglutide mediates an anti-inflammatory effect that helps protect β-cells from the immune-mediated attack that leads to T1D. Furthermore, our results highlight the value of human-islet-microtissue-immune injury models in the discovery and evaluation of novel compounds for the treatment of T1D.

Research Category

Novel Technologies

Clemens Harer (Medical University of Graz)

Abstract Title

Periodic cycles of fasting-mimicking diet in mice are associated with intermittent islet remodeling

Authors

Clemens Harer, Jelena Krstic, Laurin Herbsthofer, Barbara Ehall, Kaddour Bounab, Joakim Fraz, Petra Kotzbeck, Andreas Prokesch, Thomas R Pieber

Purpose

Periodic cycles of fasting improve metabolic markers and anti-cancer survival in mice and humans, as well as longevity in rodents. We recently showed that prolonged fasting (fasting mimicking diet, FMD) impairs glucose tolerance after a fasting period compared to subsequent refeeding and controls in healthy mice. Herein, we investigated the effects of fasting and refeeding after multiple cycles of FMD on the endocrine pancreas.

Methods

We administered three weekly cycles of FMD (1st day 50%-, 2nd-3rd day 10% of daily calorie intake) followed by 4 days of refeeding ad libitum after every cycle to 20 female C57BL/6 mice at 12 weeks of age. Animals were sacrificed and pancreatic tissues collected after fasting (n=9) or refeeding (n=9) of the 3rd FMD cycle. Controls (n=10) were fed a standard chow ad libitum and sacrificed after 20 days.

10 Sections of every formalin fixed paraffin embedded (FFPE) pancreatic tissue were cut approx. 2.5μm apart and the last section used for fluorescent multiplexed immunohistochemistry (Fm-IHC).

Fm-IHC staining was achieved using the BOND RX Fully Automated Research Stainer from Leica Biosystems. For primary antibodies we used Insulin (1:60000, Abcam ab181547), Glucagon (1:10000, Abcam ab92517), Somatostatin (1:12000, Abcam ab111912), PDX1 (1:400, Cell Signalling Technology Europe #5679), BRN4 (1:100, Novus Biologicals NBP1-89934) and PP (1:30000, Abcam ab255827). In addition to the Dako EnVision+ System-HRP 2nd anti-rabbit antibody, we used reagents contained in the Opal 7-Color Automation IHC Kit (Akoya Biosciences SKU NEL821001KT). The required microscope scanning platform were Vectra3® and Vectra® Polaris™. Spectral unmixing was done in Phenochart™ from Akoya Biosciences. Image analysis was performed in the software Halo® by Indica Labs to generate cell-based segmentation data of areas of interest that were consequently exported. Next, we applied Cell2Grid, an Fm-IHC image compression algorithm, followed by cell-based rules for islet detection and staging, enabling quantitative high-throughput analysis as well as compiling all data in a digital database ("IsletViewer").

Summary of Results

Islets of fasted mice tended to have (mean differences to control-, refed mice ±SE (%)) higher β-cell fraction than controls or refed mice (3.5±1.7, 3.2±1.7), higher relative β-cell count (1.330±2.393, 3.229±2.304) and lower relative count of alpha cells (3.411±1.591, 2.549±1.532). Furthermore, an increase of overall islet number after fasting (mean differences ±SE (islet count)) compared to controls (10.556±7.076) and refeeding (3.333±0.459) was observed. Moreover, islet size (mean differences ±SE (pixels/islet)) seemed to increase after fasting (67.691±53.166) and even more so after refeeding (93.011±54.330), compared to controls.

Conclusions

Islet data from our study not only support previous findings that prolonged fasting increases β-cell fraction and relative number of β-cells in islets of fasted mice, but also points towards an increase in islet size and higher islet count, that even increases after refeeding. These findings support the previous notion, that an increase of β-cells after fasting is linked to impaired secretory capacity of β-cells, therefore leading to temporary impaired glucose tolerance.

Research Category

Beta Cell Physiology and Dysfunction

Gaetano Santulli (Albert Einstein College of Medicine)

Abstract Title

Endothelial extracellular vesicles enriched in specific microRNAs predict new-onset diabetes in COVID-19

Authors

Uma Kansakar, Stanislovas Jankauskas, Pasquale Mone, Jessica Gambardella, Giuseppe Fiorentino, Fahimeh Varzideh, Bruno Trimarco, Gaetano Santulli

Purpose

While initial reports of COVID-19 primarily focused on pulmonary manifestations of the disease, other organs including heart, kidney, brain, as well as the pancreas, have been noted as affected by this disease, making COVID-19 a systemic disorder. The relationship between diabetes mellitus (DM) and COVID-19 is twofold: indeed, diabetic patients have an increased risk to incur in severe COVID-19 outcomes and, on the other hand, new-onset DM, development or worsening of hyperglycemia, and complications of preexisting DM have been reported in COVID-19.

SARS-CoV-2 affects not only the epithelial cells of the lung parenchyma via angiotensin converting enzyme 2 (ACE2), but also endothelial cells (ECs) across the whole body, thus leading to generalized damage and inflammation, known as endotheliitis. The functional link between COVID-19 and endothelial dysfunction, which could explain the multi-organ manifestations of the disease, has been confirmed by several investigators, including our group. Moreover, ultrastructural analyses of tissues from deceased COVID-19 patients have shown the actual presence of SARS-CoV-2 in ECs of different organs alongside alterations in the microvascular architecture.

Methods

Since the pandemic outbreak, we have collected human blood samples from COVID-19 patients and controls. We We isolated circulating extracellular vesicles (EVs) derived from ECs (EC-EVs) and we examined their microRNA (miRNA) content via RNA-sequencing, subsequently validated via RT-qPCR.

Summary of Results

We first performed a pilot study on 120 subjects, observing that when comparing EC-EVs miRNAs between COVID-19 patients that develop diabetes (DIAB-COVID+) after SARS-CoV-2 infection to COVID-19 patients that did not develop diabetes (NON-DIAB-COVID+) and control non-COVID subjects, including both non-diabetic (NON-DIAB-COVID–) and diabetic (DIAB-COVID–) individuals, one specific miRNA emerged as significantly upregulated in the first group. We subsequently confirmed these findings in a larger population (P<0.001). Then, we performed a prospective study in non-diabetic subjects, hospitalized for COVID-19, with a median follow-up of 6 months. We found that circulating levels of this miRNA within EC-EVs were significantly upregulated (P<0.001) in patients with vs without new-onset diabetes. Using a stepwise multiple regression analysis, adjusting for age, sex, BMI, hypertension, dyslipidemia, smoking status, and D-dimer, the association with new-onset diabetes in COVID-19 patients was confirmed (P<0.001).

Conclusions

We demonstrate for the first time that a specific miRNA contained in circulating EVs released by endothelial cells is able to reliably predict the risk of developing new-onset diabetes mellitus after having contracted COVID-19. Our findings are also relevant when considering the emerging importance of post-acute sequelae of COVID-19 systemic manifestations even months after viral negativization (Long-COVID).

Research Category

Angela Lombardi (Albert Einstein College of Medicine)

Abstract Title

Effect of ketone bodies in Type 1 Diabetes

Authors

Stanislovas Jankaukas, Uma Kansakar, Crystal Nieves Garcia, Jessica Gambardella, Pasquale Mone, Yaron Tomer, Gaetano Santulli, Angela Lombardi.

Purpose

Type 1 Diabetes (T1D) is characterized by progressive beta cell failure, eventually resulting in insulin deficiency and hyperglycemia leading to long-term complications. Currently no curative therapeutic or prevention modalities exist to reverse or prevent the autoimmune destruction of the islets, and the disease can only be managed with insulin replacement therapy. Thus, developing interventions aimed at achieving normoglycemia and improving outcomes in diabetic patients, minimizing substantial side effects, is urgently needed. To address this unmet need, we propose to leverage the therapeutic properties of ketone bodies to prevent and/or improve diabetes-associated ailments of the pancreatic beta cell. Of note, although excessive production of ketone bodies leads to life-threatening ketoacidosis in diabetic patients under glucose lowering treatment, emerging lines of evidence suggest that modest levels of ketone bodies play adaptive and beneficial roles. Specifically, a functional role as key signaling molecule is increasingly acknowledged for beta-hydroxybutyrate (BHB), the most abundant ketone circulating in the human body. However, therapeutic applications of BHB have never been considered in pancreatic beta cell dysfunction.

Methods

The effects of BHB has been tested in vivo in an established model of T1D, the non-obese diabetic (NOD) mouse model. To explore whether BHB can prevent diabetes in vivo, NOD mice have been fed a standard or BHB-enriched diet. A mouse Luminex Multiplex Assay Panel has been used to evaluate the serum levels of pro-inflammatory cytokines in both groups. Moreover, the effects of BHB has been assessed ex vivo in murine islets isolated from NOD mice fed with both diets and in human islets isolated from healthy donors treated with a cocktail of pro-inflammatory cytokines (i.e. IL-1, TNF-; IFN-) with or without BHB pretreatment. The effect of BHB on apoptosis, mitochondrial health and selective autophagy processes have been also evaluated.

Summary of Results

In our preliminary studies, we found that BHB treatment in vivo significantly increased survival and delayed diabetes onset in the NOD model, protecting pancreatic islets from apoptosis and reducing insulitis. Furthermore, islet yield and total pancreatic insulin were markedly higher in BHB treated mice compared to those in standard diet. Also, serum levels of pro-inflammatory cytokines were significantly lower in mice fed with a BHB-enriched diet compared to control diet. Considering that the loss of mitochondrial integrity is one of the main mechanisms underlying the apoptosis of beta cells in T1D, ex vivo experiments performed in murine and human islets showed that BHB had a protective effect on apoptosis and mitochondrial fitness. Indeed, we observed that BHB significantly decreased the percentage of TUNEL positive nuclei and the production of ROS in both systems. In line with these results, BHB preserved mitochondrial membrane potential, attenuated the impaired mitochondrial respiration and mitochondrial fragmentation and rescued mitochondrial biogenesis. Finally, we saw a significant increase in the mitophagy flux in murine and human islets pretreated with BHB.

Conclusions

Ketone bodies represent natural compounds that our body produces to cope with stress conditions. In particular, the ketone body BHB is involved in a variety of molecular signaling functions, in addition to its role as a glucose-sparing energy carrier, that may influence a broad range of human diseases. We anticipate that the experiments described in this abstract will add significantly to our understanding of the pathobiology of T1D. Indeed, if our preliminary studies are confirmed BHB might emerge as a glucose-lowering agent that, acting synergistically with pharmacotherapy, could be able to improve the quality of life in patients with T1D.

Research Category

Beta Cell Physiology and Dysfunction

Heikki Hyoty (Tampere University)

Abstract Title

Maternal virus antibodies reduce the risk of early coxsackievirus B1 infections and islet autoimmunity.

Authors

Amir-Babak Sioofy-Khojine, Jussi Lehtonen, Jutta Laiho, Leena Puustinen, Jorma Toppari, Riitta Veijola, Johanna Lempainen, Kalle Kurppa, Mikael Knip, Heikki Hyoty

Purpose

Coxsackievirus B (CVB) infections have associated with the initiation of islet autoimmunity (IA) and type 1 diabetes (T1D). A vaccine against these viruses would help to evaluate causality by testing whether it can reduce the risk of T1D. The first CVB vaccine has recently passed the first-in-human trial (PROVENT trial) and one of the key questions in the further development is the age when the first CVB infections occur. This information would help to optimize the timing of vaccinations. In addition, it would help to estimate possible impact of maternal vaccinations. Maternal CVB antibodies, induced by vaccines or natural infection, and transferred to the fetus via placenta (IgG) and breast milk (IgA), represent a "vaccine of the nature" and could potentially prevent early CVB infections. This study addresses these questions having the following specific aims:
• To identify the age when children experience their first CVB infection.
• To study the prevalence of protective CVB antibodies in pregnant mothers.
• To study the kinetics of disappearance of maternal CVB antibodies in the child.
• To find out whether maternal CVB antibodies modulate the risk-association between CVB infections and IA.

Methods

The study focused on CVB1 infection, since this CVB type has shown association with the initiation of IA in the prospective birth cohort study in Finland (DIPP study). Neutralizing antibodies were analysed against CVB1 in the cord-blood sera and in the serial plasma samples taken at the age of 3, 6 and 12 months from 683 DIPP children who were followed from birth, including 231 case children who developed multiple islet autoantibodies and 451 individually matched control children. CVB1 infections were diagnosed by seroconversions in CVB1 antibodies during the follow-up. Effect of maternal CVB1 antibodies on the risk of post-natal CVB1 infections and CVB1-IA association was analysed using conditional logistic regression. Since maternal CVB antibodies are transferred to the child also via breast milk, duration of breast-feeding was analyzed as a co-variate.

Summary of Results

CVB1 infections were frequent already at the young age; 59% of infections occurred before the age of 3 months, 25% between 3-6 months and 17% between 6-12 months. Maternal CVB1 antibodies were found in the cord-blood in 24% of children

disappearing by the age of 12 months. 74% of children who lacked maternal CVB1 antibodies had CVB1 infection already before the age of 6 months compared to 47% of children who were positive for maternal CVB1 antibodies (p<0.001). A higher proportion of case children compared to control children belonged to the group with the weakest protection against CVB1 being breast-fed for less than 6 months and being negative or having only low levels of maternal CVB1 antibodies (32% vs. 24%; p=0.05). Similarly, 53% of the case children compared to 43% of control children were exclusively breast-fed for <3 months and had negative/low levels of maternal CVB1 antibodies (p=0.025).

Conclusions

The first CVB1 infections occur early suggesting that children should be vaccinated already before the age of six months. Maternal antibodies decreased risk of early CVB infections and modulated the CVB-IA association. This effect was strongest in young children who still had high maternal CVB antibody titers. The results support the idea of testing the ability of CVB vaccines to reduce the risk of CVB infections and IA.

Research Category

Type 1 Diabetes Etiology & Environment

Joanna Filipowska (University of British Columbia)

Abstract Title

LGR4 deficiency is detrimental for beta cells in basal and stress-induced conditions

Authors

Joanna Filipowska, Nagesha G Kondegowda, Nacy L. Rivera, Peng Wang, Adolfo Garcia-Ocaña, Rupangi C. Vasavada

Purpose

Loss of functional insulin-producing β-cells and inability to regenerate them play a key role in the onset of both Type 1 and Type 2 diabetes. Therefore, understanding what is required to maintain healthy β-cells and how to enhance their regeneration and survival is crucial in treating this disease. GPCRs (G protein-coupled receptors) comprise the largest group of receptors regulating key cellular processes in all tissues. They also are therapeutic targets for multiple diseases, including diabetes. LGR4 (Leucine-rich repeat-containing G protein-coupled receptor 4), a member of the GPCR subfamily B, is the fourth most abundant GPCR in human islets, and is expressed in pancreatic β-cells. LGR4 classically binds R-spondins to potentiate Wnt signaling particularly during development. Recently, another ligand, RANKL (Receptor activator of NF-κB)/RANK ligand), was identified to bind LGR4 receptor in osteoclast precursor cells. In many tissues, including heart, skin, liver, kidney, bone, LGR4 has regenerative, anti-inflammatory, and anti-apoptotic functions. However, the functional significance of this receptor in the pancreatic beta cell remains unknown. We hypothesized that LGR4 plays an important role in maintaining pancreatic β-cell homeostasis.

Methods

To study the role of Lgr4 in vitro, we knocked out Lgr4 in INS1 cells (rat insulinoma) using Lgr4 siRNA, and in mouse islets using Lgr4 floxed (Fl/Fl) islets transduced with Cre recombinase adenovirus (Ad)under basal conditions and in the presence of cytokines. We examined the effect of Lgr4 overexpression in INS1 cells, mouse and human islets using AdLgr4. To study the effect of Lgr4 knockout in vivo, we generated β-cell-specific conditional knockout mice (cko) (Lgr4Fl/Fl; INS1KICre+). We assessed the effect of Lgr4 cko in males and females, under basal conditions and under stress [high fat diet (HFD), multiple low dose streptozotocin (MLDS) and aging].

Summary of Results

Lgr4 mRNA levels are negatively regulated by cytokines, in rodent and human islets. Knockdown of Lgr4 is detrimental for β-cell survival and proliferation in basal conditions in INS1 cells and mouse islets. Lgr4-deficient INS1 cells treated with cytokines show increased cell death, and a significant decrease in NFκB p65 (RELA) phosphorylation (Serine 468), responsible for pathway deactivation. Blocking NFκB activity significantly decreases β-cell death in Lgr4-deficient INS1 cells under basal and cytokine-treatment, suggesting LGR4 suppresses NFκB pathway in β-cells. In contrast, overexpression of Lgr4 in INS1 cells, mouse and human islets protects β-cells against cytokine-induced cell death. β-cell-specific Lgr4 cko mice, under basal conditions, exhibit normal blood glucose homeostasis, but have significantly increased β-cell death compared to wild-type (WT) controls, in both male and female mice. However, β-cell proliferation is reduced only in female but not male cko mice in basal conditions. When male cko mice are exposed to stressors such as HFD, they have significantly reduced β-cell proliferation and increased death compared to WT mice; and with MLDS treatment exhibit increased β-cell death, compared to WT-MLDS-treated mice. Upon aging, only female but not male, Lgr4cko mice exhibit significantly impaired glucose tolerance at one year of age which gets worse over time.

Conclusions

Our data indicate LGR4 is critical for maintaining β-cell health in basal and stress-induced conditions, likely by suppressing NFκB pathway activity.

Research Category

Beta Cell Physiology and Dysfunction

Miguel Medina-Serpas (University of Florida)

Abstract Title

Spatially resolved transcriptional profiling of human pancreatic lymph node

Authors

Miguel Medina-Serpas, Maigan Brusko, Leandro Batzano-Nogueira, Todd Brusko

Purpose

In these investigations, we perform spatial transcriptomics (Visium Gene Expression Assay, 10x Genomics) on pancreatic lymph nodes (pLN) isolated from a cohort of non-diabetic controls (n = 3) and a single autoantibody positive (AAB+) case (GAD65+; n = 1) to begin assessing the transcriptional profile of the major functional compartments of the pLN, including the T-cell zone (TCZ) and lymphoid follicles. We intend to expand on this initial cohort to further assess the transcriptional differences within the relevant functional compartments of the lymph node which are associated with primary seroconversion.

Methods

Visium Spatial Gene Expression Assay

Summary of Results

This initial cohort consists of 3 nondiabetic controls (median age = 19.3 years old) and a single GAD65+ autoantibody positive donor (age = 21.7 years old). Using spatial transcriptomics, we assessed the gene expression of the T-cell zone (TCZ) and

lymphoid follicle homing genes (TCZ = CCR7, SELL; Follicles = CXCR5, CXCL13) in addition to genes associated with T-cell activation (TCZ = CD69, IL2RA, HLA-DRB1), costimulation (ICOS:ICOSLG, CD40:CD40LG, PDCD1:CD275; TNFRSF4:TNFSF4) and differentiation (TCZ = TBX21, GATA3, RORC, BCL6; Follicle = PRDM1, BACH2, XBP1, IRF4) within their respective functional compartments. Spatial transcriptional profiling of the TCZ revealed no changes in the expression of TCZ homing genes CCR7 and SELL (L-Selectin). We then assessed the expression of genes associated with T-cell activation (CD69, IL2RA, HLA-DRB1) and the lineage defining transcription factors (TBX21, GATA3, RORC, BCL6) and observed increased expression of activation associated markers CD69 and IL2RA and the follicular helper T-cell (Tfh) defining transcription factor BCL6 in our AAB+ donor compared to non-diabetic controls. With respect to the lymphoid follicles, we noted an increased expression of the follicular homing gene CXCR5, and costimulatory genes involved in cognate T- and B-cell interactions such as TNFRSF4, ICOSLG, and CD40 in our AAB+ donor. Furthermore, with respect to the expression of genes associated with B-cell activation and plasma cell differentiation, we detected elevated gene expression of PRDM1 (BLIMP1), XBP1, and IRF4 within the lymphoid follicles of our AAB+ donor when compared to non-diabetic controls.

Conclusions

These findings suggests that T-cell activation is enhanced within the TCZ and that increased expression of transcription factor BCL6 and chemokine receptor CXCR5 suggests skewing towards a Tfh-like phenotype in the AAB+ case. Furthermore, transcriptional assessment of the lymphoid follicles revealed elevated expression of several genes involved in follicular homing, costimulation, and B-cell differentiation in the AAB+ case when compared to non-diabetic controls.

Research Category

Immunology

Siddhartha Sharma (Scripps Research)

Abstract Title

Measuring anti-islet autoimmunity in mouse and human by profiling peripheral blood antigen specific CD4 T cells

Authors

Siddhartha Sharma, Xuqian Tan, Josh Boyer, Don Clarke, Anne Costanzo, Brian Abe, Lisa Abe, Lisa Kain, Marie Holt, Adrienne Armstrong, Marynette Rihanek, Andrew Su, Cate Speake, Peter Gottlieb, Michael Gottschalk, Jeremy Pettus, Luc Teyton

Purpose

The endocrine pancreas is one of the most inaccessible organs of the human body. In a genetically susceptible population, autoimmune attack of beta cells leads to type 1 diabetes and a lifelong need for exogenous insulin replacement. Monitoring disease progression is limited to the measurement of circulating anti-islet antibodies which despite a recognized diagnostic value, remain poorly predictive at the individual level in a fundamentally CD4 T cell dependent disease. Sampling and evaluating the activation state of antigen specific, CD4 T cells in peripheral blood could change preclinical diagnosis and monitoring of therapeutic interventions.

Methods

Here, we coupled insulin peptide-Major Histocompatibility Complex tetramers with single cell techniques to profile circulating, anti-insulin CD4 T cells in mice and humans

Summary of Results

While percentages of circulating, insulin-specific CD4 T cells were poorly informative, the state of activation of anti-insulin T cells measured by RNA and protein profiling was diagnostic of normalcy versus disease progression, independently of anti-islet antibodies status. CD4 T cell signatures were so distinct that they could be used to predict disease status and progression in at-risk individuals with high accuracy.

Conclusions

These results support the concept that antigen specific CD4 T cells can be used to monitor autoimmunity in real time. This advance should fundamentally change our approach to the diagnosis and therapeutic interventions in the pre-clinical phase of anti-islet autoimmunity.

Research Category

Novel Biomarkers