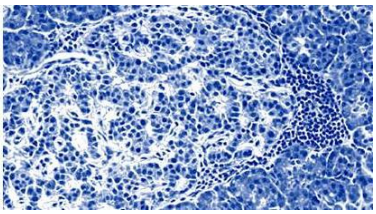
nPOD
Network for Pancreatic Organ Donors with Diabetes



Early Alpha Cell Dysfunction During the Development of Type 1 Diabetes

AUTHORS

Nicolai M Doliba[1,2], Maria L Golson[1,3], Andrea V Rozo[1,5], Long Gao [1,3], Daniel Traum[1,3], Chengyang Liu[1,4], the HPAP consortium, Ali Naji[1,4], Franz M Matschinsky[1, 2], Klaus H Kaestner[1,3], Doris A Stoffers[1,5]

[1]Institute for Diabetes, Obesity and Metabolism; [2]Department of Biophysics and Biochemistry; [3]Department of Genetics; [4]Department of Surgery; [5]Department of Medicine, Perelman School of Medicine

PURPOSE

The presence of islet autoantibodies (AAB) is currently the best biomarker for the future development of type 1 diabetes (T1D), with T1D developing in just 15% of single AAB+ individuals, but a much higher rate in individuals who progress to positivity for two or more AAB. Thus, the single AAB positive state may represent a key window for intervention to prevent progression to T1D. Here we functionally and metabolically phenotype human islets from deceased non-diabetic, single AAB+ and T1D donors to identify early defects in AAB+ donor islets that could serve as biomarkers for disease development and potential nodes for therapeutic intervention.

METHODS

Human islets were received from the accredited Human Islet Resource Center at the University of Pennsylvania. We characterized the physiology and metabolic state of 20 human islet preparations isolated from 7 control-, 9 AAB+- and 4 T1D-donors. A perifusion protocol was designed to simultaneously assess insulin and glucagon secretion. After 2-3 days of culture, islets are pre-perifused with substrate-free medium. Next, a physiological amino acid mixture (AAM) is added to stimulate glucagon secretion. Then, low and high glucose (3 and 16.7 mM) are added to stimulate insulin secretion and to inhibit glucagon secretion. IBMX is then added to maximally increase cAMP and stimulate secretion of both hormones. Finally, a brief washout period with substrate-free medium removes all stimulants, and 30mM KCl is added to depolarize the islet cells and quantify the readily releasable pool of secretory granules. Simultaneous oxygen consumption rate (OCR) and hormone release were measured by a perfusion system in conjunction with a phosphorescence quenching apparatus that allows precise measurement of VO_2 difference between the inflow and outflow. Intracellular Ca^{2+} was imaged after Fura2 loading. Bulk RNASeq was performed on sorted alpha and beta cell populations.

SUMMARY OF RESULTS

We find that while T1D islets show a fully preserved insulin secretory response in the rare remaining beta cells to low and high glucose and to cAMP elevation, alpha cell glucagon secretion was significantly reduced and the ability of glucose to inhibit glucagon secretion was lost. Insulin secretion profiles were also similar between AAB+ and control cases, whereas islets from single AAB+ donors already demonstrated aisgnificant defect in glucose suppression of glucagon secretion and a pronounced augmentation of IBMX-stimulated glucagon but not insulin secretion. AAM, low and high glucose stimulated oxygen consumption in islets from control and AAB+ donors to a similar extent; glucose was unable to stimulate oxygen consumption in T1DM islets. Notably, the oxygen consumption baseline was significantly higher in T1DM islets, and AAB+ islets had an elevated baseline intermediate between control and T1D islets. All groups of islets responded to the mitochondrial uncoupler FCCP, indicating good coupling between respiration and oxidative phosphorylation. Whole islet Ca^{2+} signaling was similar in control and AAB+ islets; however, the increase in intracellular Ca^{2+} due to low and high glucose was diminished in T1D islets. In keeping with the selective functional defect in AAB+ alpha cells, RNASeq analysis revealed ~100 differentially regulated genes in AAB+ alpha cells, but only 6 differentially regulated genes in AAB+ beta cells.

CONCLUSIONS

Taken together, results with islets isolated from non-diabetic control, AAB+ and T1D affected donors resulted in the following fundamental observations: (1) the remaining beta cells in islets from T1D donors have normal glucose-responsive insulin secretion, (2) islets from T1D donors have increased mitochondrial respiration and abnormal calcium signaling that may reflect their alpha cell predominant composition, (3) islets from AAB+ donors with normal glucose-stimulated insulin secretion already have defective glucose suppression of glucagon secretion, as well as an elevated mitochondrial respiration intermediate between normal and T1D. Thus, we find that alpha cell dysfunction precedes the beta cell insulin secretory deficit during the progression of type 1 diabetes, thereby offering a new biomarker for T1D and new targets for potential therapeutic intervention.