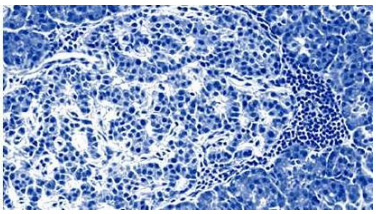
nPOD
Network for Pancreatic Organ Donors with Diabetes



MHC class II expression in human pancreatic tissue sections

AUTHORS

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PURPOSE

Type 1 diabetes (T1D) is an autoimmune disease in which insulin-producing ß cells are damaged by the immune system. However, the immunological mechanisms that lead to T1D have remained incompletely understood. It's been suggested that MHC class II can play a role in T1D pathogenesis, i.e. certain mutations in genes encoding for MHC II molecules are correlated to different risks of developing T1D, and MHC II can be upregulated in ß cells exposed to the proinflammatory cytokine cocktail. But its specific role in T1D is still undefined. The aim of this project is to characterize and quantify the expression of MHC class II in islets of human pancreatic tissue sections, obtained from the network of pancreatic organ donors (nPOD).

METHODS

Antibodies for HLA class II (DP, DQ, DR), HLA class I (A, B, C), CD68 and CD31 were optimized in Formalin Fixed Paraffin Embedded (FFPE) sections of human tonsils. Different multicolor immunofluorescent imaging panels were optimized in FFPE sections of tonsils and pancreas. Human pancreas sections from one non-diabetic (#6373) and two T1D (#6212, #6209) donors were stained with Insulin, MHC II and MHC I. The images were acquired with a Zeiss AxioScan Z1 slide scanner and an LSM780 Confocal microscopy system for higher resolution.

SUMMARY OF RESULTS

We have successfully optimized a multicolor immunofluorescence imaging strategy to precisely locate and quantify the expression of HLA class II in pancreas sections. In T1D case #6209, we observed a high MHC II expression in the insulin-containing islets (ICI) that also exhibited MHC I hyperexpression. The high expression of MHC II was mainly located surrounding the islets. In another T1D case, #6212, we couldn't identify any ICI, and neither MHC II nor MHC I hyperexpression. In the non-diabetic donor, we found very few cells expressing MHC II, and these cells were evenly distributed in the exocrine and the endocrine tissue. In all of the cases, we didn't identify any colocalization between insulin and MHC II. However, the colocalization of MHC II and MHC I was higher in the T1D case #6209 compared with the non-diabetic case (52.3% vs 8.8%).

CONCLUSIONS
HLA class II is highly expressed in most of the insulin containing islets of T1D patient #6209, but, so far, ß cells do not seem to be their main source. For that reason, CD68 and CD31 were optimized in pancreatic sections in order to further test which cells are producing MHC II. At this moment, we are about to complete the optimization and decide on a strategy for the final multicolor immunofluorescence panel. As soon as the last panel is completely optimized, we will stain 5 non-diabetic, 5 pre-diabetic, and 5 T1D cases.