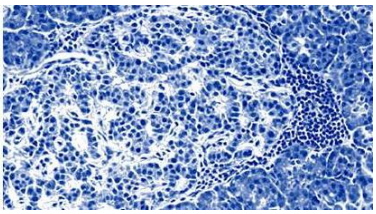
nPOD
Network for Pancreatic Organ Donors with Diabetes



Presence of anti-viral immune response markers in the pancreas and its association with type 1 diabetes

AUTHORS

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PURPOSE

Previous investigations have provided evidence for an association of type 1 diabetes (T1D) with enterovirus (EV) infections. EVs can infect beta cells and could induce chronic inflammation accompanied by activation of cellular processes, which may compromise beta cell function and survival. Our objective was to investigate the presence or absence of markers associated with viral infections (EV capsid protein VP1 and double stranded RNA (dsRNA)), anti-viral immune response proteins (HLA-I, MxA and PKR) and immune infiltration in the islets of non-diabetic, autoantibody positive (AAb+) and T1D organ donors.

METHODS

Pancreatic sections from 10 controls, 6 T1D, and 4 AAb+ (2 single and 2 double) donors were analyzed. Frozen sections were stained for insulin, glucagon, HLA-I, MxA, PKR, dsRNA and CD3 by immunofluorescence staining at the Institute of Diabetes Research (Helmholtz Center) in Munich, Germany. In addition, formalin-fixed paraffin-embedded sections were stained for VP1 and HLA-I at the University of Exeter Medical School (Exeter, UK). The number of insulin and glucagon positive islets as well as the presence of HLA-I, MxA, PKR and dsRNA were analyzed manually. All markers were combined and each islet was classified based on the presence of 1, 2, 3 or 4 markers. Islet infiltration and protein VP1 were analyzed using the software QuPath (University of Edinburgh, Division of Pathology), an open source software for whole slide image analysis.

SUMMARY OF RESULTS

The majority of the islets in non-diabetic donors had normal HLA-I expression while a small percentage had a slight elevation. AAb+ cases behaved similarly but one double AAb+ donor presented HLA-I hyperexpression in a few islets. All T1D donors presented islets with HLA-I hyperexpression (5 to 25 % of the islets). Interestingly, HLA-I expression was also high in the acinar tissue. In T1D donors, VP1+ cells (weak and strong) could be detected in more than

20% of the islets while only 2 of the non-diabetic donors showed weak VP1 positivity. In most of the T1D cases, islets that were positive for MxA and PKR could be detected in a small percentage of islets. Moreover, the combination analysis of HLA-I, MxA, PKR and dsRNA revealed that a significantly higher percentage of islets in T1D donors presented 1 or more of these markers in the same islet. Islet T cell density was also high in half of the T1D donors and all of them had a few islets with more than ≥ 6 CD3+ cells (defined as insulitis). A detailed analysis revealed that 82% of the islets with high infiltration in T1D and 86% in AAb+ donors were positive for one or more markers compared to 25 % of the highly infiltrated islets in non-diabetic donors. In addition, multiple markers were detected more frequently in insulin containing islets (ICIs).

CONCLUSIONS

Previous studies have shown that a 'viral signature' exists in type 1 diabetes and involves interferon responses that could be sustained during prolonged periods of time. These include the up-regulation of markers such as PKR, MxA and HLA-I, and the potential release of chemokines able to attract immune cells to the islets leading to insulitis. In this scenario, the hyperexpression of HLA-I molecules could promote antigen presentation to autoreactive T cells, favoring beta cell recognition and, ultimately, destruction. Our data suggest that the detection of markers associated to increased ER stress, viral infection and anti-viral responses in pancreatic islets is associated with T1D. This, together with the detection of the enterovirus capside protein VP1 could indicate the presence of a low level, persistent, viral infection and/or a chronic anti-viral immune and cellular response. In addition, our analysis suggests that this signature appears early in the disease process and could be detected in AAb+ donors. Future work will also investigate the presence of enterovirus specific T cell responses at different stages of the disease process.