Complement Activation in Type 1 Diabetes: Analysis of Pancreatic Tissue from nPOD Cases

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Purpose: Decades ago, antibody-mediated complement activation was considered to have a pathogenic role in type 1 diabetes (T1D). Despite this, interest in the potential pathogenic role for autoantibodies and B lymphocytes in T1D declined. However, recent studies have brought the focus back to a role for humoral immune mechanisms (eg, gene associations with several complement proteins, therapeutic interventions with B lymphocyte-directed agents, etc). Therefore, we investigated whether evidence of complement activation could be found in pancreatic tissue from organ donors with established T1D and/or donors without diabetes positive for one or more islet autoantibodies (mIAA, IA-2, GAD65, ZnT8), thought to represent the early stage of the disease.

Methods: Immunohistochemical (IHC) techniques were used to measure density of the complement activation product C4d (mouse α-human C4d IgG1, clone 10-11) in pancreata from the different donor groups [no diabetes (n=11), T1D (n=11), no diabetes autoantibody-positive (n=5)]. IHC staining was ranked by a pathologist blinded to the donor groups and C4d density was quantified by analyzing whole-section images from digitally scanned slides using ImageScope software.

Summary of Results: Regardless of donor group or staining density, C4d immunoreactivity was primarily restricted to blood vessels. C4d density was significantly higher, as assessed both by pathologist ranking (T1D: 21.3±1.4, no diabetes: 8.5±1.2; mean±SE, $p<0.001$) and computer-aided image analysis (T1D: 25.2±4.1%, no diabetes: 2.1±0.5%; mean±SE, $p<0.001$), on pancreatic sections from donors with T1D compared to donors without diabetes. No significant differences were found between autoantibody-positive (rank: 10.2±3.7, density: 2.7±1.3%) and autoantibody-negative nondiabetic donors.

Conclusions: Our results suggest that complement activation is occurring via the classical (antibody-mediated) pathway within pancreatic tissue from long-standing T1D donors, a finding that may be related to the persisting pro-inflammatory environment in T1D pancreata, vascular effects of long-term hyperglycemia, or donor group differences in acute responses to stress-hyperglycemia just prior to death. Future analyses will focus on colocalizing approaches to determine whether C4d-positive blood vessels are associated with islets in specific donors and/or donor groups, C4d immunostaining on additional nPOD cases with type 2 diabetes to serve as hyperglycemia controls, and measures of average blood glucose (fructosamine).