
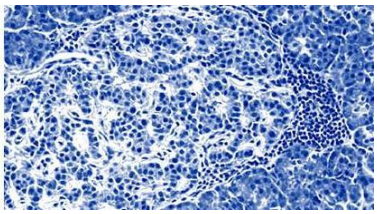

Characterization of a Hybrid Insulin Peptide (HIP) as an Autoantigen in Human Type 1 Diabetes

AUTHORS

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PURPOSE

Relatively little is known about the primary peptide epitopes targeted by the autoimmune response during the development of type 1 diabetes (T1D) in humans. Using mass spectrometry, we have shown that in both mouse and human islets insulin fragments become covalently linked via a peptide bond to other beta cell peptides, leading to the generation of hybrid insulin peptides (HIPs). We established that HIP-reactive CD4 T cells can trigger disease in NOD mice, indicating that HIPs are major autoantigens in this animal model . Furthermore, we determined that HIP-reactive CD4 T cells are present in the peripheral blood of recent onset T1D patients and in the residual islets of organ donors with T1D. We sought here to further characterize a specific HIP as an autoantigen in human T1D.

METHODS

Mass spectrometric analysis of islets from human donors was used to identify endogenous HIPs. PBMCs from T1D patients and controls were assessed by ELISpot analysis for reactivity to HIPs, and a HIP-reactive CD4 T cell clone was isolated and used for epitope mapping.

SUMMARY OF RESULTS

A novel HIP was identified in human islets by mass spectrometry. PBMCs from a subset of T1D patients responded to this peptide. A CD4 T cell clone isolated from one of these patients responded to a synthetic version of the HIP at low nanomolar concentration but not to the non-hybrid peptide constituents. Screening of a panel of truncated peptides revealed that the core epitope for this HIP-reactive clone centered around the hybrid peptide junction.

CONCLUSIONS
Through a combination of immunological and mass spectrometry-based analyses, we have identified a HIP antigen that may play an important role in the etiopathogenesis of human T1D. This work can act as a springboard for future efforts to elucidate the role of HIPs as antigens in T1D and identify biomarkers and therapeutic targets in this disease.