Monoclonal Expansions of TCR in a Diabetic Pancreas

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Purpose: T1D is an autoimmune disease characterized by the selective destruction of insulin-producing cells located within the Langerhans islets. Pathology is associated with CD4+ and CD8+ T cells infiltration around the pancreatic islets. The study of the TCR repertoire is an indirect measure of the T cell diversity that is taking part of the effector and regulatory immune response inside the islets. Several investigators have described the TRBV family repertoire of the NOD mouse intrapancreatic infiltrates from but not in humans, where the limited availability of affected-pancreatic tissue has been a handicap to performe such studies. We had the opportunity to study the TRBV repertoire of intrapancreatic T cells of a diabetic pancreas at onset (case 1) that was characterized by Somoza et al (J. Immunol 1994; 153: 1360) (Codina-Busqueta et al. J. Immunology 2011; 186: 3787).

Methods: TCR analysis was performed by multiplex RT-PCR analysis. PCR products were directly analyzed by spectratyping. Some of the products were further cloned and sequenced.

Summary of Results: Five monoclonal expansions were identified and their CDR3 sequenced: Vb1 (CASSVSTTDTQYF), Vb7 (CASSQVAGAGTGELFF), Vb11 (CASSDPGTQETQYF), Vb17 (CATSPLGMNNEQFF) and Vb22 (CASSEAQQGYSGELFF). All these clones were also expanded in the total digested tissue sample except for the Vb11 clone that was only found at low frequency, not showing a pattern of monoclonal expansion. When further analyzing the Vb11 TRBV family, using total digest, spleen and PBMCs from the same patient, we found that the distribution of Vb11 peaks did not show a normal pattern, because only a few dominant peaks with similar area were visible, corresponding to CDR3 sizes of 10, 11, 12, 14 and 16aa, respectively. Of 34 sequenced clones, only six different sequences were identified, all at a frequency range of 9-26%, without any evidence of one dominant sequence. This was not the case for any other TRBV family, where a normal distribution of CDR3 sizes was always found, with several sequences for each CDR3 size. All Vb11 peaks present in the pancreas sample but not detectable in the islets, were also detected in the spleen of the same patient, but the monoclonal 12aa CDR3 clone identified in the islets has so far not been sequenced from any of the spleen samples analyzed.
The T cells isolated from the total digest were also expanded and oligoclonal lines were generated. A large number of Vb11+ positive cell lines could be isolated, many of which shared one of the sequences found in the pancreas, but not the expanded clone from the islets. This sequence was not found in the patient's spleen.

Conclusions: These data suggest that in this particular case of T1D, the Vb11 family of TRBV can be relevant for the disease, showing an unusual pattern of CDR3 size distribution, with several dominant expansions, some of which appear to be directly involved in islet recognition. The mechanisms involved and the functional relevance of these cells are under study.