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Islet-associated T-cell receptor-β CDR sequence repertoire in prediabetic NOD mice reveals antigen-driven T-cell expansion and shared usage of VβJβ TCR chains

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ABSTRACT

Autoimmune destruction of pancreatic islets in the nonobese diabetic (NOD) mouse is driven by T cells recognizing various autoantigens mostly in insulin-producing beta-cells. To investigate if T-cell accumulation in islets during early insulitis is clonally predetermined, we compared the complementarity determining regions (CDR3) of T-cell receptor (TCR)β-chains present in islet-infiltrating T cells in young prediabetic NOD mice. High-throughput sequencing of TCRβ-chain DNA extracted from islets of 7-wk old NOD mice revealed a biased TCRβ-chain repertoire in all mice, as a restricted number of clones (17–36 clones) was highly overrepresented and made over 20% of total islet repertoire in each mouse. Among these clones, various Vβ and Jβ families were present but certain VβJβ combinations such as TRBV19-0-TRBJ2-7 and TRBV13-3-TRBJ2-5 were highly shared between individual mice. On TCRβ-chain CDR sequence level, many islet clones (72–146) were shared between at least two individual mice. None of them was among expanded clones in both, suggesting considerable stochasticity in the interactions between TCR and peptide-MHC, even with a limited range of autoantigens. A comparison of islet-CDR3-sequences with CRD-sequences from other tissues revealed clonal overlap with pancreatic lymph node and gut, but these repertoires did not overlap together. Our results suggest that antigen-specific T cells are expanded in pancreatic lymph node and islets, but different specificities expand in individual mice. Some islet-infiltrating T-cell specificities may have a distinct origin shared with gut-infiltrating T cells.

1. Introduction

Insulin-dependent (type 1) diabetes (T1D) is an autoimmune disease which results from gradual destruction of insulin-producing β-cells in pancreatic islets. This is associated with a prodromal period characterized by production of autoantibodies (e.g. anti-insulin, anti-GAD65 and anti-IA2 antibodies) and accumulation of mononuclear leukocytes in pancreatic islets, i.e. insulitis. CD8+ and CD4+ (cytotoxic and helper) T cells are the most prevailing cells infiltrating in islets at T1D onset, and autoantigen-specific T cells are present in circulating blood of individuals who progress to T1D. This supports the notion that islet destruction is due to an autoimmune process driven by autoantigen-specific T cells.

The nonobese diabetic (NOD) mouse is a widely studied model of spontaneous autoimmune diabetes. T cells from (pre)diabetic NOD mice efficiently transfer diabetes to healthy recipients, and transfer of monospecific T cells recognizing only one given autoantigen induce insulitis and diabetes in the recipient. CD4+ and/or CD8+ T cells specific for insulin (Wong et al., 1999), IGRP (islet-specific glucose-6-phosphatase catalytic subunit-related protein (Tsai et al., 2008)) and the recently characterized autoantigen of BDC2.5 cells, chromogranin A (Stadinski et al., 2010; Crawford et al., 2011), are all present in pancreatic islets beginning from early insulitis and they all induce insulitis or diabetes to varying degrees upon transfer. Insulitis is believed to evolve by intra- and intermolecular epitope spreading (Kaufman, 2003; Luo et al., 2010) resulting in accumulation of T cells with a growing number of different TCR specificities in islets.

To characterize T cells and their antigen specificities in early insulitis, many studies have investigated the expression of the variable regions of TCRβ-chains in islet-inflitrating T cells in prediabetic NOD mice. TCR Vβ, Dβ and Jβ usage of GAD-specific T cells in islets

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of NOD mice was found to be skewed, and T cells infiltrating grafted islets were found to express predominantly a restricted set of TCR Vβ chains (Diz et al., 2012). Quinn et al. reported that T cells specific for an immunodominant peptide of GAD65 preferentially utilize the Vβ4, Dβ2.1 and Jβ2.7 gene segments, and that T cells expressing these are present in early islet infiltrates. Insulin-specific T cells appear to rise from distinct TCRα-chain recombinations (Nakayama et al., 2012), and CD8T cells specific for insulin (Wong et al., 1999) and IGRP (Wong et al., 2006) show restricted usage of TCR α and TCR β chain variable regions. The associated TRBV and TRBJ genes to bind insulin and IGRP have been identified earlier from transgenic mouse models and are Vβ6S1 (TRBV19) and Jβ2.3 (TRBJ2-3) for insulin (Wong et al., 2009) and Vβ8.1 (TRBV13-3), Vβ10 (TRBV4), Jβ2.4 (TRBJ2-4) and Jβ2.7 (TRBJ2.7) for IGRP (Wong et al., 2006). Spectratyping analysis of pancreatic lymph node T cells in prediabetic and diabetic NOD mice revealed perturbations in CDR3 length in defined TCR Vβ families [Vβ5.1 (TRBV12-2), Vβ9 (TRBV10), Vβ10 (TRBV4), Vβ15 (TRBV20)] (Marrero et al., 2012) supporting the notion, that among these families, T cells with given antigen-specificities have expanded.

One issue of particular importance in the pathogenesis of T1D is whether there is a common "triggering" autoantigen in the disease to which islet-destructive immune response initially arises. NOD mice genetically modified not to express native insulin peptides are protected from diabetes and insulitis (Nakayama et al., 2005). In human T1D, several in vitro T-cell clones expanded from pancreatic lymph nodes of three long-term diabetic patients were found to be specific to a single insulin peptide (A1-15) and to carry certain Vβ rearrangements, albeit different in different individuals (Kent et al., 2005). These findings suggest that anti-islet autoimmunity is initiated and perhaps also maintained by insulin-specific T cells using restricted TCR elements. While pancreatic lymph node is the prime site for initiation and perpetuation of anti-islet autoimmunity, islet-specific T cells activated in gut-associated lymphoid tissue can also accumulate in islets by virtue of their gut-imprinted homing receptors (Hanninen et al., 2007). T cells infiltrating islets during early insulitis frequently express gut-associated homing receptor α4β7 which also mediates T-cell infiltration in islets (Hanninen et al., 1996a, b; Yang et al., 1997). The notion that dietary and/or microbial antigens in the gut could act as molecular mimics to ignite T cells with reactivity also to a given islet antigen has been the focus of a number of studies (Knip and Simell, 2012; Vaarala, 2012). If such T cells were to home to pancreatic islets as well as back to the gut, comparison of expanded T-cell clonotypes in islets and the gut might reveal shared clonal expansions between these tissues.

Analysis of frequencies of T-cell clonotypes on T-cell population level has become possible with high-throughput sequencing technology. Antigen-specificity of a given T cell is determined by rearrangements of its TCR, and structures most important for antigen recognition are those encoded by the junctional sequences in the V, D, and J genes, as they constitute the CDR3 of the TCR chains. High-throughput sequencing of the CDR3 regions of TCR β-chains can thus give explicit information of T-cell repertoire in terms of frequencies of individual T-cell clones in a given tissue. We utilized this technique in order to investigate T-cell clonotypes in islets of individual NOD mice during early insulitis, and to look for clonotypes shared with T cells in the gut. Our results indicate that islet T-cell repertoire is biased towards highly expanded clones mostly not shared between mice, but sharing two VJ-recombinations, TRBV19-0-TRBJ2-7 and TRBV13-3-TRBJ2-5. Several clones present in islets are also found in pancreatic lymph node, suggesting their origin in the latter. Mostly distinct from those found in pancreatic lymph node, several other clones present in islets are found in gut tissues, suggesting a distinct origin for these islet-clones.

2. Materials and methods

2.1. Mice and sample collection

We used non-obese diabetic NOD/ShiLtJ mice purchased from Jackson laboratories, USA. The mice were bred and maintained in our animal facilities under SPF conditions. To analyze early T-cell repertoire in islets and to compare that with gut and pancreatic lymph node repertoire, we first determined the level of insulitis in 5 and 6-week old female NOD mice. As mice at this age showed only scant insulitis, we chose to use 7-week-old mice to ensure sufficient collection of infiltrated islet-tissue for DNA isolation. Pancreas, pancreatic lymph node (PaLN), ileum, colon, and blood were collected from three individual mice at the age of 7 weeks and snap-frozen in liquid nitrogen immediately after sacrifice.

2.2. Laser capture microdissection

Pancreas, snap frozen in liquid nitrogen at collection, was kept at −70 °C until sectioning. 16 μm cryosections were prepared in 60 section batches; discarding 200 μm in every 200 μm cut. Sections were attached to pretreated PEN membrane glasses (Zeiss, Germany). Sections were fixed onto the glasses with cold methanol for 5 min. A modified H&E staining was used to visualize the pancreatic islets as follows: rinse sections with H_2O, incubate in Mayer's hematoxylin solution (Sigma, USA) 2 min, rinse with H_2O until no coloring of water, dip in alcoholic Eosin Y solution (Sigma, USA), rinse with H_2O until no coloring of water, incubate 2 min 70% EtOH, incubate 2 min in fresh 70% EtOH, air dry before microsectioning. Islets with infiltrating cells were cut with IR laser and catapulted to P.A.L.M. opaque adhesive caps (Zeiss, Germany). Minimum 480 microsections from each pancreas were sectioned and pooled. Immediately after collection the microsections were submerged in a lysis buffer. Islets with no insulitis were not microsectioned and are omitted from the samples.

2.3. gDNA extraction

The genomic DNA (gDNA) was extracted from the microsections with MasterPure DNA purification kit (Epicentre Biotechnologies, USA). Due to extremely low amount of starting material we modified manufacturer's instructions by reducing all buffer volumes to 20% of original volumes and final elution volume down to 12 μl. The amount of enzymes and incubation times were used as instructed by the manufacturer. Extraction of gDNA from the blood was performed with standard silica membrane based extraction system according to manufacturer's instructions (GENEJet Genomic DNA purification kit, Fermentas, USA). Tissue samples were shipped frozen to Adaptive Biotechnologies (Seattle, WA) for processing and TCR sequence analysis.

2.4. TCR analysis

TCRb CDR3 regions were amplified and sequenced by Adaptive Biotechnologies as described earlier (Marrero et al., 2013). Analyses of the sequence data for TCRB CDR3 sequences and TRBV-J recombinations and their comparisons were all performed using ImmunoSEQ-software provided online (Adaptive Biotechnologies Corp.).

2.5. Statistical analysis

All statistical analyses were performed using GraphPad Prism 5 software (La Jolla, USA). Student's *t*-test was used to compare two groups and 1-way Anova with Dunnett's post-hoc test when