A31 MOLECULAR AND CELLULAR EVOLUTION OF FUNCTIONAL TERTIARY LYMPHOID STRUCTURES IN SALIVARY GLANDS OF NOD MICE

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Tertiary lymphoid structures (TLSs) are common features of chronic inflammatory diseases including Sjogren's syndrome (SS). We recently showed that these ectopic structures acquire secondary lymphoid organs properties and are capable of supporting B cell activation and autoantibody production including expression of activation-induced cytidine deaminase (AID) and Ig class switching. Dissecting TLSs dynamics in humans is technically and ethically challenging. Thus, we used the NOD mouse, a spontaneous model of autoimmune sialoadenitis, to characterise the cellular and molecular basis of autoreactive B cell activation and evolution of functional ectopic lymphoid structures (ELS) in the chronically inflamed NOD salivary glands.

Submandibular glands from 110 female NOD mice from 4 to 35 weeks of age were collected. Paired snap-frozen samples were analysed by immunohistochemistry (IHC) for T and B lymphocytes (CD3/CD20) in order to evaluate cell infiltration and the degree of B/T cell segregation. ELS were detected by staining for FDC-M1 (follicular dendritic cell networks), GL7 (germinal centre B cells) and AID (marker for ELS functionality). Characterisation of B cell subsets within the infiltrates was carried out by immunostaining and by fluorescence activated

cell sorter (FACS) analysis with CD19, CD21, CD23, B220, IgD, IgM, CD1d and CXCR5 antibodies. Quantitative TaqMan real-time PCR was performed to investigate the mRNA expression of ELS-related genes. Sex/age matched Balb/c and C57BL/six mice were used as controls.

NOD infiltrates in glands displayed progressive features of ELS from week 8, with 75% of mice developing B/T cell segregation, FDC networks and GL7+ ectopic germinal centres from week 20. Evolution of TLSs was closely associated with mRNA upregulation of genes regulating ELS organisation and function such as lymphoid chemokines CXCL13/CCL19 and their receptors CXCR5/CCR7, lymphotoxins and B cell survival factors BAFF and APRIL. In agreement with CXCL13/CXCR5 mRNA expression, B cells in infiltrates display strong CXCR5 expression and were mostly characterised by a follicular phenotype (B220+/IgD+/IgMlow/CD23/CD21low) as demonstrated by both IHC and FACS analysis on isolated cells. Finally, functionality of ELS was demonstrated by expression of AID mRNA and protein within FDC networks, which paralleled the detection of circulating SS-related autoantibodies.

This work provided the first in depth characterisation of cellular and molecular mechanisms underlying the evolution of functional TLSs within submandibular infiltrates of NOD mice. Overall, these data support the critical importance of ELS formation in chronic autoimmune inflammation and identified NOD mice as a suitable model to test therapeutic strategies aimed at modulating B cell functionality.

Comments These data strongly support the hypothesis that B cells can be activated within TLSs in the target organ and promote in situ autoantibody response.