ANALYSIS OF GRANZYME B EXPRESSION OF PERIPHERAL B CELLS IN SJÖGREN'S SYNDROME

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INTRODUCTION

B cell hyperactivity, altered B cell subset composition and elevated serum levels of soluble interleukin-21 (IL-21) was published in primary Sjögren's syndrome (pSS). The serin protease Granzyme B (GrzmB), furthermore interaction of invariant NKT (iNKT) cells with B cells may also have potential significance in autoimmune processes. B cell GrzmB secretion is inducible by IL-21 and B-cell receptor (BCR) engagement. Our goal was to investigate the expression of GrzmB in peripheral B cells and CD5+ B cell subsets of patients with pSS, the level of B cell IL-21 receptor (IL-21R) expression and the contribution of iNKT cells to IL-21 production in pSS.

PATIENTS AND METHODS

Twenty pSS patients and 12 healthy controls were included. B cell intracellular GrzmB expression was determined after stimulation of peripheral blood mononuclear cells (PBMC) with recombinant IL-21 and anti-BCR antibody for 20 hours. In the last four hours Brefeldin A was added. IL-21R (CD360) expression was determined in unstimulated PBMC. In both cases the cells were surface labelled with CD5-FITC and CD19-PC5, and intracellular staining was performed with anti- GrzmB –PE or CD360-PE.

iNKT cell IL-21 expression was analysed after 6 hour stimulation with PMA/Ionomycin in the presence of Brefeldin A and incubating the cells with 6B11-PE, CD3-PC7 and then IL-21-APC. Coulter Fix and Perm kit was used for intracellular staining. Cells were analysed on FACSCalibur or Coulter FC500 flow cytometer.

RESULTS

The CD5+ B cells, but not all CD19+ B cells of patients with pSS showed an elevated baseline GrzmB expression compared to control samples ($P<0.05$; Mann-Whitney test).

In stimulated samples, the specific GrzmB expression (stimulated - unstimulated) on CD19+ B cells enhanced, but not significantly, and increased significantly on CD5+CD19+ B cells of pSS patients ($P<0.05$; Mann-Whitney test).

The intracytoplasmic IL-21 expression of iNKT cells elevated significantly in pSS patients group ($P<0.05$; Student t-test). There was no difference in the expression level of IL-21R on CD19+B and CD5+CD19+ B cell subsets in pSS patients.

CONCLUSIONS

Endogeneous IL-21 may able to stimulate in vivo the CD5+ B cell GrzmB expression in pSS patients. On the basis of our result the IL-21 may play a role in the pathogenesis of Sjögren's syndrome by increasing GrzmB in B cells and can induce the autoregulation of the CD5+ B cells. Our data suggest that in pSS the IL-21R expression of B cells probably have no effect, but the production of IL-21 by iNKT cells can contribute to the regulation of B cell GrzmB expression.