T cell receptor expression in Sjögren's syndrome

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Abstract

T lymphocytes expressing the γ/δ T cell receptor and B lymphocytes expressing CD5 are known to occur in expanded numbers in the peripheral blood of patients with primary Sjögren's syndrome. The cellular infiltrates for the surface phenotypic markers for α/β and γ/δ T cell receptors, CD4, CD8, CD45, and CD5 were examined in lip biopsy specimens from two patients with primary Sjögren's syndrome, six with secondary Sjögren's syndrome, and seven healthy controls. Most of the Sjögren's lip biopsy cellular infiltrates were T lymphocytes of the CD4 subset expressing the α/β T cell receptor (mean 70%). The low prevalence of γ/δ T cell receptor bearing cells in lip biopsy specimens is maintained in Sjögren's syndrome (mean 1.5%), and thus it seems unlikely that these lymphocytes bearing the γ/δ T cell receptor have a major role in the immunopathology of Sjögren's syndrome. Over 70% of cells within the lesional infiltrate of primary and secondary Sjögren's syndrome expressed the CD5 and CD45 cell surface molecules.

Sjögren's syndrome is an autoimmune disease characterised histologically by a lymphocytic infiltration of lachrymal and salivary glands with concomitant destruction of acinar tissue and proliferation of ducts, leading to oral and ocular dryness. Previous studies showed that most infiltrating cells were activated T lymphocytes expressing class II antigen major histocompatibility complex determinants.¹⁻³ Co-ordinate levels of lymphocytes with fetal phenotypes in peripheral blood have been described in patients with Sjögren's syndrome,⁴ and B lymphocytes expressing CD5 are known to occur in expanded numbers in the peripheral blood of patients with primary Sjögren's syndrome.⁵ To obtain more insight into the role of T cells with fetal phenotypes in the pathology of Sjögren's syndrome we examined in more detail the active involvement of T lymphocytes and studied T cell receptor expression within the cellular infiltrate of lip biopsy specimens.

Materials and methods

Lip biopsy specimens were obtained from two patients with primary and six with secondary Sjögren's syndrome. Of the six patients with secondary Sjögren's syndrome, two had systemic lupus erythematosus, three had rheumatoid arthritis, and one had myositis. Each of the patients met the criteria for the diagnosis of

Sjögren's syndrome. Seven control labial biopsy specimens were obtained with written consent and hospital ethical committee approval from subjects during major dental work or from patients initially suspected of having Sjögren's syndrome but whose symptoms proved transitory and who had no autoantibodies. The biopsy tissues were stained with the relevant mouse monoclonal antibody as described by Hsu *et al.*⁶ Briefly, cryosections (5 μ m) were cut and air dried overnight. These were fixed in cold (4°C) acetone for 10 minutes and air dried before immunostaining. The sections were stained with 50 μ l of a 1 in 10 dilution of a panel of the following primary mouse IgG antihuman monoclonal antibodies; anti-T-cell receptor α , anti-T-cell receptor δ (T-Cell Sciences, Cambridge, MA), anti-CD4, anti-CD8, anti-CD45R, and anti-CD5 (Dakopatts, United Kingdom). As background controls, irrelevant mouse IgG were used instead of the specific antibodies. Sections were incubated overnight at 4°C, treated with 0.5% hydrogen peroxide for 20 minutes, and washed with TRIS buffered saline. Peroxidase conjugated rabbit antimouse polyclonal antibody (Dakopatts) was added to the sections for 30 minutes. After washing the sections with TRIS buffered saline the reaction was developed with diaminobenzidine, counterstained with haematoxylin, and the sections were then mounted. The biopsy sections were assessed 'blind'. The cells were counted with an ocular counting square (20 $\times$ 20) and an oil immersion objective ($\times$ 1000 magnification). Within the same field for each section the infiltration of mononuclear cells was counted for positive staining cells and expressed as a percentage of non-stained cells.

Results

Peroxidase positive, specifically stained cells were brown and thus readily distinguishable from unstained cells under a light microscope (figs 1 and 2). The table shows the results using the six monoclonal antibodies for phenotypic analyses of the cellular infiltrate. Infiltrating lymphocytes were rarely identified in lip biopsy specimens from healthy controls. Focal collections of lymphocytes were present in biopsy specimens from patients with secondary Sjögren's syndrome, whereas the specimens from those with primary disease tended to have more diffusely scattered lymphocytes. Over 70% (mean 76%) of infiltrating T lymphocytes in biopsy specimens from patients with Sjögren's syndrome were positive for the α/β T cell receptor and were of the CD4 phenotype. Only

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Immunohistochemical analysis of lip biopsy specimens in Sjögren's syndrome. Results are given as the mean percentage of total mononuclear infiltrate (range)

Source of lip biopsy specimen	Phenotypic marker expressed					
	α/β TCR*	γ/δ TCR	CD4	CD8	CD5	CD45
Primary Sjögren's syndrome (n=2)	78 (75-80)	2.5 (2-3)	80 (75-86)	25 (20-30)	81 (75-88)	81 (80-88)
Secondary Sjögren's syndrome (n=6)	76 (70-80)	1 (1-2)	68 (60-80)	14 (5-20)	77 (70-80)	77 (70-85)
Controls (n=7)	0	0	0	0	0	0

*TCR=T cell receptor.

Figure 1 Labial biopsy specimen from a patient with Sjögren's syndrome, stained with anti-T cell receptor δ showing scanty cell staining.

Figure 2 Labial biopsy specimen from a patient with Sjögren's syndrome, stained with anti-T cell receptor α showing multiple cell staining.

a minority of T cells (mean 1.5%) expressed the γ/δ T cell receptor. Over 75% of mononuclear cells within the infiltrate of lip biopsy specimens from patients with both primary and secondary Sjögren's syndrome expressed the CD5 and CD45 cell surface molecules.

Discussion

B cell hyperreactivity is a major feature of Sjögren's syndrome, but the triggering events

which lead up to it remain enigmatic.⁷ Given that T cells are known to influence B lymphocytes in general, it seems reasonable to postulate that at least part of the trigger in Sjögren's syndrome may involve T cell subsets. To study this we previously made an immunohistochemical study of labial biopsy specimens from patients with Sjögren's syndrome and showed that the predominant cell in the infiltrates bears the T cell helper/inducer phenotype.¹ The same observation was again evident in this study and has also been confirmed by others.² Most of these cells also express HLA-DR antigens, implying that they are in the activated state. In the last three years it has become apparent that there are at least two types of T cell receptor, designated α/β and γ/δ . The latter are usually expressed early during T cell ontogeny in the fetal thymus.⁸ They have been identified in increased numbers in the peripheral blood and synovial tissues of patients with early rheumatoid arthritis,⁹ however, and also recently in the peripheral blood of patients with Sjögren's syndrome.⁴ This latter observation suggested that the increased expression of lymphocytes with the fetal phenotypes might have an important role in the pathogenesis of Sjögren's syndrome.

In this study, however, we show that T cells expressing the α/β T cell receptor overwhelmingly predominated in the lesional tissue. In fact, less than 3% of cells expressed the γ/δ T cell receptor in the eight patients with Sjögren's syndrome studied. Although we did not study serial lip biopsy specimens and thus might have missed a change in the T cell receptor status, it seems on the present quantitative evidence less likely that the T cells bearing the γ/δ T cell receptor are critical to the induction or maintenance of the lesion in Sjögren's syndrome. As it is becoming evident that only a small percentage of cells within a lesion need to be activated,^{10 11} however, the data presented here do not exclude an active role for T cells bearing the γ/δ T cell receptor. Further in depth ultramorphological studies of these lesional T cells may provide further insight.

Previous reports have indicated an increase in B lymphocytes expressing CD5 and a decrease in T lymphocytes expressing CD5 obtained from the peripheral blood of patients with Sjögren's syndrome.¹² The high percentage of cells expressing CD5 and CD45 cell surface molecules within lesional tissue found in both primary and secondary Sjögren's syndrome in this study needs further definition of the cell types bearing these molecules and their possible role in pathogenesis.

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