Sjögren’s Syndrome in Systemic Lupus Erythematosus and Rheumatoid Arthritis: Immune Effector Cells in Salivary Glands

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Summary. A simultaneously capturing azo dye method for acid α-naphthyl acetate esterase was used to characterize the cellular infiltrate in labial salivary glands in 25 patients with Sjögren’s syndrome (SS). There was no significant difference in the T-pattern lymphocyte percentage in situ between the untreated group with SS and the group treated with 10 mg prednisone/day. There was a significant correlation (P < 0.05) between the T-pattern lymphocyte percentage in situ and the focus-score value. In secondary (2°) SS in cases of systemic lupus erythematosus (SLE) and rheumatoid arthritis, respectively, 55% ± 4% (range 41–69) and 43% ± 7% (range 15–80) of all inflammatory cells in the periductal lymphocyte-rich infiltrates were T-pattern lymphocytes. In other SS patients the corresponding value was 28% ± 7% (range 4–50). The T-pattern lymphocyte percentage in situ was dependent on the disorder associated with SS (P = 0.07). The present results indicate the dominance of T-lymphocytes in situ in 2° SS with SLE and suggest that there are differences in cell-mediated immunity in different clinical subgroups of SS.

Key words: Sjögren’s syndrome – Rheumatoid arthritis – Systemic lupus erythematosus – Salivary glands – Lymphocytes

Introduction

Xerostomia, keratoconjunctivitis sicca and some autoimmune disease are the components of Sjögren’s syndrome (SS) and two of these are sufficient for diagnosis to be made [1]. However, because half of the patients with symptoms suggestive of SS have other disorders, such as local glandular disorders or anxiety depression syndromes, or are on parasympatholytic drugs [2], objective tests are also necessary. The cardinal feature of the SS, lymphocyte and plasma-cell infiltrates in exocrine glands [1, 2] can be disclosed by biopsy. Minor salivary glands are a convenient site for biopsy, which also has differential diagnostic value for diseases such as sarcoidosis [3] or lymphoproliferative disorders [4]. Using this test as a basis for diagnosis, information has already been obtained about the clinical, serological and genetic differences between patients who have sicca syndrome alone (1° SS) and those who have associated rheumatoid arthritis (2° SS in RA), with the result that the clinical syndrome is in the process of being subclassified into smaller entities [5]. Recently, SS has been frequently associated with systemic lupus erythematosus (SLE) [6, 7], but the immunocompetent cells participating in the local pathogenesis in 2° SS with SLE have not been identified. Furthermore, it is not known whether SS can be divided into subentities on the basis of T/B lymphocyte counts in salivary glands. This prompted us to study the immune effector cells in situ in 2° SS with SLE and in SS with other clinical associations.

Materials and Methods

Patients and Biopsies. For a definite diagnosis of SS, at least two of the following were required: (1) keratoconjunctivitis sicca (Schirmer’s test, Rose-Bengal staining or biomicroscopic examination); (2) xerostomia (labial salivary gland biopsy and in 16 patients sialography); (3) one of the connective tissue or autoimmune diseases [1]. In 2° SS the associated clinical disorder was SLE (8) nine patients, aged 57 ± 4 years, range 37–74), rheumatoid arthritis (RA; 9) nine patients, aged 59 ± 3 years, range 40–71) and juvenile RA, mixed connective-tissue disease, chronic aggressive hepatitis or mixed cryoglobulinaemia (one patient each); three patients had 1° SS aged 52 ± 7 years, range 34–76). One patient did not have SS.

Citanest-Octapressin (Astra, Södertälje, Sweden) was used for local anaesthesia. Three to five salivary glands were removed through a 2-cm-long horizontal incision on the inner side of the lower lip, 1 cm from the vermilion border, right of the midline. Biopsy specimens were fixed in Baker’s formol calcium (20 h), rinsed in Holt’s solution (6 h), dehydrated in acetone (10 h), cleared in xylene (22 h), all at +4 °C, before embedding in paraffin at +55 °C (1–2 h); 6-μm sections of paraffin blocks were prepared. Acid α-naphthyl acetate esterase (ANAE) activity was unaffected by this kind of processing [10].

Histological Methos. ANAE was demonstrated by a simultaneously capturing azo dye method. Briefly, tissue specimens deparaffinized first in xylene and then in acetone (10 min each at
Fig. 1. Labial gland of a patient suffering from secondary Sjögren’s syndrome (2° SS) associated with systemic lupus erythematosus (SLE). This infiltrate contains more than 50 cells and is therefore called a focus (×400).

+4°C) were incubated in a medium consisting of 40 ml 0.067 mol/l phosphate buffer (pH 5.3), 2.4 ml hexazoized pararosaniline, and 10 mg α-naphthyl acetate (Sigma, St. Louis, Missouri, USA) in 0.4 ml acetone. The mixture was adjusted to pH 5.8 using 1 M NaOH [10]. In addition, every specimen was stained in an incubation medium consisting of 35 ml 0.067 mol/l phosphate buffer (pH 7.6), 2.4 ml hexazoized pararosaniline and 16 mg α-naphthyl acetate in 2 ml ethylene glycol monomethyl ether (Merck, Darmstadt, Federal Republik of Germany). The mixture was adjusted to pH 6.1 using 0.067 mol/l KH₂PO₄ or Na₂HPO₄ [11]. Tissue sections were incubated at room temperature for 2 h. Counterstaining was performed in a 1% aqueous solution of toluidine blue. The sections were dehydrated in methanol, acetone and cleared in xylene, (10 min each) and mounted.

Inflammatory cells were counted using the focus-score method (focus score is the number of foci greater than 50 mononuclear cells/4 mm² of labial salivary gland; Fig. 1). ANAE staining disclosed three different staining patterns: one or more cytoplasmic dots (T-pattern lymphocytes), diffuse cytoplasmic activity (M-pattern) or no activity at all (non-T, non-M pattern). Cells were counted from periductal lymphocyte-rich infiltrates using an ocular counting square (20 squares × 20 squares) and an oil-immersion objective (×1,000 magnification). The ocular counting square was calibrated with a stage micrometer. The person counting the cells did not know the source of the biopsy (RA, SLE or some other disease). Counting of the T-pattern lymphocytes in the same section four times gave a coefficient of variation of 3%.

Statistical Procedures. Standard error of the mean and range were used to express dispersion. The significance of differences between mean values was tested using a t-test and analysis of covariance. Linear regression analysis was used to study the relationship between two variables.

Results

Biopsy specimens of the labial salivary glands displayed a reduction of secretory parenchyma by fibrosis, fat and inflammatory cell infiltrate. Lymphocytes were located in large infiltrates around the salivary ducts, whereas plasma cells were located peripherally to the lymphocyte foci and situated between the glandular acini.

M-pattern cells formed 4% ± 1% (range 0–15) of all inflammatory cells in the periductal lymphocyte-rich foci. The correlation between T-pattern lymphocytes and non-T, non-M-pattern lymphocytes was −0.977.

In 2° SS in SLE or RA 55% ± 4% (range 41–69) and 43% ± 7% (range 15–80), respectively, of all inflammatory cells in the periductal lymphocyte-rich infiltrates were T-pattern lymphocytes (Figs. 2 and 3). In other SS patients the corresponding value was 28% ± 7% (range 4–50). One patient with auto-immune thyroiditis had sicca symptoms,