Expansion of identical B-cell clones in the bilateral parotid glands and their circulation in the peripheral blood in a patient with Sjögren’s syndrome

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Abstract We encountered a patient with Sjögren’s syndrome (SS) associated with bilateral parotid salivary gland swelling. Histological analysis of biopsy specimens from both parotid glands showed myoepithelial islands and infiltration of small- and intermediate-sized lymphocytes but no cytological atypia. Using reverse transcriptase-polymerase chain reaction and subsequent single-strand conformational polymorphism analysis, monoclonal B-cell expansion was detected in samples from both right and left parotid glands, bone marrow, and peripheral blood (PB). Our case suggests that the circulating clonal lymphocytes represent clones that can repopulate tissue sites and may contribute to B-cell lymphomagenesis. Detection of monoclonal B cells in PB is therefore considered to be important in monitoring the disease course of SS.

Key words B lymphocytes · Single-strand conformational polymorphism · Sjögren’s syndrome · Immunoglobulin variable region

Introduction

Sjögren’s syndrome (SS) is an autoimmune disease characterized by lymphocytic infiltration of lacrimal and salivary glands leading to xerophthalmia and xerostomia. This disorder may exist as a primary condition or may be associated with other autoimmune diseases. In addition to the clinical and laboratory evidence of systemic autoimmune disease, SS patients also have a high frequency of paraproteins in their sera and urine and a high frequency of non-Hodgkin’s B-cell lymphoma. The continual antigenic stimulation of B cells within the salivary gland has been proposed to induce clonal expansion, which may subsequently undergo karyotypic alteration to become a non-Hodgkin’s lymphoma. The study of rearranged immunoglobulin (Ig) gene DNA by a Southern blot analysis demonstrated clonal expansion in the salivary gland tissue of SS patients with pseudolymphoma before the occurrence of malignancy. B-cell clonal expansion has also been observed in the early infiltrates of the minor labial salivary glands of SS patients.

We describe here an SS patient with bilateral parotid salivary gland swelling and a monoclonal M protein. We performed a B-cell clonality analysis of multiple tissues from the patient and also discuss the possible role of clonal B cells in peripheral blood (PB) in B-cell lymphomagenesis.

Case report

In September 1995, a 70-year-old Japanese woman noticed xerostomia. In October 1996, she developed bilateral parotid salivary gland swelling. She was admitted to our hospital on October 23, 1996. Laboratory studies disclosed the following values: hemoglobin concentration, 11.7 g/dl; white blood cell count, 5130/mm3; platelet count, 203104/mm3; erythrocyte sedimentation rate, 3 mm/h; C-reactive protein, negative; rheumatoid factor, negative; CH50, 36.1 U/ml (normal, 30.0–40.0); C3, 105 mg/dl (normal, 85–135); C4, 57 mg/dl (normal, 33–65); IgG, 1377 mg/dl (normal, 1125–1738); IgA, 180 mg/dl (normal, 179–349); and IgM, 787 mg/dl (normal, 26–252). Both liver and renal function tests were normal. The skeletal muscle enzyme levels were normal. Serum antinuclear antibody by indirect immunofluorescence was positive at 1:5120 with speckled nuclear staining. Anti-dsDNA antibody, anti-Sm antibody, anti-
and PB in the detected in samples from right and left parotid glands, BM, clones from different samples as well as to detect clonal B-cell populations were detected as discrete bands. This smear in the RT-PCR-SSCP analysis, whereas clonal SSCP bands showed a similar mobility, thus suggesting the identity of B-cell populations. To confirm the clonal identity, we performed a sequence analysis of the dominant bands in all samples. The CDR3 sequences were identical among different samples in each \( \mu \) and \( \lambda \) RT-PCR-SSCP analysis (Fig. 1C). To further confirm that the dominant band in the RT-PCR-SSCP analysis was derived from clonal B cells, a conventional sequence analysis was performed. In a \( \mu \) sequence analysis, we used a mixture of family-specific consensus leader primers and a \( \mu \) constant region primer to amplify cDNA from the left parotid gland. In a \( \lambda \) sequence analysis, the same cDNA was amplified using a mixture of \( V_{\mu}I, II, \) and III family-specific consensus framework (FR)1 primers and a \( \lambda \) constant region primer. The resulting PCR products were ligated in the pBluescript SK+ (Stratagene, La Jolla, CA, USA), and \( VH \) and \( V_{\lambda} \) sequences were determined. Each amplified DNA was found to give a major \( V \) gene sequence that was present in 9 of the 10 clones in a \( \mu \) sequence analysis and 10 of the 10 clones in a \( \lambda \) sequence analysis (data not shown). The CDR3 sequences of the major \( V \) gene sequences of the \( \mu \) and \( \lambda \) analyses were identical to those obtained from the dominant bands from the \( \mu \) and \( \lambda \) RT-PCR-SSCP analyses, respectively.

Clonal signals were detected in the \( \mu \) and \( \lambda \) isotypes. Therefore, the monoclonal B cells detected in the RT-PCR-SSCP analysis appeared to correspond to the cells producing IgM (\( \mu \)) M protein. Serial dilution experiments, in which the MNCs from B-chronic lymphocytic leukemia were serially diluted into the MNCs from a healthy subject, revealed that the RT-PCR-SSCP analysis could detect 1 malignant cell in 100 MNCs. The sensitivity of the RT-PCR-SSCP analysis is similar to that of a Southern blot analysis. The reason that we could detect clonal populations in BM by a RT-PCR-SSCP analysis but not by a Southern blot analysis was not clear. If the clonal B cells we detected produced and secreted M protein, they may contain a larger amount of Ig RNA than immature, mature, or memory B cells. In such cases, the RT-PCR-SSCP analysis may become more sensitive.

**Discussion**

De Vita et al. performed a B-cell clonality analysis of synchronous and metachronous samples from six patients with SS. In one of their patients, the expansion of the same dominant clone was detected in synchronous tissue (lymph node, parotid, and gastric fundus biopsy specimens). This patient developed high-grade gastric lymphoma with diffuse large B cells after a 16-month follow-up. Using a novel clonal analysis, we demonstrated the expanded B-cell clones from the right and left parotid glands from our patient with SS to be identical. Although no definite evidence of associated lymphoma was seen in the parotid glands of our patient, the detection of the identical monoclonal B-cell population in bilateral parotid glands suggests that this patient should be followed carefully. The monoclonal B-cell population was circulating in morphologically normal PB and was also present in the BM. It is known that clonal cells