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CASE REPORT

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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.[1] B-cell clonal expansion has also been observed in the early infiltrates of the minor labial salivary glands of SS patients.[2]

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.

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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/mm^3; platelet count, 20×10^4/mm^3; 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-

ribonucleoprotein antibody, and circulating immune complex were negative. Anti-ssDNA antibody was positive at 29.7 AU/ml (normal, <25). Precipitin antibody against Ro (SS-A) was positive, but anti-La (SS-B) was negative. Serum immunoelectrophoresis demonstrated an IgM (μλ) M-component.

Bone marrow (BM) aspiration showed no abnormal findings. Schirmer's test and Saxon's test were positive. Sialography demonstrated punctate sialectasis. Gallium scintiphotography revealed an abnormal accumulation of bilateral parotid glands. A computed tomographic scan and an ultrasonographic examination of the abdomen showed no significant abnormalities. A histological analysis of the biopsy specimens from both parotid glands showed myoepithelial islands and an infiltration of small- and intermediate-sized lymphocytes lacking cytological atypia. Immunohistochemical analysis of the left parotid gland revealed a slight increase of μλ-positive lymphocytes. The histological findings were compatible to SS, and no definite evidence of malignant lymphoma was observed.

Prednisolone, 20 mg/day, was started on December 16, 1996. The bilateral parotid salivary gland swelling disappeared after a few weeks. After the patient was discharged, her prednisolone dosage was gradually tapered. In November 1997, when the prednisolone daily dose was 5 mg, bilateral parotid gland swelling recurred. Even though prednisolone 10 mg/day was administered for 6 months, salivary gland size increased. Therefore, cyclophosphamide therapy (50 mg every other day) was added, and thereafter the swelling gradually disappeared. At present, no evidence of associated lymphoma has been observed, based on physical examinations and laboratory studies including chest X-ray films and an ultrasound study of the abdomen.

We prepared genomic DNA and RNA from biopsy specimens from the right and left parotid glands and from mononuclear cells (MNCs) from PB and BM. To examine the Ig gene rearrangement, a Southern blot analysis was performed using a 5.6-kb JH or a 361-bp Cλ2 DNA fragment as a probe. IgH gene rearrangement was observed in the samples from the right and left parotid glands (Fig. 1A). However, regarding the BM sample, neither IgH gene rearrangement (Fig. 1A) nor λ IgL chain rearrangement was detected (data not shown). We also performed a reverse transcriptase-polymerase chain reaction (RT-PCR)-single-strand conformation polymorphism (SSCP) analysis as described.[3,4] PB MNCs from healthy individuals revealed a smear in the RT-PCR-SSCP analysis, whereas clonal B-cell populations were detected as discrete bands. This method allowed us to determine the identity of B-cell clones from different samples as well as to detect clonal B-cell expansion. In addition, using RNA as materials, we could also determine the isotype of the B-cell clone.

In this analysis, monoclonal B-cell expansion was detected in samples from right and left parotid glands, BM, and PB in the μ and λ RT-PCR-SSCP analyses (Fig. 1B). All SSCP bands showed a similar mobility, thus suggesting the expanded B-cell clones in right and left parotid glands as well as the PB and BM to have identical complementarity determining region-3 (CDR3) sequences. 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 μ and λ 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 μ sequence analysis, we used a mixture of family-specific consensus leader primers[5] and a μ constant region primer to amplify cDNA from the left parotid gland. In a λ sequence analysis, the same cDNA was amplified using a mixture of VλI, II, and III family-specific consensus framework (FR)1 primers[6] and a λ constant region primer. The resulting PCR products were ligated in the pBluescript SK^- (Stratagene, La Jolla, CA, USA), and VH and Vλ 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 μ sequence analysis and 10 of the 10 clones in a λ sequence analysis (data not shown). The CDR3 sequences of the major V gene sequences of the μ and λ analyses were identical to those obtained from the dominant bands from the μ and λ RT-PCR-SSCP analyses, respectively.

Clonal signals were detected in the μ and λ isotypes. Therefore, the monoclonal B cells detected in the RT-PCR-SSCP analysis appeared to correspond to the cells producing IgM (μλ) 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.[3] 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.[7] 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

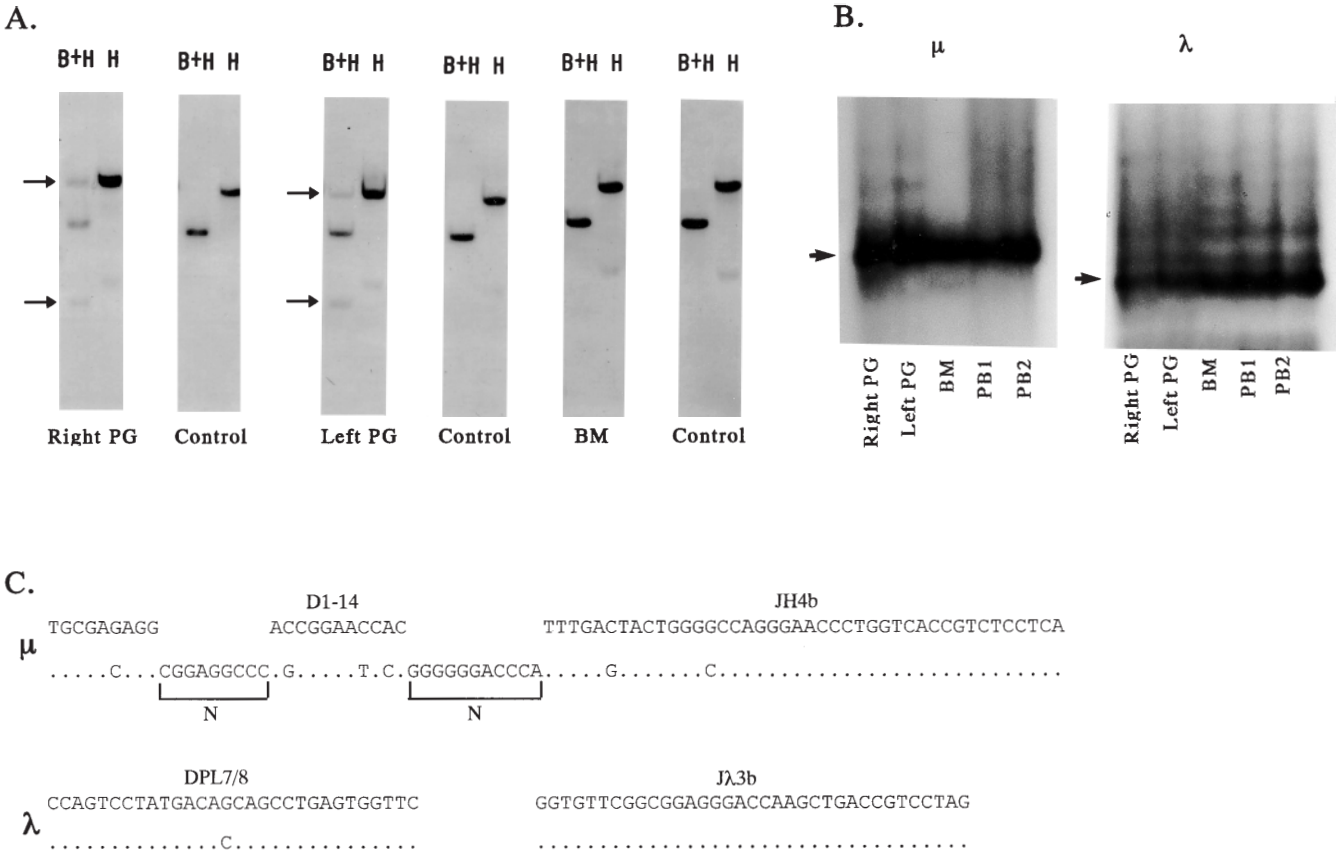

Fig. 1. **A** DNA from the right parotid gland (*PG*), left PG, and bone marrow (*BM*) mononuclear cells (MNCs) were digested with enzymes *Bam*HI and *Hin*dIII or *Hin*dIII alone and reacted with biotin-labeled 5.6-kb JH probe. *Arrows* adjacent to the lanes indicate the positions of rearranged IgH gene DNA. In control experiments, DNA from the peripheral blood (*PB*) MNCs from healthy individuals was analyzed. **B** RT-PCR-SSCP analysis using RNA from right PG, left PG, BM MNCs, and PB MNCs. Samples PB1 and PB2 were obtained immediately after and 3 months after admission, respectively. *Arrows* adjacent to the lanes indicate the positions of a single dominant band, which was common in all samples in both the μ and λ analyses. The primers used for the μ RT-PCR-SSCP analysis were a VH FR3 consensus primer (5′-ACACGGCC/TG/CTGTATTACTG-3′) and a μ constant region primer (5′-GGAGAAAGTGATGGAGTCGGG-3′). The primers used for the λ RT-PCR-SSCP analysis were Vλ FR3 consensus primer (5′-AGGAT/CGAGGCTGAT/CTATTACTG-3′) and λ constant region primer (5′-AGTGTGGCCTTGTTGGCTTG-3′). Internal μ (5′-AATTCTCACAGGAGACGAG-3′) and λ (5′-AGGAGGGT/CGGGAACAGAGTGA-3′) constant region oligonucleotides were used for the probes. **C** Slices containing the dominant bands were excised from the polyacrylamide gel in all samples. The eluted DNA was then reamplified and sequence analyzed. The nucleotide sequences of the DNA fragments derived from the dominant bands in the μ and λ RT-PCR-SSCP analyses are shown (*lower line*). Each of the μ and λ nucleotide sequences was compared with the most homologous germline gene segments (*upper line*). *Dots* indicate sequence identity

can circulate in the blood of patients with plasma cell proliferative diseases, which include monoclonal gammopathy of undetermined significance (MGUS), smoldering multiple myeloma, and multiple myeloma. Using the allele-specific oligonucleotide PCR, clonal cells can be found at very low levels in the blood in most patients with MGUS.[8] SS with a monoclonal protein in the serum belongs to the entity of MGUS. However, no previous studies have examined the relationship between B-cell clones in salivary gland lesions and in the PB. In our patient, expanded B-cell clones, which appeared to produce monoclonal protein, were present in both parotid glands and in the PB. These circulating clonal lymphocytes may represent clones that can repopulate tissue sites and contribute to B-cell lymphomagenesis. The detection of monoclonal B cells in the PB is thus considered to be important in monitoring the disease course of SS.

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