High Frequency of t(14;18) Translocation in Salivary Gland Lymphomas from Sjögren's Syndrome Patients

By Eva K. Pisa, Pavel Pisa, Ho-I1 Kang, and Robert I. Fox

From the Department of Rheumatology and Immunology, Research Institute of Scripps Clinic, La Jolla, California 92037

Summary
Sjögren's syndrome (SS) is a chronic autoimmune disorder characterized by lymphocytic infiltration of the salivary and lacrimal glands. These patients have a markedly increased frequency of developing non-Hodgkin's lymphoma in their salivary glands and cervical lymph nodes. Translocations of proto-oncogene bcl-2 t(14;18) were observed in five of seven SS-associated lymphomas by Southern blot analysis. Using primers specific for chromosomes 14 and 18, translocation of the proto-oncogene bcl-2 was detected by polymerase chain reaction (PCR) in all five lymphomas positive by Southern blot analysis. Among SS patients lacking clinical evidence of coexistent lymphoma, no bcl-2 translocations were detected in 50 consecutive salivary gland biopsies. Of particular interest, pre-lymphoma biopsies were available from the seven SS patients who subsequently developed lymphoma and these DNA samples lacked detectable t(14;18) translocations even though they exhibited oligoclonal rearrangements of their immunoglobulin genes. We conclude that the great sensitivity of PCR can help us in detecting early onset of lymphoma in SS patients and aid in understanding the transition from autoimmunity to lymphoma.
lymphoma biopsy, they might contain the same karyotypic translocations that are detected in the subsequent lymphoma. This possibility is shown schematically in Fig. 2 A, where rare "lymphoma cells" subsequently undergo clonal expansion to become an overt lymphoma. To test this hypothesis, we examined pre-lymphoma biopsies from five SS patients whose subsequent lymphoma contained t(14;18) translocations detectable by PCR. The great sensitivity of PCR would make possible detection of rare cells bearing the translocation. Of importance, each of these pre-lymphoma SS biopsies previously has been shown to contain oligoclonal rearrangements of heavy and light chain Ig genes (22), and thus it is possible to determine whether bcl-2 translocation is detectable at the time of oligoclonal expansion of B-cells. An alternative hypothesis (Fig 2 B) is that the pre-lymphoma salivary gland does not contain B cells with karyotypic abnormalities and that oligoclonal B cell expansion precedes karyotypic translocation. Among the five SS pre-lymphoma biopsies, we did not find bcl-2 translocations. These results suggest that lymphoma develops in SS patients as a multistep process where oligoclonal B-cell expansion precedes t(14;18) translocation.

Materials and Methods

Patients with SS were seen at Scripps Clinic and Research Foundation (La Jolla, CA). All patients had definite SS with keratoconjunctivitis sicca, xerostomia, class 4-positive minor salivary gland biopsies, autoantibodies including rheumatoid factor and ANA (titers >640), and the presence of anti-SS-A/SS-B antibodies (15, 26). Among 200 SS patients followed for >5 yr, 14 developed non-Hodgkin’s lymphoma involving cervical lymph node or salivary gland. The time interval between initial diagnosis of SS and the appearance of lymphoma was at least 3 yr. All lymphomas were B cell based on immunohistological study and Southern blot analysis of DNA for heavy and light chain rearrangement (22). 13 of 14 lymphomas were IgM-x and one lymphoma was IgA-λ. In seven of these patients, previous biopsies of major salivary glands or lymph nodes had been performed and showed "reactive" changes but not overt lymphoma (17); therefore, these biopsies are referred to as "pre-lymphoma". Bone marrow aspirates from iliac crest were available from these seven patients. In two cases, combination chemotherapy was unsuccessful and patients died of septicemia; complete autopsy tissue was analyzed including thoracic and abdominal lymphoid tissues.

DNA was examined from other tissues including minor salivary gland biopsies from 50 consecutive SS patients who had no clinical evidence of lymphoma. 30 minor salivary gland biopsies from patients lacking autoimmune disease, 10 salivary gland biopsies from patients with benign adenoma or adenocarcinoma, 10 lymph node biopsies from patients with systemic lupus erythematosus, 10 lymph node biopsies from patients with rheumatoid arthritis, 10 tonsillar lymph node biopsies from immunologically normal individuals, and seven lymph node biopsies containing "follicular" non-Hodgkin’s lymphoma.

Genomic Southern Blot Analysis. Procedures for extraction of DNA from tissues and cell lines, Southern blot analysis of DNA, and radiolabeling of probes have been described (22, 27). High molecular weight DNA was digested with HindIII according to manufacturers instruction (New England Biolab, Beverly, MA). Chromosome 18-specific probes pFL1 and pFL2, detecting mbr and mcr, respectively (Fig. 1) (7, 28), were obtained from Dr. M. Cleary (Stanford University, Stanford, CA).

A B cell line lymphoma, SU-DHL6, containing known translocation t(14;18) involving mbr region was obtained from Dr. Allan Epstein (University of Southern California, Los Angeles, CA). Samples of lymphoma tissue from a patient with known translocation t(14;18) involving mcr region was obtained from Dr. M. Cleary (Stanford University, Stanford, CA).

Polymerase Chain Reaction. Amplification of DNA involving bcl-2 translocation was performed by PCR using a oligonucleotide primer specific for a consensus sequence of Ig heavy chain J segment and a primer specific for mbr or mcr (29, 30). The PCR assay was performed with a DNA Thermal Cycler (Cetus Corp., Emeryville, CA), using 1 μg DNA, 100 pmol of each primer, 2.0 mM MgCl₂, 20 mmol of each dNTP, 2.5 U Taq polymerase (Cetus Corp.), and 35 cycles of amplification. One-tenth of the amplified product was electrophoretically separated in 1.6% agarose gel and transferred by alkaline blot method onto nylon membranes, which were hybridized with a 32P end-labeled oligonucleotide probe at 42°C for 16 h. Autoradiography was performed for 4–24 h at ~80°C using X-Omat AR film (Eastman Kodak Co., Rochester, NY) with a single intensifying screen.

Results

To demonstrate the sensitivity and specificity of the PCR reaction for bcl-2 t(14;18) translocations involving the mbr

![Figure 1](https://example.com/figure1.png)
region, DNA from the lymphoma cell line SU-DHL-6 was mixed with normal genomic human DNA before PCR amplification. Using a 4-h autoradiogram (Fig. 3 A), 10^{-5} \mu g of SU-DHL-6 DNA easily could be detected in a mixture containing 1 \mu g of normal DNA. This corresponds to approximately one cell translocation per 10^6 of uninfected cells. Fig. 3 B shows a similar reconstitution experiment using lymphoma DNA that exhibits t(14;18) translocation involving mcr segment.

DNA from seven SS lymphomas was analyzed and a bcl-2 translocation involving the mbr region was detected in three of seven patients by PCR methods (Fig. 4, lanes 6, 8, and 10). In comparison, lanes 1 and 2 are negative controls, and lane 3 contains DNA from a cell line (SU-DHL6) with known mbr t(14;18) translocation. In pre-lymphoma biopsies from each SS patient, mbr translocations were not detectable (Fig. 4, lanes 5, 7, and 9).

DNA from SS lymphomas with breakpoints in the mcr of chromosome 18 is shown in Fig. 5, lanes 6, 8, and 10. Again, the prelymphoma biopsies from SS patients did not demonstrate any detectable mcr translocation (Fig. 5, lanes 5, 7, and 9). One patient (SS-1) showed translocations with both mbr and mcr breakpoints on chromosome 18 (Fig. 4, lane 6; Fig. 5, lane 10). To rule out the possibility that a negative result in some patient samples was due to inefficiency or complete failure of the PCR reaction, a single copy gene (HLA-DQα) was successfully amplified in all samples (data not shown).

To confirm the PCR results and to search for additional t(14;18) translocations that may not be detected by PCR, DNA from each of the lymphomas was digested with restriction enzyme HindIII and hybridized with PFL-1 and PFL-2 probes to mbr and mcr regions, respectively (Fig. 6). The results of restriction fragment length analysis using PFL-1 and PFL-2 probes were in correlation with the PCR results, and no additional SS lymphoma with bcl-2 translocation was detected. Fig. 6, A shows representative Southern blots from lymphomas with mcr translocation (lanes 4 and 6) and the absence of...
translocation in the pre-lymphoma biopsies (lanes 3 and 5). Similarly, Fig. 6B shows mcr translocation in a lymphoma from a SS patient (lane 4). Of particular interest, patient SS-1, who exhibited both mcr and mbr translocations using PCR, had detectable bcl-2 rearrangement on Southern blot only with mcr probe pFL-2 (Fig. 6A, lane 6).

Finally, DNA from salivary gland and lymph node tissues of normals, patients with autoimmune disease, and patients with solid tumors was studied. These included minor salivary gland biopsies from 50 SS patients lacking lymphoma, 10 lymph nodes from patients with rheumatoid arthritis or systemic lupus erythematosus, and 10 adenocarcinoma salivary gland tumors. These DNA samples did not exhibit detectable bcl-2 translocation by PCR analysis.

Discussion

Patients with SS have increased risk of developing lymphomas involving the cervical lymph nodes and salivary glands (1). We now show that five of seven of these tumors had bcl-2 t(14;18) translocations. From a clinical and histologic point of view, it is often difficult to distinguish "pseudolymphoma" (pre-lymphoma) in SS patients from definite non-Hodgkin's lymphoma (21, 22, 31). Therefore, analysis of bcl-2 translocations in tissue biopsies will aid diagnosis. Since only a small amount of tissue is required for PCR analysis, diagnostic samples may be obtained by percutaneous biopsy or fine needle aspiration. This is in contrast to the current need for an open surgical biopsy to remove enlarged salivary glands.
and the associated risk of damage to the facial nerves. A negative result (i.e., no detectable translocation) would not eliminate the diagnosis of lymphoma, but a positive finding would alert the internist and surgeon to the increased chance of lymphoma. It has recently been reported that patients with B cell lymphomas with t(14;18) translocations have a poorer response to therapy (32). Therefore, detection of bcl-2 translocation at the earliest stage would give more opportunities for clinical intervention to prevent lymphoma progression.

The finding of bcl-2 translocation in SS lymphomas also helps clarify the steps in transition from autoimmunity to lymphoproliferation. At the stage of pre-lymphoma, the majority of lymphocytes infiltrating the salivary gland are polyclonal T cells (17). However, 15-20% of these salivary gland lymphocytes are B cells that express follicular B cell-associated antigens (33), exhibit oligoclonal rearrangements of their Ig genes (22), and have an increased proportion of cells undergoing DNA replication (17). The present study demonstrates that these pre-lymphoma B cells lack detectable bcl-2 translocations. We propose that these B cells undergo cell division as a result of stimulation by activated T cells, their growth factors, and/or autoantigen. As a result of the cell division within the salivary gland, the B cells have increased opportunity for bcl-2 translocation and resulting lymphoma. Other factors that may contribute to lymphoma genesis in SS patients may include the high levels of growth factors produced in the salivary gland (34) and Epstein-Barr virus that has latency at this site (35, 36).

The lymphomas in SS patients occur predominantly in the salivary glands and therefore we favor the occurrence of the bcl-2 translocations at this peripheral site. This is in contrast to the hypothesis of Bakhshi et al. (12), who suggested that bcl-2 translocations could occur only among pre-B cells in the bone marrow. Although we can not rule out that the translocation first occurred in the marrow and the neoplastic cells subsequently migrated to the salivary glands, this possibility seems less likely since lymphomatous cells were not detected at other lymphoid sites such as abdominal lymph nodes, spleen, or bone marrow in SS lymphoma patients where autopsy was performed. Also, it remains possible that rare circulating pre-B cells underwent bcl-2 translocation within the microenvironment of the salivary gland. However, Cotter et al. (13) have demonstrated bcl-2 translocations among mature B cells in tissue culture, and our results suggest that a similar process has occurred in vivo at an inflammatory site.

Surprisingly, one patient's lymphoma contained both an mbr and an mcr translocation using PCR. Based on Southern blot analysis, the proportion of cells that contained the mcr translocation was much higher than the proportion of cells with the mbr translocation. It is not yet possible to determine whether the mbr translocation occurred in a cell with a prior mcr alteration or on a distinct B cell. However, DNA sequence analysis of these bcl-2 translocations is in progress to clarify the basis of this unusual finding.

In conclusion, our results demonstrate the presence of bcl-2 translocations in non-Hodgkin's lymphoma occurring in SS patients. These translocations were not detected in the pre-lymphoma biopsies from the same patients. Thus, detection of bcl-2 translocations may aid in improved early detection of lymphoma and improved treatment of SS patients. Although the function of the bcl-2 gene product is not yet clear (37, 38), the translocation juxtaposes the bcl-2 gene with Ig heavy chain locus, increases B cell survival, and may lead to increased chance of neoplastic transformation (11, 28, 29, 39, 40).

We are indebted to Drs. Michael Cleary for the pFL-1 and pFL-2 probes, Allen Epstein for the SU-DHL-6 cell line, Teresa Compton for helpful discussions, and to Ms. Bonnie Towle for typing the manuscript.

This is Publication No. 6396-IMM from the Research Institute of Scripps Clinic. This study was supported by research grants ROI AR-33983 and MO1 RR-00833 from the National Institutes of Health. Eva K. Pisa and Pavel Pisa were supported in part by grants from the Scripps-Stedham Foundation, the Hennings Foundation, the Swedish Cancer Society, and the Swedish Medical Research Council.

Address correspondence to Robert I. Fox, Department of Rheumatology and Immunology, Scripps Clinic and Research Foundation, 10666 North Torrey Pines Road, La Jolla, CA 92037. Eva and Pavel Pisa's present address is the Department of Immunology, Karolinska Institute, Stockholm, Sweden.

Received for publication 17 June 1991.

References
Nucleotide sequence of a uniquely expressed human T cell receptor β chain variable region gene (VB) in Sjögren's syndrome. **Nucleic Acids Res.** 17:455.


