ANATOMIC PATHOLOGY

Original Article

Analysis of Immunoglobulin Heavy Chain Gene Rearrangement in Myoepithelial Sialadenitis by Polymerase Chain Reaction

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Myoepithelial sialadenitis (MESA) can often be difficult to distinguish from low grade B-cell lymphoma of mucosa-associated lymphoid tissue (MALT). The authors have previously studied a series of 25 patients with MESA and identified histologic and immunologic features predictive of extrasalivary lymphoma (ESL). The authors have now analyzed formalin-fixed, paraffin-embedded salivary gland tissue from 21 of these patients (and 1 new patient) for immunoglobulin heavy chain (IgH) gene rearrangement by a semi-nested polymerase chain reaction (PCR) technique to compare monoclonality by IgH PCR with clinical outcome (median follow-up 6.7 years, range 3 months-19.4 years) and the paraffin section immunophenotype. The PCR technique employed consensus primers from variable (FR3A) and joining regions (L.JH, VLJH) of the IgH gene. A monoclonal PCR product was detected in 16 of 28 specimens from 13 of 22 patients. By Fisher’s exact test, a monoclonal PCR pattern did not correlate (P > .05) with development of ESL, broad strands of monocytoid B-cells, plasma cell light chain restriction by immunoperoxidase, or CD43 coexpression on monocytoid B cells by immunoperoxidase. This study suggests that the majority of MESA lesions harbor monoclonal B-cell populations and that clonality does not predict progression to clinically overt lymphoma. Acquired salivary gland MALT, in the form of MESA, may progress to a process that is clonal but not necessarily malignant. Extranodal lymphoma develops in a minority of patients with this lesion. (Key words: Myoepithelial sialadenitis; Immunoglobulin gene rearrangement; Polymerase chain reaction; Non-Hodgkin’s lymphoma) Am J Clin Pathol 1996;106:498-503.

MATERIALS AND METHODS

Case Reports

Cases were retrieved from the archives of the University of Michigan (UM) Department of Pathology and the
personal consult files of one of us (B.S.) via SNOMED search for major salivary glands coded as Sjögren’s syndrome, benign lymphoepithelial lesion, or Mikulicz disease. Formalin-fixed, paraffin-embedded tissues were used from cases that demonstrated heavy lymphoid infiltration with destruction of acinar tissue and formation of epimyoepithelial islands diagnostic of MESA. Specimens from 22 patients were included based on the availability of suitable tissue. Twenty-one patients have been previously reported.10

Clinical Follow-Up

Clinical information was obtained from patient charts and communication with the referring physician when necessary.

Immunohistochemistry

Immunoperoxidase staining was performed in the Immunoperoxidase Laboratory at UM on 4 μ thick sections using a standard avidin-biotin complex technique with 3'-diaminobenzidine (Sigma, St. Louis, MO) chromogen. The commercially available antibodies used were: L26 (CD20, Dako, Carpinteria, CA), UCHL-1 (CD45RO, Dako), anti-CD3 (Dako), anti-kappa (Dako), anti-lambda (Dako), Leu22 (CD43, Becton Dickinson, Mountain View, CA), and CAM5.2 (Becton Dickinson).

Polymerase Chain Reaction

Formalin-fixed, paraffin-embedded tissue was de-waxed in xylene and digested for 4 days at 37 °C in buffer containing 0.25 μg/μL proteinase K (BRL, Bethesda, MD), 20mM Tris HCl pH 8.3, 5mM MgCl2, 100mM KCl, 1% Tween 20, and 1%NP40. DNA in the supernatant was precipitated with ethanol, quantitated spectrophotometrically and reconstituted at 1 μg/μL in TE buffer.

Semi-nested PCR was carried out using primers FR3A and LJH (round 1), and FR3A and VLJH (round 2).11,12 Briefly, the round 1 reaction contained 1 μg template DNA, 250 ng each of FR3A and LJH primers, 0.2 mM of each dNTP, and 1.5 units of Taq polymerase (Boehringer Mannheim, Indianapolis, IN) in the PCR buffer of McCarthy and colleagues.13 Reactions were performed in an automated thermocycler (Perkin Elmer, Norwalk, CT) with a total volume of 50 μL per reaction for both rounds of amplification. All reactions were performed in duplicate with separate aliquots of DNA extract. First round amplification was carried out for 30 cycles of 94 °C denaturation for 2 minutes, 60 °C annealing for 2 minutes, and 72 °C extension for 2 minutes, ending with a final extension at 72 °C for 10 minutes. A 1 μL aliquot of first round product was used as the template DNA for round 2. Twenty cycles of amplification were performed in round 2, using the same reaction conditions as in round 1, except that the VLJH primer was substituted for the LJH primer. The final PCR product was analyzed by 8% polyacrylamide gel electrophoresis (PAGE) with visualization by ethidium bromide staining. Positive and negative controls were Raji cell line DNA, reactive tonsillar DNA, and water (no template DNA added).

We defined a monoclonal pattern as one or two dominant bands (representing one or two rearranged alleles) in the appropriate size range on 8% PAGE with at least one of the bands reproducible on duplicate analysis. These criteria were implemented because of the appearance of occasional unexplained bands that were not reproducible on repeat amplification.14 Polyclonal cases yielded no dominant bands, but instead showed a smear or “ladder” pattern in which multiple bands were present throughout the size range of expected PCR product (approximately 80–120 bp). In each PCR experiment, positive control Raji cell line DNA gave a strong signal while amplification without template DNA yielded no PCR product.

To determine the sensitivity of detecting a monoclonal population in a polyclonal background, we performed a dilutional assay in which Raji cell line DNA was progressively diluted with tonsillar DNA and subjected to PCR for IgH gene rearrangement as above. We were able to detect a monoclonal band at the level of 2% Raji cell line DNA diluted in reactive tonsillar DNA.

Statistics

Data were analyzed using the Fisher’s exact test to correlate PCR patterns with development of histologically confirmed extrasalivary lymphoma, paraffin section immunophenotype (specifically, CD43 coexpression on B cells and monotypic plasma cell immunoglobulin light chain staining), and presence of broad strands of monocytoid B cells as defined previously.16 Statistics were performed on a specimen basis except when correlating PCR result and ESL, which was done on a patient basis. In cases in which there were multiple specimens from a single patient, a patient was considered to have a monoclonal pattern if any one of the specimens met the criterion for monoclonality by PCR.

RESULTS

Clinical

Clinical features of all but one of the patients have been reported previously and are presented in Table 1.
The additional patient was a 77-year-old woman with a history of rheumatoid arthritis with a submandibular swelling (patient 22, Table 1). The group of 22 patients for which paraffin tissue blocks were available consisted of 3 men and 19 women with a median age of 56.5 years (range 22–77 years). Clinical information was available in 18 patients with a median follow-up of 6.7 years (range 3 months–233 months). Five patients developed extrasalivary lymphoma as previously reported.\(^ {10}\)

### Immunohistochemistry and Polymerase Chain Reaction

Paraffin section immunohistochemistry was performed previously for 21 of the 22 patients.\(^ {10}\) Nineteen of 28 specimens from 16 of the 22 patients coexpressed CD43 on B cells as evidenced by both Leu22 and L26 immunoreactivity in and around lymphoepithelial lesions. Immunoglobulin light chain staining revealed monocytic plasma cell staining in 8 of the 28 specimens from 4 of the 22 patients.

All specimens contained amplifiable DNA. Polymerase chain reaction analysis for immunoglobulin heavy chain gene rearrangement resulted in a monoclonal pattern in 16 of the 28 specimens from 13 of the 22 patients tested. Twelve specimens were monoclonal only by PCR and 4 were monoclonal both by PCR and immunohistochemistry. Four additional specimens (1B, 2, 4A, and 4C, Table 1) were found to have a light chain restriction in plasma cells by immunohistochemistry but were negative for gene rearrangement by PCR.

Sequential biopsies were available from four patients (Table 1, patients 1, 4, 13, 14). For patient 1, identically sized PCR products were obtained from a sample of the parotid and a subsequent sample of the submandibular gland. Similarly, sequential parotid biopsies from patient 14 yielded identically sized PCR products. For patient 13, sequential biopsies of parotid and submandibular glands yielded monoclonal PCR products of different sizes. Only one of three salivary gland biopsies from patient 4 yielded a monoclonal PCR product. Examples of monoclonal PCR patterns are shown in Figure 1. Clinical, histologic, immunophenotypic, and PCR data for all of the patients are summarized in Table 1.

### Statistical Analysis

Fisher's exact test showed that a monoclonal PCR pattern did not significantly correlate at the \( P < .05 \) level with the development of extrasalivary lymphoma.
monotypic immunoglobulin light chain staining in plasma cells, CD43 coexpression on B-cells, or broad strands of monocytoid B-cells (Table 2). When taken together, clonality (as implied by monotypic plasma cell immunoglobulin light chain staining and/or monoclonality by PCR) did not correlate with ESL \( (P = .11) \).

**DISCUSSION**

Myoepithelial sialadenitis often presents a problem to the surgical pathologist because the lymphoid infiltrate can be difficult to distinguish from low grade B-cell lymphomas of MALT (MALTomas). These extranodal lymphomas usually have an indolent clinical course with a tendency to remain localized at MALT sites. In some cases, dissemination to lymph nodes or transformation to a high grade lymphoma may occur.\(^6\),\(^7\),\(^15\)

We previously reported a series of 25 patients with MESA and identified histologic and immunophenotypic features that correlated with the development of extrasalivary lymphoma on clinical follow-up (since extrasalivary lymphoma is a reliable indicator of clinical malignancy). Broad interconnecting strands of monocytoid B-cells surrounding epimyoepithelial islands and monotypic plasma cell immunoglobulin light chain staining by immunoperoxidase were features that correlated with the development of lymphoma outside the salivary gland.\(^10\) However, not all patients that developed ESL showed these features and not all patients whose specimens showed broad strands of monocytoid/marginal zone B-cells developed ESL.

To further characterize these cases and to seek other predictors of ESL, we undertook analysis of these lesions for IgH gene rearrangement by PCR, a method applicable to archival tissues with a reported sensitivity of 65% to greater than 90%.\(^11\),\(^13\),\(^14\),\(^16\),\(^21\) A monochronal PCR pattern was found in biopsies from 13 of the 22 patients. These results are in keeping with the general finding that many cases of MESA, when analyzed for gene rearrangements, do indeed show evidence of monoclonality.\(^9\),\(^22\) In fact, due to recognized sensitivity limitations of the PCR technique, our results may have falsely underestimated the incidence of monoclonality among the MESA cases we studied. Unlike most other series, we were able to obtain extended clinical follow-up on many of these patients. Only a minority of cases (5 cases) had extrasalivary lymphoma and there was no statistical correlation between the demonstration of clonality in the salivary gland and the finding of extrasalivary lymphoma. Furthermore, PCR clonality did not correlate with the histologic presence of broad strands of monocytoid B-cells (a feature noted by us to correlate with development of ESL in patients with MESA\(^10\)), monotypic immunoglobulin light chain staining in plasma cells, or with coexpression of CD43 on B cells.

Two prior studies have used molecular diagnostic techniques to examine B-cell clonality in MESA. Fishleder and colleagues\(^9\) demonstrated B-cell monoclonality by Southern blotting in 8 of 8 cases of MESA and suggested that clonal expansion in this lesion had a role in the increased risk for subsequent lymphoma. More recently Diss and associates\(^22\) detected IgH gene rearrangement in 25 of 45 cases of MESA using a technique similar to ours; they detected light chain monotypism by immunohistochemistry alone in an additional 8 cases. Seven of their patients had sequential biopsies available for PCR analysis; persistence of the same clone was demonstrated in sequential samples from six of these individuals. The authors concluded that the presence of a B-cell monoclonal heralds the onset of lymphoma.\(^22\)

**TABLE 2. STATISTICAL CORRELATION OF PCR CLONALITY WITH DEVELOPMENT OF EXTRASALIVARY LYMPHOMA, MONOTYPIC LIGHT CHAIN STAINING, CD43 COEXPRESSION ON B CELLS, AND BROAD STRANDS OF MONOCYTOID B CELLS**

<table>
<thead>
<tr>
<th>PCR clonality</th>
<th>Extrasalivary Lymphoma</th>
<th>Monotypic Light Chain Staining of Plasma Cells</th>
<th>CD43 Coexpression on B Cells</th>
<th>Broad Strands of Monocytoid B Cells</th>
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<td></td>
<td>(P = 0.60)</td>
<td>(P = 0.70)</td>
<td>(P = 0.70)</td>
<td>(P = 0.99)</td>
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PCR = polymerase chain reaction; NS = not significant.

* Using Fisher's exact test.
The above discussion raises the question of whether demonstration of clonality in a single biopsy of MESA is sufficient for a diagnosis of lymphoma. From our results, we propose that demonstration of clonality by PCR is not sufficient for a diagnosis of lymphoma in MESA. The concept that monoclonality does not necessarily denote malignancy has been proposed for other conditions such as lymphomatoid papulosis, angioimmunoblastic lymphadenopathy, and post-transplant lymphoproliferative disorders.\textsuperscript{23-30} We suggest caution in the diagnosis of lymphoma in the setting of MESA when relatively sensitive techniques such as IgH PCR are performed to prove clonality. Given the generally indolent clinical course of MALT lymphomas and the morbidity that can be associated with local and/or systemic therapy, this caution seems reasonable.

As outlined previously,\textsuperscript{10} demonstration of monotypic plasma cell staining in cases of MESA warrants a diagnosis of lymphoma. In cases with histologic features predictive of development of ESL (ie, broad confluent strands of monocytoid B cells), but with polytypic plasma cells by paraffin section immunohistochemistry, additional studies for monoclonality are probably indicated and if positive would favor a diagnosis of lymphoma. However, it is important to note that one of our patients (patient 13, Table 1) with broad strands of monocytoid B cells and monoclonality by PCR did not develop ESL during the follow-up period of 72 months.

Cases without histologic features predictive of ESL and with polytypic plasma cells by paraffin section immunohistochemistry should be closely followed, because long periods of time can elapse between the diagnosis of MESA and the development of ESL.\textsuperscript{4} From a clinical perspective, gene rearrangement studies are probably not required in this situation, because many patients may harbor a small monoclonal and do not appear to develop ESL over extended periods of time. However, from an academic viewpoint, genotypic analysis of sequential specimens from such patients may help us understand the biology of the disease. Perhaps the persistence of a monoclonal in sequential biopsies is a strong predictor of the development of ESL. This contention is supported by our patient 1, who had two biopsies which showed the same size PCR product and who subsequently developed ESL.

In summary, we have extended the histologic, immunophenotypic, and clinical characterization of a series of patients with MESA using PCR studies for immunoglobulin heavy chain gene clonality. Monoclonality by IgH PCR in cases of MESA did not correlate with histologic features predictive of ESL, clinical development of ESL, plasma cell light chain restriction in paraffin sections, or coexpression of CD43 on B-cells. Future studies should be directed at other predictors of ESL in MESA. It has been postulated that additional molecular events, such as mutations in oncogenes or tumor suppressor genes, may confer a malignant phenotype upon a clonal population of lymphocytes in MESA. Until these additional events are defined, it will continue to be difficult to predict with certainty which MESA lesions will behave like malignant lymphoma.

REFERENCES


