EXTRANODAL LYMPHOID INFILTRATES, i.e., those that originate and/or present outside of the major lymphoid tissue-bearing sites, often represent a difficult clinical and pathological diagnostic dilemma. This is particularly true for small lymphocytic infiltrates, which constitute the majority of the ocular adnexal and pulmonary lymphoid neoplasms and which also occur in the gastrointestinal tract, skin, salivary glands, and other sites. The histopathologic criteria commonly used to distinguish benign and malignant extranodal small lymphocytic infiltrates are often incapable of reliably making this distinction, sometimes resulting in inaccurate patient prognostication. Cell marker analyses have shown that extranodal small lymphocytic infiltrates are largely divisible into monoclonal B cell, presumably malignant, small (well-differentiated) lymphocytic lymphomas and polyclonal, presumably benign, pseudolymphomas. However, a recent prospective study suggested that disseminated malignant lymphoma occurs with equal frequency in patients with ocular adnexal lymphoid neoplasms belonging to each category. This finding suggests that immunophenotypic analysis also may be incapable of reliably prognosticating patients with extranodal small lymphocytic infiltrates. Moreover, clinical, morphological, and immunophenotypic approaches have not provided significant insight into the pathogenetic relationship between extranodal pseudolymphoma and malignant lymphoma, between extranodal and nodal lymphoid neoplasms, and among multiple extranodal lymphoid infiltrates occurring in the same individual.

The antigen recognition molecules of B and T cells, immunoglobulin (Ig) and T cell receptor (TCR) gene loci, respectively, are encoded by genetic loci that undergo somatic recombinations (rearrangements) to become functionally active in mature lymphocytes. We and other investigators have demonstrated that clonal rearrangements of the Ig and the T cell receptor β chain (TCRβ) gene loci are accurate and objective molecular genetic markers of the lineage and clonality of B and T cells, respectively. The demonstration of clonal Ig and TCRβ gene rearrangements by Southern blot hybridization analysis has determined the lineage and the clonality of a variety of lymphoproliferative disorders and has become an important adjunct in the diagnosis, classification, and investigation of lymphoid neoplasias. However, immunogenotypic analysis has not been systematically applied to an examination of extranodal small lymphocytic infiltrates.

We investigated the organization of the immunoglobulin heavy chain (IgH) and the T cell receptor beta chain (TCRβ) gene loci in 20 ocular adnexal and four extracocular lymphoid neoplasms obtained from 18 patients presenting with an ocular adnexal lymphoid neoplasm. Fifteen ocular adnexal and four extracocular lymphoid neoplasms occurring in 13 patients were classified by morphological examination and immunophenotypic analysis as monoclonal B cell lymphomas. Each one of these 19 lymphoid neoplasms exhibited clonal IgH gene rearrangements upon hybridization of EcoRI- or HindIll-digested DNA to a heavy-chain joining region (JHγ)-specific DNA probe. The bilateral ocular adnexal monoclonal B cell neoplasms occurring simultaneously in two individuals exhibited identical clonal IgH gene rearrangements, which indicated their derivation from an identical B cell clone. The ocular adnexal and the extraocular monoclonal B cell neoplasms occurring in two of three patients also exhibited identical clonal IgH gene rearrangements, which suggested that they too were derived from an identical B cell clone. Five ocular adnexal lymphoid neoplasms were classified by morphological examination and immunophenotypic analysis as benign, polyclonal pseudolymphomas. Three of these five ocular adnexal lymphoid neoplasms exhibited clonal IgH gene rearrangements, which suggested the presence of monoclonal B cell populations that escaped detection by morphological and immunophenotypic examination. None of the 24 pathological samples exhibited clonal TCRβ gene rearrangements upon hybridization of EcoRI- or BamHI-digested DNA to a TCRβ gene DNA probe. The results of these studies demonstrate the value of Southern blot hybridization analysis for clonal IgH and TCRβ gene rearrangements in the diagnosis, classification, and investigation of extranodal lymphoid neoplasms originating and/or presenting in the ocular adnexa.

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<th>Presentation and Findings</th>
<th>Histopathology</th>
<th>Clonality</th>
<th>Treatment</th>
<th>Clinical Course</th>
<th>Eventual Outcome</th>
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<td>1</td>
<td>62</td>
<td>M</td>
<td>36 mo earlier RUL pseudolymphoma; systemic evaluation negative; no therapy</td>
<td>Proptosis, firm nodular RUL mass; systemic evaluation negative</td>
<td>RLH</td>
<td>Polyclonal</td>
<td>Incisional Bx; local irradiation</td>
<td>Asymptomatic</td>
<td>NED: 29 mo</td>
</tr>
<tr>
<td>2</td>
<td>55</td>
<td>F</td>
<td>None</td>
<td>7-mo Hx of painless, enlarging LUL; ptosis on LUL; firm mass adherent to orbit; systemic evaluation negative</td>
<td>RLH</td>
<td>Polyclonal</td>
<td>Excisional Bx only</td>
<td>Asymptomatic</td>
<td>43 mo: RUL lymphoid tumor; Bx: DPDL, monoclonal B; local irradiation</td>
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<tr>
<td>3</td>
<td>64</td>
<td>F</td>
<td>None</td>
<td>6-mo Hx of LLL swelling with protrusion of left eye; palpable mass on lower orbital rim with slight proptosis and upward displacement of globe; systemic evaluation negative</td>
<td>RLH</td>
<td>Polyclonal</td>
<td>Subtotal excision only</td>
<td>Asymptomatic</td>
<td>NED: 56 mo</td>
</tr>
<tr>
<td>4</td>
<td>51</td>
<td>F</td>
<td>Undocumented Hx of &quot;benign lymphoma&quot; of lung treated by irradiation</td>
<td>36-mo Hx of LLL fullness and 6-mo Hx of LLL swelling; palpable firm LUL fullness; systemic evaluation negative</td>
<td>RLH</td>
<td>Polyclonal</td>
<td>Excisional Bx only</td>
<td>Asymptomatic</td>
<td>NED: 43 mo</td>
</tr>
<tr>
<td>5</td>
<td>59</td>
<td>F</td>
<td>None</td>
<td>30-mo Hx of LUL swelling, recent ptosis; CAT scan: left orbital mass; systemic evaluation negative</td>
<td>RLH</td>
<td>Polyclonal</td>
<td>Excisional Bx; local irradiation</td>
<td>Asymptomatic</td>
<td>NED: 44 mo</td>
</tr>
<tr>
<td>6</td>
<td>54</td>
<td>M</td>
<td>None</td>
<td>72-mo Hx of right eye prominence; proptosis; systemic evaluation negative</td>
<td>SL</td>
<td>Monoclonal</td>
<td>Excisional Bx; local irradiation B</td>
<td>Asymptomatic</td>
<td>NED: 12 mo</td>
</tr>
<tr>
<td>7</td>
<td>64</td>
<td>F</td>
<td>None</td>
<td>4-mo Hx of left ocular proptosis; Firm, rubbery mass on LLL; systemic evaluation negative</td>
<td>IL</td>
<td>Monoclonal</td>
<td>Excisional Bx; local irradiation B</td>
<td>Asymptomatic</td>
<td>NED: 39 mo</td>
</tr>
<tr>
<td>8</td>
<td>63</td>
<td>F</td>
<td>Kaposi’s sarcoma of lower extremities</td>
<td>2-mo Hx of LUL swelling; firm, nontender, mobile LUL mass; systemic evaluation negative</td>
<td>SL</td>
<td>Monoclonal</td>
<td>Excisional Bx; local irradiation B</td>
<td>Kaposi’s sarcoma lesions increase in number</td>
<td>Dead: 46 mo, metastatic lung carcinoma</td>
</tr>
<tr>
<td>9</td>
<td>43</td>
<td>M</td>
<td>None</td>
<td>1-mo Hx of RUL ptosis; palpable mass on RUL, RUL ptosis; systemic evaluation negative</td>
<td>DLNC</td>
<td>Monoclonal</td>
<td>Excisional Bx; systemic chemotherapy for 6 mo</td>
<td>Asymptomatic</td>
<td>NED: 28 mo</td>
</tr>
<tr>
<td>10</td>
<td>81</td>
<td>M</td>
<td>None</td>
<td>12-mo Hx of right superior conjunctival mass with occasional blurred vision; firm, salmon-colored lesion on right epibulbar surface extending into superolateral fornix; systemic evaluation negative</td>
<td>SL</td>
<td>Monoclonal</td>
<td>Excisional Bx; local irradiation B</td>
<td>Asymptomatic</td>
<td>NED: 36 mo</td>
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<tr>
<td>11</td>
<td>81</td>
<td>M</td>
<td>2-yr Hx of generalized lymphadenopathy; Bx diagnosis: DPDL</td>
<td>2-mo Hx of painless RUL swelling; firm, salmon-colored mass of superotemporal quadrant; CAT scan: extensive abdominal lymphadenopathy</td>
<td>DLNC</td>
<td>Monoclonal Incisional Bx; local irradiation; systemic chemotherapy</td>
<td>Systemic dissemination</td>
<td>Dead: 16 mo, disseminated lymphoma</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>56</td>
<td>F</td>
<td>None</td>
<td>4-mo Hx of RUL mass; firm, rubbery, mobile RUL mass; refused systemic evaluation</td>
<td>SL</td>
<td>Monoclonal Excisional Bx; refused further therapy</td>
<td>12 mo: asymptomatic LUL mass; Bx: lymphoma; serum IgMx spike, refused therapy; 18-mo CAT scan: gastric mass; Bx: lymphoma; systemic chemotherapy</td>
<td>Alive with systemic disease: 26 mo</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>64</td>
<td>F</td>
<td>None</td>
<td>2-mo Hx of RUL mass; firm RUL mass; CAT scan: generalized lymphadenopathy, hepatosplenomegaly</td>
<td>SL</td>
<td>Monoclonal Excisional Bx; systemic chemotherapy</td>
<td>Asymptomatic</td>
<td>NED: 14 mo</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>63</td>
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<td>None</td>
<td>6-mo Hx of LUL ptosis; LUL fullness with ptosis; generalized lymphadenopathy, hepatosplenomegaly, IgG A monoclonal gammopathy; LN Bx: lymphoma</td>
<td>FLC</td>
<td>Monoclonal Incisional Bx; local irradiation; systemic chemotherapy</td>
<td>17 mo: generalized lymphadenopathy; systemic chemotherapy</td>
<td>Dead: 29 mo, disseminated lymphoma</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>51</td>
<td>F</td>
<td>None</td>
<td>18-mo Hx of left eye prominence; palpable mass above orbital rim, beneath LLL; CAT scan: mediastinal and retroperitoneal lymphadenopathy</td>
<td>SL</td>
<td>Monoclonal Incisional Bx; systemic chemotherapy</td>
<td>CAT scan: decreased lymphadenopathy</td>
<td>Alive with systemic disease: 12 mo</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>67</td>
<td>F</td>
<td>None</td>
<td>4-mo Hx of LLL mass; firm, nontender LLL mass; generalized lymphadenopathy</td>
<td>SL</td>
<td>Monoclonal Excisional Bx; local irradiation</td>
<td>CAT scan: abdominal lymphadenopathy; Bx: lymphoma; LN Bx: lymphoma</td>
<td>Dead: 15 mo, disseminated lymphoma</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>70</td>
<td>F</td>
<td>None</td>
<td>4-mo Hx of bilateral LLL and RUL swellings; firm LLL mass and visible RUL mass with globe displacement</td>
<td>IL</td>
<td>Monoclonal Bilateral excisional Bx; bilateral local irradiation</td>
<td>CAT scan: retroperitoneal lymphadenopathy; LN Bx: lymphoma; BM Bx: lymphoma; systemic chemotherapy</td>
<td>Alive with systemic disease: 24 mo</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>86</td>
<td>F</td>
<td>None</td>
<td>12-mo Hx of RUL swelling and ptosis, LUL and LLL lumps; palpable RUL, LUL, LLL masses with RUL ptosis; bilateral cervical, axillary lymphadenopathy; LN Bx: lymphoma; BM Bx: negative</td>
<td>F + DSC</td>
<td>Monoclonal Incisional Bx; local irradiation</td>
<td>Asymptomatic</td>
<td>Alive with systemic disease: 24 mo</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: RUL, right upper lid; RLL, right lower lid; LUL, left upper lid; LLL, left lower lid; RLH, reactive lymphoid hyperplasia; SL, small lymphocytic lymphoma; IL, intermediate lymphocytic lymphoma; FLC, follicular large cell lymphoma; DLNC, diffuse large, noncleaved cell lymphoma; F + DSC, follicular and diffuse small cleaved cell lymphoma; DPDL, diffuse poorly differentiated lymphocytic lymphoma; Hx, history; Bx, biopsy; LN, lymph node; BM, bone marrow; NED, no evidence of disease; CAT, computed axial tomography.
tomic lymphoma, patients with simultaneous bilateral ocular lymphoid neoplasms, and patients who were discovered to have concurrent ocular adnexal and nonocular lymphoid neoplasms at the time of presentation. The results of these studies demonstrate the utility of immunogenotypic analysis in the diagnosis, classification, and investigation of extranodal lymphoid neoplasms.

MATERIALS AND METHODS

Patients. Eighteen patients presenting with an ocular adnexal lymphoid neoplasm were included in this study (Table 1). These patients were randomly selected from among cases of ocular adnexal lymphoid neoplasia that had been previously well characterized and in which adequate numbers of cells were available for Southern blot hybridization analysis.

Specimens. One sample of heparinized peripheral venous blood and representative portions of the surgical biopsy specimens of 20 ocular adnexa and three lymph nodes, all suspected of involvement by malignant lymphoma, were obtained from these 18 patients. These specimens were collected under sterile conditions during the course of standard diagnostic procedures. A mononuclear cell suspension of greater than 95% viability and free of erythrocytes, dead cells, and cellular debris was prepared from each sample by Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) density gradient centrifugation. Immunophenotypic analysis was performed at the time of mononuclear cell isolation before cryopreservation. Excess cells were cryopreserved in vapor-phase liquid nitrogen at -170°C in the presence of dimethylsulfoxide and fetal calf serum.

Histopathology. Hematoxylin-eosin-stained sections were prepared from the paraffin blocks from each biopsy specimen corresponding to the tissue investigated by immunogenotypic and molecular genetic analysis. Each ocular lymphoid neoplasm was classified as an inflammatory pseudotumor, reactive lymphoid hyperplasia, or malignant lymphoma according to previously established histopathologic criteria. The malignant lymphomas were subclassified according to the working formulation of the non-Hodgkin’s lymphomas. All additional relevant biopsy specimens also were reviewed, even though these tissues might not have been subjected to special studies.

Immunophenotypic analysis. The proportion of cells in each pathological sample that formed sheep erythrocyte rosettes, expressed surface Ig (slg) and/or a variety of B- and T cell–associated differentiation antigens, Ia, B1, B4, Leu-4, T1, T3, T4, and T8, was determined in suspensions of viable mononuclear cells by using previously described techniques.

Molecular genetic analysis. DNA was extracted from each of the 24 pathological samples by standard techniques. Twenty micrograms of DNA was digested with the appropriate restriction endonuclease and analyzed for Ig and Tβ gene rearrangements by Southern blot hybridization. Hybridization was performed in 50% formamide, 3 x standard saline citrate buffer (SSC) at 37°C for 16 hours. The filters were washed in 0.2 x SSC and 0.5% sodium dodecyl sulfate, pH 7.0, at 60°C for two hours. DNA clones representative of different portions of the Ig and Tβ gene loci were 32P labeled by nick-translation for use as probes. The range of specific activities obtained with this DNA-labeling technique is 2 to 5 x 108 dpm/μg. We have determined, by DNA dilution experiments, that clonal populations representing 5% of the total DNA may be detected by using nick-translated probes and these hybridization and washing conditions. The IgH gene locus was studied by hybridization of EcoRI- and HindIII-digested DNA to a J region (JH)-specific probe, a BamHI-HindIII 6-kilobase fragment representative of the entire JH region (kindly provided by Dr Stanley Korsmeyer, National Institutes of Health). The Tβ gene locus was analyzed by hybridization of EcoRI- and BamHI-digested DNA to a Tβ gene probe that hybridizes to both constant region alleles (a gift from Dr Tak Mak, Ontario Cancer Institute).

RESULTS

Clinical features. The age, sex, pertinent prior history, clinical presentation and findings, histopathologic and immunogenotypic diagnosis, form of therapeutic intervention, subsequent clinical course, and eventual outcome of each of the 18 patients included in this study is summarized in Table 1. The patients ranged from 43 to 86 years of age (mean age, 63 years). Six patients were male, and 12 patients were female. Sixteen patients (Table 1, case nos. 1 to 16) presented with unilateral, and two patients (Table 1, cases 17 and 18) presented with simultaneous bilateral, ocular adnexal lymphoid neoplasms. Both patients with bilateral and five of the 16 patients (Table 1, case nos. 11 to 15) with unilateral, ocular adnexal lymphoid neoplasms were discovered to have disseminated systemic lymphoma at the time of presentation. One of these five patients (Table 1, case no. 11) had a history of generalized lymphadenopathy and carried a prior lymph node biopsy diagnosis of “diffuse, poorly differentiated lymphocytic lymphoma.” The remaining 11 patients had no evidence of systemic lymphoma based upon radiological and, in some instances, bone marrow examination at the time of presentation. However, patient no. 1 had undergone an excisional biopsy of a histopathologically benign right upper lid mass 3 years earlier. Systemic evaluation was negative at that time, and therapy had never been initiated. Patient no. 4 had an undocumented history of a “benign lung lymphoma” that had been treated by irradiation.

Immunophenotypic and histopathologic analysis. We performed immunophenotypic analysis on each of the 20 ocular adnexal lymphocytic neoplasms occurring in these 18 patients and also on three samples of lymph node and one sample of peripheral blood obtained from three of these 18 patients (Tables 2 and 3). The results of these analyses determined that 15 ocular adnexal lymphoid neoplasms occurring in 13 patients (Table 1, case nos. 6 to 18) were monoclonal B cell proliferations. This includes six patients with unilateral ocular lymphoid neoplasms limited to the ocular adnexa, the two patients with simultaneous bilateral ocular adnexal lymphoid neoplasms, and all five patients discovered to have nonocular lymphoid neoplasia. The unila-

Table 2. Results of Immunophenotypic and Molecular Genetic Analysis of Five Ocular Adnexal Pseudolymphomas

<table>
<thead>
<tr>
<th>Patient</th>
<th>Ia</th>
<th>slg</th>
<th>λ</th>
<th>T1</th>
<th>T3</th>
<th>T4</th>
<th>T8</th>
<th>IgH</th>
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<td>R</td>
</tr>
<tr>
<td>4</td>
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<td>12</td>
<td>14</td>
<td>51</td>
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<td>R</td>
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<tr>
<td>5</td>
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<td>16</td>
<td>44</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>R</td>
</tr>
</tbody>
</table>

Numbers indicate the percentage of cells expressing the particular cell marker.

Abbreviations: G, germline; R, rearranged; ND, not done.
teral ocular adnexal lymphoid neoplasms occurring in the five remaining patients (Table 1, case nos 1 to 5) were polyclonal lymphoid proliferations.

Each of the 15 ocular adnexal monoclonal B cell neoplasms contained ≥60% B cells expressing either κ or λ light chains. The immunoglobulin isotype was IgMDκ in 7, IgMκ in three; and IgMDλ, IgMLκ, and IgGLκ in one case each, respectively. We did not determine the Ig heavy chain class in two cases; one of these cases expressed λ and 1 case expressed κ light chains. The bilateral ocular adnexal lymphoid neoplasms occurring in a single individual expressed an identical Ig isotype. Each nonocular lymphoid neoplasm examined expressed the same Ig isotype as the ocular adnexal monoclonal B cell neoplasm occurring in the same patient.

We classified each of the 15 ocular adnexal monoclonal B cell neoplasms as a malignant lymphoma on the basis of morphologic criteria. We subclassified these 15 malignant lymphomas as small lymphocytic, intermediate lymphocytic, diffuse large noncleaved cell, follicular and diffuse small cleaved cell, and follicular large cell. The bilateral ocular adnexal lymphoid neoplasms occurring in a single individual were identical morphologically. In addition, each monoclonal lymphoid neoplasm was similar morphologically to the ocular adnexal monoclonal B cell neoplasm occurring in the same patient.

Each of the five ocular adnexal polyclonal lymphoid neoplasms contained an admixture of T and B cells. The proportion of T cells ranged between 44% and 69% on the basis of T1 and/or T3 antigen expression. The T cell population consisted of T4+ and T8+ cells in ratios ranging from 3.2 to 8.6. The B cell population consisted of κ and λ light chain–positive B cells in ratios ranging from 0.5 to 1.6. We interpreted these five ocular adnexal lymphoid neoplasms as reactive lymphoid hyperplasias on the basis of morphologic criteria.

**Molecular genetic analysis.** We investigated the organization of the IgH and the Tβ gene loci in each of the 20 ocular adnexal lymphoid neoplasms occurring in these 18 patients. Each of the 15 ocular adnexal lymphoid neoplasms that we determined to be a monoclonal B cell proliferation by immunophenotypic analysis exhibited clonal IgH gene rearrangements upon hybridization of EcoRI- or HindIII-digested DNA to a JH-specific DNA probe. In addition, the ocular adnexal monoclonal B cell neoplasm occurring in each individual exhibited a unique IgH gene rearrangement pattern consistent with a derivation from a distinctive B cell clone (representative data are illustrated in Fig 1). The bilateral ocular adnexal lymphoid neoplasms occurring simultaneously in each of two individuals (Table 1, case nos. 17 and 18) exhibited identical clonal IgH gene rearrangement patterns (Fig 2), which suggested that they are derived from the identical B cell clone and do not represent separate primary neoplasms. None of these monoclonal B cell neoplasms exhibited clonal Tβ gene rearrangements upon hybridization of EcoRI- or BamHI-digested DNA to a Tβ gene DNA probe (data not shown). We had previously demonstrated that approximately 11% of lymphoid neoplasms exhibit both clonal IgH and Tβ gene rearrangements, ie, they are bigenotypic. The absence of bigenotypic neoplasms in this series suggests that extranodal lymphoid neoplasms may be bigenotypic less often than are nodal lymphoid neoplasms.

We also examined one peripheral blood and three lymph node samples obtained from three of the patients discovered to have concurrent ocular adnexal and nonocular lymphoid neoplasia (Table 1, case nos. 14 to 16). We demonstrated by immunophenotypic analysis that each of these samples contained
tained a monoclonal B cell proliferation expressing the same immunoglobulin isotype as the B cells constituting the patient's ocular adnexal lymphoid neoplasm. Molecular genetic analysis with two different restriction endonucleases, EcoRI and HindIII, demonstrated that the nonocular lymphoid neoplasms in patients no. 14 and no. 16 exhibited a pattern of clonal \(\text{IgH}\) gene rearrangement bands that was identical to that expressed by the ocular adnexal lymphoid neoplasms occurring in the same patients (Fig 3). Thus, the ocular adnexal and the nonocular lymphoid neoplasms occurring in these two patients appear to be derived from the same B cell clone and do not represent separate primary lymphoid neoplasms. However, the lymph node obtained from patient no. 15 exhibited a clonal \(\text{IgH}\) gene rearrangement pattern that was distinct from that of the patient's ocular adnexal lymphoid neoplasm (Fig 3). This case may represent a biclonal B cell lymphoma.31 None of these nonocular lymphoid neoplasms exhibited clonal \(\text{T}\) gene rearrangements upon hybridization of \(\text{EcoRI}\)- or \(\text{BamHI}\)-digested DNA to a \(\text{J}^\text{H}\) probe. Rearrangement bands are indicated by arrows. Lanes C show the \(\text{IgH}\) gene germline configuration without rearrangement bands. The bilateral ocular adnexal lymphoid neoplasms occurring simultaneously in each individual exhibited identical clonal \(\text{IgH}\) gene rearrangement patterns, which suggested that each neoplasm was derived from the identical B cell clone and that they do not represent separate primary lymphoid neoplasms. We had interpreted five unilateral ocular adnexal lymphoid neoplasms (Table 1, case nos. 1 to 5) to be benign, polyclonal pseudolymphomas based upon histopathologic examination and immunophenotypic analysis. None of these five pathological samples exhibited clonal \(\text{T}\) gene rearrangements upon hybridization of \(\text{EcoRI}\)- or \(\text{BamHI}\)-digested DNA to a \(\text{T}\) specific DNA probe (data not shown). However, two of these five pathological samples (Table 1, case nos. 4 and 5) exhibited clear and easily recognizable clonal \(\text{IgH}\) gene rearrangement bands upon hybridization of \(\text{EcoRI}\)- or \(\text{HindIII}\)-digested DNAs to a \(\text{J}^\text{H}\) specific probe (Fig 4). A third sample exhibited a faint nongermline band upon analysis of \(\text{EcoRI}\)-digested DNA and three faint, barely perceptible, \(\text{IgH}\) gene rearrangement bands upon analysis of \(\text{HindIII}\)-digested DNA, which suggested \(\text{B}\) cell oligoclonality (Fig 4). The two remaining pathological samples (Table 1, case nos. 1 and 2) exhibited the germline \(\text{IgH}\) DNA configuration (Fig 4). Thus, three of the five ocular adnexal pseudolymphomas that we investigated contained either oligoclonal or conspicuous mono-
were alive with evidence of systemic lymphoma at 1
were also polyclonal at the molecular genetic level, the

Fig 3. Southern blot hybridization analysis of IgH gene in DNAs extracted from ocular adnexal monoclonal B cell neoplasms and nonocular lymphoid neoplasms occurring concurrently in the same individual. Lanes C contain human fibroblast DNA. The number above each of the other lanes corresponds to the case number in Table 1, and the letters a, b, and c indicate different anatomic sites. The DNAs were digested with EcoRI and HindIII and hybridized to a JH probe. Rearrangement bands are indicated by arrows. Lanes C show the IgH gene germline configuration without rearrangement bands. The ocular adnexal and nonocular lymphoid neoplasms occurring concurrently in patient nos. 14 and 16 exhibit identical clonal IgH gene rearrangement patterns which suggested that they are derived from the identical B cell clone. The lymphoid neoplasms occurring in patient no. 15 exhibit distinctive clonal IgH gene rearrangement patterns.

clonal B cell populations by molecular genetic analysis that were not recognizable by morphological examination and immunophenotypic analysis.

Eventual patient outcome. Thirteen of the 18 patients had an ocular adnexal lymphoid neoplasm that we determined to be monoclonal B cell in nature by immunophenotypic analysis. Five of these 13 patients were asymptomatic, without evidence of ocular or nonocular lymphoid neoplasia at 12, 14, 28, 36, and 39 months, respectively. Four patients were alive with evidence of systemic lymphoma at 12, 14, 24, and 26 months, respectively. Three patients died of systemic malignant lymphoma 15, 16, and 29 months after their presentation with an ocular adnexal lymphoid neoplasm. One patient died of metastatic bronchogenic carcinoma 46 months after presentation but had no evidence of ocular or nonocular lymphoid neoplasia at postmortem examination.

Five of the 18 patients had an ocular adnexal lymphoid neoplasm that we determined to be polyclonal by immunophenotypic analysis. The neoplasms in two of these patients lacked clonal IgH gene rearrangement bands and thus lack detectable monoclonal B cell populations. Case nos. 4 and 5 display clearly visible clonal IgH gene rearrangement bands, which indicate the presence of monoclonal B cell populations. Case no. 3 contains faint, barely perceptible IgH gene rearrangement bands, which suggests the presence of multiple clonal B cell expansions. The clonal B cell populations in case nos. 3 to 5 were not detectable by morphological examination and immunophenotypic analysis.
neoplasm in one patient contained faint oligoclonal bands, and the neoplasms in the two remaining patients contained clearly identifiable clonal B cell expansions by molecular genetic analysis. The two patients with ocular adnexal lymphoid neoplasms that were immunophenotypically polyclonal but monoclonal B cell by molecular genetic analysis were alive, asymptomatic, and without evidence of ocular or extraocular lymphoid neoplasias at 29 and 44 months, respectively. The patient with oligoclonal bands had no evidence of systemic disease after 56 months of follow-up. One of the two patients with a polyclonal lymphoid proliferation was alive and well without evidence of disease at 43 months, whereas the other patient developed a monoclonal B cell proliferation in the contralateral orbit 43 months after the initial presentation.

DISCUSSION

Southern blot hybridization analysis using specific Ig and Tβ gene DNA probes allowed us to determine the lineage and clonality of 20 extranodal lymphoid neoplasms originating and/or presenting in the ocular adnexa of 18 patients and four nonocular lymphoid neoplasms occurring in three of these same patients. The results of molecular genetic analysis were correlated with the results of morphological and immunophenotypic examination of the same samples and with the clinical course of each patient. The results of these studies provide several observations with significant clinical and biologic implications concerning extranodal lymphoid neoplasia. First, ocular adnexal lymphoid neoplasms composed of monomorphic collections of small cytologically mature appearing lymphocytes exhibiting Ig light-chain isotypic exclusion clearly represent clonal B cell expansions. This is true for lymphoid neoplasms originating in and remaining limited to the ocular adnexa as well as those associated with disseminated systemic lymphoma. Second, simultaneous bilateral ocular adnexal monoclonal B cell neoplasms may be derived from the identical B cell clone and do not necessarily represent separate primary tumors. Third, the ocular adnexal and nonocular lymphoid neoplasms occurring concurrently in the same individual may be derived from the same B cell clone. Fourth, many ocular adnexal lymphoid neoplasms currently classified as benign pseudolymphomas may contain clonal B cell expansions demonstrable by molecular genetic analysis, which are not recognizable by histopathologic and/or by immunophenotypic examination. This finding does not necessarily imply that these pseudolymphomas are in reality malignant lymphomas because clonality cannot be equated with malignancy. However, this finding does support the contention that monoclonal B cell lymphomas may arise in extranodal polyclonal pseudolymphomas.

The determination of the benignancy or malignancy of an extranodal lymphoid neoplasm has been based upon traditional, long-established morphological criteria. It is generally accepted that cellular polymorphism and cytological maturity favor benignancy whereas monomorphism and cytological atypia favor malignancy. Extralymphotic neoplasms have been traditionally classified as benign pseudolymphomas because of their apparent cytological maturity and lack of atypia by morphological examination. However, many extranodal small lymphocytic neoplasms resemble nodal small (well-differentiated) lymphocytic lymphomas morphologically. Moreover, the cells frequently express low-density monotypic slg and the T1 antigen, similar to the cells of nodal small (well-differentiated) lymphocytic lymphomas. These observations have led some observers to suggest that many extranodal small lymphocytic neoplasms actually represent small (well-differentiated) lymphocytic lymphomas.

Molecular genetic analysis is a considerably more objective and accurate approach than morphological examination and immunophenotypic analysis in determining the lineage and clonality of lymphoid populations. Our molecular genetic studies demonstrate that lymphoid neoplasms resembling nodal small (well-differentiated) lymphocytic lymphomas morphologically and immunophenotypically but that are localized to the ocular adnexa exhibit clonal IgH gene rearrangements. These studies also demonstrate that the various ocular adnexal and nonocular lymphoid neoplasms resembling nodal small (well-differentiated) lymphocytic lymphomas morphologically and immunophenotypically that occur as simultaneous bilateral ocular adnexal or concurrent ocular and nonocular lymphoid neoplasias in a single patient nearly always express an identical pattern of clonal IgH gene rearrangements, which suggests that they are derived from one distinctive B cell clone. This is strong and compelling evidence that the multiple lymphoid tumors exhibiting the small (well-differentiated) lymphocytic lymphoma morphology occurring in each individual represent one malignant lymphoma that disseminated. These findings support our contention that extranodal lymphoid neoplasms morphologically identical to nodal small (well-differentiated) lymphocytic lymphoma legitimately fall into the spectrum of small (well-differentiated) lymphocytic lymphomas. Taken together, the clinical, morphological, immunophenotypic, and now the molecular genetic evidence suggests that small lymphocytic neoplasms that occur in extranodal sites such as the ocular adnexa represent the extranodal counterpart of nodal small (well-differentiated) lymphocytic lymphomas whether they are localized to an extranodal site or are disseminated.

It can be correctly argued that the molecular genetic determination of B cell clonality does not in itself imply malignancy, ie, complete transformation, nor does it necessarily imply that a lymphoid neoplasm will behave in a clinically aggressive manner. Nodal small (well-differentiated) lymphocytic lymphomas consistently exhibit clonal IgH gene rearrangements, but they are often clinically indolent and only slowly progressive. The results of the studies presented here suggest that the majority of ocular adnexal small lymphocytic lymphomas remain limited to the ocular adnexa and also are clinically indolent; only a minority are associated with disseminated systemic lymphoma. The ability to predict which small lymphocytic lymphomas will be clinically aggressive may have to await recognition of oncogenic marker(s) associated with their malignant transformation.

It is well known that benign pseudolymphomas occur in
each of the anatomic sites in which malignant B cell lymphomas also originate.\textsuperscript{1} For example, chronic lymphocytic thyroiditis is commonly seen in association with malignant lymphomas that originate in the thyroid gland.\textsuperscript{20} In addition, Sjögren's syndrome and benign lymphoepithelial lesions often antedate the development of monoclonal B cell lymphomas in the salivary glands.\textsuperscript{49} Therefore, it has been suggested that extranodal benign pseudolymphomas may serve as precursor lesions and/or be associated with the development of extranodal malignant lymphoma in some instances.\textsuperscript{41} Support for this hypothesis comes from the recognition of "clonal proliferation centers" within benign lymphoepithelial lesions of the salivary gland.\textsuperscript{42}

In this respect, one of the most interesting observations of this study was that many ocular adnexal lymphoid neoplasms classified as benign pseudolymphomas may contain clonal B cell expansions that are not recognizable morphologically and/or immunophenotypically. In fact, only two of the five ocular adnexal pseudolymphomas that we investigated lacked clonal B cell populations and were truly polyclonal. These results strongly suggest that clonal B cell expansions may commonly occur within benign extranodal pseudolymphomas. This finding provides considerable further support for the contention that monoclonal B cell lymphomas may originate in benign extrafolial pseudolymphomas. However, longitudinal multiparametric studies of patients with extranodal lymphoid neoplasms are necessary to substantiate this hypothesis.

It could be argued that we did not identify these small clonal B cell populations immunophenotypically because they were selectively lost during their extraction from the tissue samples. However, our cell isolation techniques have been successfully used in the phenotypic identification of clonal B cell populations for several years and are carefully monitored at each step to avoid cell loss. Mononuclear cell viability was consistently \(\geq 90\%\), and appreciable cell loss was not noted in any of the cases included in this study. Moreover, molecular genetic analysis of 45 benign, hyperplastic lymph nodes handled in an analogous manner revealed an occult clonal B cell population in only one instance (data not shown). Therefore, we do not believe that the discrepancy between morphological examination, immunophenotypic analysis, and molecular genetic determination of clonality resides at the technical level. Rather, we believe that the molecular genetic detection of these B cell clones is due to the exquisite sensitivity of the Southern blot technique, which previously has been shown to detect DNA that is representative of \(\leq 5\%\) of the total DNA in a given sample.\textsuperscript{10,13}

We previously reported that ocular adnexal pseudolymphomas commonly contain increased numbers of T4 antigen--positive T cells.\textsuperscript{43} We previously had not been able to determine the clonality of this cell population because there is no reliable phenotypic marker to determine T cell clonality. However, we were now able to investigate the clonality of this T cell population by using a specific DNA probe that detects clonal T\(\beta\) gene rearrangements. Using this approach, we demonstrated that the large number of T cells present in some ocular adnexal lymphoid neoplasms do not exhibit clonal T\(\beta\) gene rearrangements and therefore do not represent clonal T cell proliferations.

It should be clear from the studies presented here that molecular genetic analysis provides a useful, accurate, and objective approach to evaluate the lineage and clonality of extranodal lymphoid neoplasms. This approach should be useful in identifying patients with extranodal monoclonal B cell proliferations that cannot be identified by morphological examination and/or by immunophenotypic analysis and that have been designated indeterminate by some investigators.\textsuperscript{44} For example, immunogenotypic analysis should be particularly helpful in examining small biopsy samples of extranodal lymphoid proliferations, specimens in which a large inflammatory response masks a malignant lymphoma, and specimens in which there is only partial involvement by malignant lymphoma. This approach will also be useful in determining clonality in extranodal lymphomas that exhibit atypical histopathology, ie, contain prominent germinal centers, a polymorphous cellular component, or cells that are cytologically mature. The identification of patients with extranodal monoclonal B cell proliferations should provide useful prognostic information and may be helpful in identifying patients in which there is a possibility of conversion from a small (well-differentiated) lymphocytic lymphoma to a diffuse aggressive lymphoma. Furthermore, the identification of a clonal B cell population by its distinctive pattern of Ig gene rearrangements provides a useful genetic marker with which to follow a patient's future lymphoid proliferations.

However, our demonstration of the presence of clonal B cell populations in some morphologically benign and immunophenotypically polyclonal pseudolymphomas also raises important clinical questions concerning the role of antigen receptor gene rearrangement analysis in the diagnosis of malignant lymphoid neoplasia. None of the three patients in this series carrying such lesions developed overt clinical evidence of lymphoid malignancy at 29, 44, and 56 months. We have previously also identified patients with the acquired immunodeficiency syndrome--related complex without clinical evidence of lymphoid malignancy at 29, 44, and 56 months. We have previously also identified patients with the acquired immunodeficiency syndrome--related complex without clinical evidence of lymphoid malignancy who have hyperplastic lymph nodes containing clonal B cell expansions demonstrable by Southern blot hybridization analysis.\textsuperscript{45} These results support a widespread belief that clonality should not be equated with malignancy. It will be necessary to investigate additional cases of extranodal pseudolymphoma and to carefully monitor such patients longitudinally to determine the clinical and biologic significance of these subclinical clonal B cell populations. Nonetheless, the results of these studies demonstrate the value of Southern blot hybridization analysis for clonal Ig and T\(\beta\) gene rearrangements in the diagnosis, classification, and investigation of extranodal lymphoid neoplasms originating and/or presenting in the ocular adnexa.

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Immunoglobulin and T cell receptor beta chain gene rearrangement analysis of ocular adnexal lymphoid neoplasms: clinical and biologic implications

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