Extranodal Noncutaneous Lymphoid Hyperplasias Represent a Continuous Spectrum of B-Cell Neoplasia: Demonstration by Molecular Genetic Analysis

Daniel M. Knowles, Eleni Athan, Angela Ubriaco, Lois McNally, Giorgio Inghirami, Rosemary Wieczorek, Michael Finfer, and Frederick A. Jakobiec

We investigated 16 lymphoid proliferations occurring in the ocular adnexa, salivary glands, breast, and thyroid gland and satisfying the histopathologic and immunophenotypic criteria of benign lymphoid hyperplasia for the presence of clonal rearrangements of the antigen receptor, c-myc, bcl-1, and bcl-2 genes and Epstein-Barr virus (EBV) DNA sequences. Each of these 16 extranodal, noncutaneous lymphoid neoplasms exhibited clonal immunoglobulin heavy and/or light chain and lacked T-cell receptor (TCR) β-chain gene rearrangements. The patterns of immunoglobulin gene rearrangements included solitary and multiple barely perceptible to faint bands, solitary clear and definite bands, and solitary high-intensity bands superimposed on a background of multiple less-intense bands. Three ocular adnexal lymphoid neoplasms exhibited bcl-1 or bcl-2 gene rearrangements. None of the 16 lymphoid neoplasms contained EBV DNA sequences. Two patients developed a histopathologically confirmed malignant lymphoma in an extranodal site. None of the remaining 14 patients developed additional lymphoid neoplasms during a mean follow-up period of 30 months, despite conservative therapy. These results demonstrate that extranodal, noncutaneous lymphoid neoplasms meeting the histopathologic and immunophenotypic criteria for benign lymphoid hyperplasia frequently contain occult monoclonal or oligoclonal B-cell populations representing a continuous and progressive spectrum of B-cell neoplasia up to and including malignant lymphoma.

Approximately 10% to 25% of non-Hodgkin's lymphomas (NHLs) are extranodal, ie, they originate and/or present outside the major lymphoid tissue-bearing sites.1,2 The most common sites of origin are the gastrointestinal tract, skin and soft tissues, ocular adnexa, respiratory tract, and the salivary glands, but extranodal lymphomas have been reported to arise in nearly every conceivable anatomic site.1,2 A heterogeneous group of benign, lymphoid hyperplasias that were once designated "pseudolymphomas," since they mimic malignant lymphoma clinically and pathologically, also occur in the identical extranodal sites.1,2 These benign and malignant extranodal lymphoid neoplasms are of considerable clinical significance because they often represent a difficult diagnostic dilemma due to their overlapping clinical and histopathologic features and are of biologic interest because they sometimes occur in association with another, suggesting a pathogenetic relationship.1-3

We suggested a decade ago that the diagnostic dilemma posed by extranodal lymphoid neoplasms sometimes may be resolved by immunophenotypic analysis of the constituent lymphoid cell subpopulations.4 However, some prospective studies have suggested that this approach may also be incapable of reliable prognostication of patients with extranodal lymphoid neoplasms.5 Moreover, clinical, morphologic, and immunophenotypic approaches have provided limited insight into the pathogenetic relationship between benign and malignant extranodal lymphoid neoplasms.

We and other investigators have shown that Southern blot hybridization analysis for clonal rearrangements of the immunoglobulin and T-cell receptor (TCR) genes is an objective and accurate method of determining the lineage and clonality of lymphoid neoplasms6-10 and is also capable of detecting clonal B- or T-cell expansions that are not demonstrable by morphologic examination or immunophenotypic analysis.6-12 Southern blot hybridization analysis with appropriate DNA probes is similarly useful in detecting viral sequences, oncogenes, and chromosomal translocations12 and thereby investigating pathogenetic mechanisms of neoplasia.

Recently, we demonstrated that three ocular adnexal lymphoid neoplasms satisfying morphologic and immunophenotypic criteria for classification as benign lymphoid hyperplasia exhibited clonal immunoglobulin heavy chain gene rearrangements.13 This finding has significant clinical and biologic implications since it suggests that clonal B-cell populations may frequently be present within otherwise benign-appearing extranodal lymphoid hyperplasias. We chose to extend these preliminary studies by investigating a larger group of extranodal lymphoid hyperplasias from diverse sites for clonal rearrangements of the antigen receptor genes, the c-myc, bcl-1, and bcl-2 genes, and the presence of Epstein-Barr virus (EBV) sequences. In this way, we hoped to determine the frequency, origin, and nature of these clonal B-cell populations; (b) gain insight into the pathogenetic relationship between benign and malignant extranodal lymphoid neoplasms; and (c) evaluate the utility of antigen receptor gene rearrangement analysis in the differential diagnosis of extranodal lymphoid neoplasms.

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1635
<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age</th>
<th>Sex</th>
<th>Prior History</th>
<th>Clinical Presentation and Findings</th>
<th>Histopathologic Diagnosis</th>
<th>Treatment</th>
<th>Clinical Course</th>
<th>Eventual Outcome</th>
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<tbody>
<tr>
<td>1</td>
<td>55</td>
<td>F</td>
<td>None</td>
<td>Painless swelling and ptosis of LUL, 7-mo history; LUL ptosis and firm mass adherent to orbit; systemic evaluation negative</td>
<td>Diffuse lymphoid hyperplasia of orbit</td>
<td>Excisional biopsy</td>
<td>RUL lymphoid tumor, 43 mo; bx: monoclonal B-cell DPDL; systemic evaluation negative; local irradiation</td>
<td>NED: 12 mo after RUL biopsy</td>
</tr>
<tr>
<td>2</td>
<td>59</td>
<td>F</td>
<td>RUL &quot;cyst&quot; 60 mo earlier; no treatment</td>
<td>RUL mass, 4-mo history; palpable RUL mass; systemic evaluation negative</td>
<td>Follicular and diffuse lymphoid hyperplasia of orbit</td>
<td>Excisional biopsy</td>
<td>Asymptomatic, unremarkable</td>
<td>Dead at 21 mo of pancreatic carcinoma; no evidence of NHL</td>
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<td>3</td>
<td>64</td>
<td>F</td>
<td>None</td>
<td>LUL swelling and protrusion of left eye, 6-mo history; palpable mass of lower left orbital rim with slight proptosis and upward displacement of globe; systemic evaluation negative</td>
<td>Follicular and diffuse lymphoid hyperplasia of orbit</td>
<td>Subtotal excision</td>
<td>Right breast, 25 mo; bx: NPDL; systemic evaluation negative, no therapy; 6 mo, left breast bx: NPDL; systemic evaluation negative; chlorambucil</td>
<td>NED: 6 mo following left breast biopsy</td>
</tr>
<tr>
<td>4</td>
<td>65</td>
<td>M</td>
<td>RUL pseudolymphoma 36 mo earlier; systemic evaluation negative, no therapy</td>
<td>RUL mass and ptosis, 4-mo history; firm nodular RUL mass; systemic evaluation negative</td>
<td>Diffuse lymphoid hyperplasia of orbit</td>
<td>Incisional biopsy, local irradiation</td>
<td>Asymptomatic, unremarkable</td>
<td>NED: 55 mo</td>
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<tr>
<td>5</td>
<td>77</td>
<td>F</td>
<td>None</td>
<td>Diplopia, 3-mo history; palpable mass superior aspect of left orbital rim; systemic evaluation negative</td>
<td>Diffuse lymphoid hyperplasia of orbit</td>
<td>Subtotal excision, local irradiation</td>
<td>Asymptomatic, unremarkable</td>
<td>NED: 12 mo</td>
</tr>
<tr>
<td>6</td>
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<td>Undocumented history 8 yr earlier of &quot;benign lymphoma of lung&quot; treated by irradiation</td>
<td>LUL swelling, 36-mo history; palpable LUL fullness; systemic evaluation negative</td>
<td>Diffuse lymphoid hyperplasia of orbit</td>
<td>Excisional biopsy</td>
<td>Asymptomatic, unremarkable</td>
<td>NED: 47 mo</td>
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<tr>
<td>7</td>
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<td>None</td>
<td>Swelling of left eye, 4-mo history; 2 mm proptosis and palpable mass of left orbit; systemic evaluation negative</td>
<td>Diffuse lymphoid hyperplasia of orbit</td>
<td>Incisional biopsy, local irradiation</td>
<td>Asymptomatic, unremarkable</td>
<td>NED: 29 mo</td>
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<td>8</td>
<td>57</td>
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<td>Sjogren’s syndrome, Henoch-Schönlein purpura, diabetes mellitus</td>
<td>Increasing fullness LUL, 12-mo history; palpable LUL mass; systemic evaluation negative</td>
<td>Diffuse lymphoid hyperplasia of orbit</td>
<td>Incisional biopsy, local irradiation</td>
<td>Henoch-Schönlein purpura, no ocular symptoms or recurrence</td>
<td>NED: 47 mo</td>
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<tr>
<td>9</td>
<td>61</td>
<td>F</td>
<td>None</td>
<td>Increasing swelling of RUL, 4-mo history; mild proptosis; systemic evaluation negative</td>
<td>Follicular and diffuse lymphoid hyperplasia of orbit</td>
<td>Incisional biopsy, local irradiation</td>
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<tr>
<td>10</td>
<td>59</td>
<td>M</td>
<td>Left axillary lymph node, 47 mo earlier; bx: DPDL; systemic evaluation negative; treated with chemotherapy</td>
<td>RUL swelling, 8-mo history; freely mobile, nontender fleshy mass right supraorbital rim, proptosis, proptosis; systemic evaluation negative</td>
<td>Follicular and diffuse lymphoid hyperplasia of orbit</td>
<td>Incisional biopsy, local irradiation</td>
<td>Asymptomatic, unremarkable</td>
<td>NED: 4 mo</td>
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<tr>
<td>11</td>
<td>70</td>
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<td>Excision of benign lymphoepithelial lesion of left parotid gland 24 mo earlier</td>
<td>Enlarging mass of right parotid gland, 5 x 6 cm, firm right parotid gland mass; systemic evaluation negative</td>
<td>Benign lymphoepithelial lesion of parotid salivary gland</td>
<td>Subtotal excision</td>
<td>Asymptomatic, unremarkable</td>
<td>NED: 10 mo</td>
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<td>None</td>
<td>Asymptomatic left parotid salivary gland swelling which suddenly increased in size, 7-mo history; 2-cm firm, movable, nontender left parotid gland mass; systemic evaluation negative</td>
<td>Benign lymphoepithelial lesion of parotid salivary gland</td>
<td>Subtotal excision</td>
<td>Asymptomatic, unremarkable</td>
<td>NED: 12 mo</td>
</tr>
</tbody>
</table>
EXTRANODAL LYMPHOID HYPERPLASIAS

1637

for immunophenotypic analysis. Mononuclear processed promptly delivered to the laboratory in sterile conditions and immediately placed in one of these 11 samples was performed immediately. Excess cells not used in the initial immunophenotypic characterization were of five specimens and by both techniques in four specimens. We analyzed the cells in all 16 specimens for their expression of surface immunoglobulin (SIg), including κ and λ light chains, and B-cell and T-cell lineage-associated antigens using monoclonal antibodies (MoAbs) B1 (CD20) (Coulter, Hialeah, FL); Leu1 (CD5), Leu14 (CD22) (Becton Dickinson); T3 (CD3), T4 (CD4), T8 (CD8) (Ortho Pharmaceutical, Raritan, NJ); and HLA-DR and B7.9 (United Biomedical, Lake Success, NY).

MATERIALS AND METHODS

Patients. Sixteen patients who presented with an extranodal, noncutaneous lymphoid neoplasm that was interpreted as a benign lymphoid hyperplasia by standard morphologic criteria and after immunophenotypic analysis were included in this study (Table 1). The 16 patients were randomly selected from among cases of extranodal, noncutaneous lymphoid hyperplasia that had been previously well characterized and in which adequate numbers of cells and/or tissue were available for additional studies, including Southern blot hybridization analysis. Preliminary studies concerning four of these patients have been previously reported.

Pathological specimens. Biopsy specimens of the extranodal lymphoid neoplasms occurring in these 16 patients, ten ocular adnexal, three parotid salivary gland, two breast, and one thyroid gland, were collected during the course of standard diagnostic procedures under sterile conditions and immediately placed in RPMI 1640 tissue culture media. Each pathologic specimen was promptly delivered to the laboratory where one representative portion was routinely processed for histopathology and another was processed for immunophenotypic analysis. Mononuclear cell suspensions of ≥95% viability and free of erythrocytes, dead cells, and debris were prepared from 11 of these 16 specimens by Ficoll-Hypaque (Pharmacia, Piscataway, NJ) density-gradient centrifugation. Immunophenotypic analysis of the viable mononuclear cells isolated from these 11 samples was performed immediately. Excess cells not used in the initial immunophenotypic characterization were cryopreserved in vapor-phase liquid nitrogen at −170°C with dimethylsulfoxide (DMSO) and fetal calf serum (FCS). A representative portion of nine of the 16 pathologic specimens, including the five specimens in which a cell suspension was not prepared, were snap-frozen in OCT-embedding compound on circular cork disks in a mixture of isopentane and dry ice. Immunophenotypic analysis of these nine samples was performed by immunoperoxidase staining of cryostat sections prepared from the snap frozen tissue blocks. The unused portions of these tissue blocks were cryopreserved at −70°C.

Immunophenotypic analysis. The proportions of B and T cells were delineated by direct and indirect immunofluorescent cytofluorometric analysis of the isolated cells in suspension with a model 420 FACS (Becton Dickinson, Mountain View, CA) in 11 specimens, by an avidin-biotin immunoperoxidase technique in cryostat tissue sections in five specimens and by both techniques in four specimens. We analyzed the cells in all 16 specimens for their expression of surface immunoglobulin (SIg), including κ and λ light chains, and B-cell and T-cell lineage-associated antigens using monoclonal antibodies (MoAbs) B1 (CD20) (Coulter, Hialeah, FL); Leu1 (CD5), Leu14 (CD22) (Becton Dickinson); T3 (CD3), T4 (CD4), T8 (CD8) (Ortho Pharmaceutical, Raritan, NJ); and HLA-DR and B7.9 (United Biomedical, Lake Success, NY).

Molecular genetic analysis. DNA was extracted from either the cryopreserved cells or the snap-frozen tissue blocks in each of the 16 specimens by standard techniques. Aliquots of the DNA were digested with various restriction endonucleases (Bethesda Research Laboratories, Bethesda MD), electrophoresed in 0.8% agarose gel, denatured, neutralized, transferred to a nitrocellulose filter, and hybridized according to Southern as previously described. Various DNA clones were P-labeled by nick-translation or by random priming for use as probes.

The immunoglobulin heavy chain gene was investigated by hybridization of EcoRI and HindIII digested DNAs to an immunoglobulin heavy chain gene joining region (JH) probe. The immunoglobulin κ light chain gene was investigated by hybridization of BamHI-digested DNAs to a κ light chain joining region (Jκ) probe and to a λ light chain constant region (Cλ) probe. The immunoglobulin λ light chain gene was investigated by hybridization of EcoRI-digested DNAs to a λ light chain constant region (Cλ) probe. The TCR β chain (Tβ) gene was investigated by hybridization of EcoRI- and BamHI-digested DNAs to a DNA probe that hybridizes to the constant region of the Tβ gene. The organization of the c-myc gene was analyzed by hybridization of EcoRI- and HindIII-digested DNAs to the human c-myc probe MC1R3, representative of the third exon of the c-myc gene. Bel-1-Digested DNAs were analyzed for the presence of bel-1 gene rearrangements. The presence of bel-2 gene rearrangements was analyzed by hybridization of HindIII-digested DNAs to the PFL-1 probe, representing a portion of chromosome 18 at the major bel-2 breakpoint region.

Table 1. Clinical Characteristics of 16 Patients Presenting With an Extranodal, Noncutaneous Reactive Lymphoid Hyperplasia (Cont’d)

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age</th>
<th>Sex</th>
<th>Prior History</th>
<th>Clinical Presentation and Findings</th>
<th>Histopathologic Diagnosis</th>
<th>Treatment</th>
<th>Clinical Course</th>
<th>Eventual Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>70</td>
<td>F</td>
<td>None</td>
<td>Asymptomatic right parotid salivary gland mass, 2-mo history; 2-cm firm, movable, nontender salivary gland mass; systemic evaluation negative</td>
<td>Benign lymphoepithelial lesion of parotid salivary gland</td>
<td>Subtotal excision</td>
<td>Asymptomatic, unremarkable</td>
<td>NED: 48 mo</td>
</tr>
<tr>
<td>14</td>
<td>58</td>
<td>F</td>
<td>None</td>
<td>Painless left breast mass, 4-mo history; Poorly defined 4 x 3-cm mass of upper outer quadrant of left breast; systemic evaluation negative</td>
<td>Diffuse lymphoid hyperplasia of breast</td>
<td>Subtotal excision</td>
<td>Asymptomatic, unremarkable</td>
<td>NED: 14 mo</td>
</tr>
<tr>
<td>15</td>
<td>27</td>
<td>F</td>
<td>None</td>
<td>Painless, nontender, firm left breast mass, 6-mo history; Poorly defined 4-cm left breast mass; systemic evaluation negative</td>
<td>Diffuse lymphoid hyperplasia of breast</td>
<td>Subtotal excision</td>
<td>Asymptomatic, unremarkable</td>
<td>NED: 60 mo</td>
</tr>
<tr>
<td>16</td>
<td>53</td>
<td>F</td>
<td>None</td>
<td>Hashimoto’s thyroiditis, 24-mo history; Firm, tender thyroid gland increasing in size</td>
<td>Chronic lymphocytic thyroiditis</td>
<td>Subtotal excision</td>
<td>Asymptomatic, unremarkable</td>
<td>NED: 25 mo</td>
</tr>
</tbody>
</table>

LLL, left lower lid; RUL, right upper lid; LUL, left upper lid; RLL, right lower lid; NPDL, nodular, poorly differentiated lymphocytic; DPDL, diffuse, poorly differentiated lymphocytic; NM, nodular mixed; NED, no evidence of disease.
RESULTS

Clinical. The age, sex, pertinent past history, clinical presentation and findings, histopathologic diagnosis, form of therapeutic intervention, subsequent clinical course, and eventual outcome of each of the 16 patients included in this study are summarized in Table 1. The patients ranged in age from 27 to 77 years. Three patients were men and 13 patients were women. Patient 2 had a right upper eyelid “cyst” excised 5 years earlier. Patient 4 had a histopathologically benign lymphoid neoplasm of the right upper eyelid excised 3 years earlier. Systemic evaluation was negative at that time, and therapy was not initiated. Patient 6 had an undocumented history of a “benign lung lymphoma” that was treated by irradiation. Patient 8 had a history of Sjogren’s syndrome, and patient 11 had a benign lymphoepithelial lesion of the contralateral parotid salivary gland excised 2 years earlier. A diagnosis of “diffuse poorly differentiated lymphocytic lymphoma” had been rendered 47 months previously on an axillary lymph node biopsy obtained from patient 10. The patient received chemotherapy at that time despite a negative systemic evaluation. The remaining patients had no pertinent prior history. All 16 patients had a negative systemic evaluation based on bone marrow and/or radiologic examination at the time of presentation.

Histopathology. The ocular adnexal lymphoid neoplasms occurring in patients 1 and 4 through 8 were classified as diffuse lymphoid hyperplasia. Each of these lesions consisted of a diffuse, variably dense lymphoid proliferation. In each case, small lymphocytes were the predominant cell population, but variable numbers of plasma cytoid lymphocytes, immunoblasts, and mature plasma cells also were present. The ocular adnexal lymphoid neoplasms occurring in patients 2, 3, 9, and 10 were classified as follicular and diffuse lymphoid hyperplasia. These lesions displayed the above histopathologic features, but in addition contained variable numbers of well-formed reactive germinal centers (Fig 1). All ten ocular adnexal lymphoid neoplasms contained abundant vascularization lined by hyperplastic endothelium. Hemosiderin was present in four lesions.

The lymphoid neoplasms occurring in the breasts of patients 14 and 15 were predominantly diffuse, polymorphous lymphoid cell proliferations that surrounded scattered, residual breast ducts. The lesion in patient 15 contained multiple focal areas of sclerosis. Each lesion contained occasional small, poorly-outlined germinal centers surrounded by a large, interfolllicular proliferation of small mature lymphocytes, plasma cytoid lymphocytes, immunoblasts, and mature plasma cells (Fig 2). Eosinophils and neutrophils were absent.

The parotid salivary gland specimens from three patients (Table 1, cases 11 through 13) displayed squamous metaplasia of the ducts, epimyoepithelial islands, and dense diffuse and follicular polymorphous mononuclear cell infiltration by mature lymphocytes, immunoblasts, and plasma cells. These features are typical of the benign lymphoepithelial lesion of the salivary gland. The thyroid gland specimen from patient 16 exhibited oxyphilic change of the follicular epithelium and a diffuse and follicular, polymorphous, mature lymphoid cell infiltrate typical of chronic lymphocytic (Hashimoto’s) thyroiditis.

Immunophenotypic analysis. Eleven of the 16 extranodal, noncutaneous lymphoid neoplasms were studied in cell suspension by cytofluorometric analysis, permitting accurate quantitation of the constituent cellular subpopulations (Ta-
The proportion of T cells in these 11 specimens ranged between 43% and 67% on the basis of CD3 and/or CD5 antigen expression. The T-cell population consisted of CD4+ and CD8+ cells in ratios ranging from 1.1 to 3.1 in the non-ocular specimens and from 2.2 to 9.9 (mean 5.0) in the ocular adnexal specimens. The increased proportion of CD4+ T cells in the ocular adnexal polyclonal lymphoid hyperplasias is consistent with our previous observations.32 The proportion of B cells in these 11 specimens ranged between 17% and 53% on the basis of surface immunoglobulin and/or CD20 antigen expression. The B-cell population consisted of κ and λ light chain-bearing B cells in ratios ranging from 0.5 to 4.2 (mean 2.0). The remaining cells were primarily Ia (HLA-DR) antigen positive and lacked B- and T-cell-associated antigens and were interpreted as macrophages.

Four of the 11 extranodal noncutaneous lymphoid neoplasms studied in cell suspension and the five remaining extranodal, noncutaneous lymphoid neoplasms not studied in cell suspension were analyzed by immunoperoxidase staining of cryostat tissue sections. This approach precludes precise quantitation of the constituent cell populations but permits accurate assessment of cell populations in situ and thereby avoids certain problems inherent in cell suspension techniques. Each of these nine specimens similarly contained a clear mixture of T and B cells. In each case, T cells constituted ~50% to 70% and B cells ~20% to 50% of the total cell population. The T-cell population always contained both CD4+ and CD8+ cells; the CD4/CD8 ratios ranged from ~2:1 to 4:1. The B-cell population always contained both κ and λ light chain-bearing cells; the κ/λ ratios ranged from ~1:1 to 3:1. Therefore, these five and the aforementioned 11 extranodal, noncutaneous lymphoid neoplasms, interpreted as hyperplastic by histologic criteria, were considered polyclonal by immunophenotypic analysis.

Antigen receptor genes. Fifteen of the 16 extranodal, noncutaneous lymphoid neoplasms exhibited clonal immunoglobulin heavy chain gene rearrangements on hybridization of HindIII- and/or EcoRI-digested DNAs to the Jκ probe (Table 3, Fig 3). Eleven of 12 lymphoid neoplasms, including the one case lacking clonal immunoglobulin heavy chain gene rearrangements, exhibited clonal immunoglobulin κ light chain gene rearrangements on hybridization of BamHI-digested DNA to the Jκ probe and/or to the Cκ probe (Fig 4). The two cases lacking clonal immunoglobulin κ light chain gene rearrangements (Table 3, cases 2 and 15) also lacked clonal immunoglobulin λ light chain gene rearrangements on hybridization of EcoRI-digested DNAs to the Cλ probe. The organization of the immunoglobulin light chain genes was not investigated in three specimens.

Three lymphoid neoplasms, two orbital and one breast (Table 3, cases 1, 2, and 14), exhibited a solitary faint immunoglobulin heavy chain gene rearrangement band. Two of these three cases exhibited clonal immunoglobulin light chain gene rearrangements. An orbital lymphoid neoplasm and a benign lymphoepithelial lesion of the parotid gland (Table 3, cases 3 and 12) exhibited multiple, barely perceptible to faint but definite immunoglobulin heavy chain gene rearrangement bands and a hybridization smear, suggesting B-cell oligoclonality. Three orbital lymphoid neoplasms (Table 3, cases 4 through 6) exhibited solitary weak but definite immunoglobulin gene rearrangement bands, suggesting the presence of a small clonal B-cell population. Three orbital and two parotid gland lymphoid neoplasms (Table 3, cases 7 through 9, 11, and 13) exhibited one or two clear and strong rearrangement bands of the immunoglobulin heavy and light chain genes, consistent with the presence of a significant clonal B-cell population. The orbital lymphoid neoplasm occurring in patient 10 was distinctive in displaying at least three immunoglobulin heavy chain gene rearrangement bands of varying intensity on hybridization of HindIII-digested DNA to the Jμ probe, suggesting a background of B-cell oligoclonality with a predominant clone. The lymphoid neoplasm occurring in the breast of patient 15 exhibited two strong immunoglobulin heavy chain gene rearrangement bands but lacked clonal immunoglobulin light chain gene rearrangements. The specimen of chronic lymphocytic thyroiditis in patient 16 exhibited the germline immunoglobulin heavy chain gene configuration but a solitary clear and strong rearrangement band on hybridization of BamHI-digested DNAs to the Jκ probe, suggesting the presence of a clonal B-cell expansion which has undergone deletion of the joining region of the immunoglobulin heavy chain gene. Each of the 16 specimens lacked clonal Tp gene rearrangements on hybridization of EcoRI- and BamHI-digested DNAs to the Tp probe. In summary, these results suggest that each of the extranodal noncutaneous lymphoid neoplasms we investi-

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<tr>
<th>Patient No.</th>
<th>Site</th>
<th>Na</th>
<th>CD20</th>
<th>Slg</th>
<th>κ</th>
<th>λ</th>
<th>κ/λ</th>
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<th>CD8</th>
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<td>50</td>
<td>41</td>
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<td>18</td>
<td>1.2</td>
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<td>ND</td>
<td>25</td>
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<td>10</td>
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<td>42</td>
<td>29</td>
<td>7</td>
<td>4.1</td>
<td>60</td>
<td>51</td>
<td>25</td>
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ND, not done.
Numbers indicate percentage of positive cells.
Table 3. Results of Antigen Receptor Gene Rearrangement Analysis

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<th>Patient No.</th>
<th>Site</th>
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<th>Cμ</th>
<th>Jκ</th>
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<td>1R</td>
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<td>G</td>
<td>1R</td>
<td>ND</td>
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</tbody>
</table>

R, rearrangement; G, germline; ND, not done.
Numbers indicate number of rearrangement bands.

Gated contained one or more clonal B-cell populations and lacked clonal T-cell populations.

c-myc, bcl-1, and bcl-2 Gene rearrangements. The orbital lymphoid neoplasms occurring in patients 7 and 10 exhibited bcl-1 gene rearrangements on hybridization of bcl-1 digested DNAs to the bcl-1 probe (Table 4, Fig 5). The orbital lymphoid neoplasms occurring in patient 9 exhibited bcl-2 gene rearrangements on hybridization of HindIII-digested DNA to the pFL-1 probe (Table 4, Fig 5). None of the 12 specimens investigated exhibited c-myc gene rearrangements on hybridization of EcoRI and HindIII-digested DNAs to the c-myc probe.

EBV sequences. None of the 12 specimens investigated exhibited evidence of EBV sequences on hybridization of BamHI- and HindIII-digested DNAs to probes for the EBNA-1 and EBNA-2 genes, respectively (Table 4).

Clinical course and outcome. Patient 1 developed a monoclonal B-cell diffuse, poorly differentiated lymphocytic lymphoma of the right upper eyelid 43 months after excisional biopsy of the left upper eyelid lymphoid neoplasm we studied. This patient had a negative systemic evaluation, received local irradiation, and is disease-free 12 months later. Patient 3 developed nodular poorly differentiated lymphocytic lymphomas of the right and left breasts 25 and 31 months, respectively, after subtotal excision of the orbital lymphoid neoplasm we studied. This patient had no evidence of systemic lymphoma six months later. The remaining eight patients presenting with an ocular adnexal lymphoid neo-

![Fig 3](https://www.bloodjournal.org)
plasm were treated as follows: incisional biopsy and local irradiation, five; excisional biopsy, two; and excisional biopsy and local irradiation, one. None of these eight patients have evidence of extranodal or systemic lymphoma in 4 to 55 (mean 31) months of follow-up. The lymphoid neoplasms occurring in the parotid salivary gland, breast, and thyroid gland in the six remaining patients (Table 1, patients 11 through 16) were treated by subtotal excision only and none of the patients have developed evidence of extranodal or systemic lymphoma in 10 to 60 (mean 28) months.

**DISCUSSION**

The widespread application of immunophenotypic analysis in recent years has led to recognition that many extranodal small lymphocytic neoplasms that were once called benign lymphoid hyperplasias or pseudolymphomas based on morphologic criteria are monoclonal B-cell proliferations and therefore, presumably, are B-cell lymphomas. The reclassification of many of these pseudolymphomas as malignant lymphomas based on immunologic criteria has resulted in a significant reduction in the proportion of extranodal lymphoid neoplasms now designated benign lymphoid hyperplasia.  

Antigen receptor gene rearrangement analysis is a considerably more accurate, sensitive, and objective method of determining B-cell clonality than is immunophenotypic analysis. We demonstrated that all 16 extranodal noncutaneous lymphoid neoplasms (ten ocular adnexal, three parotid salivary gland, two breast, and one thyroid gland) that we had previously classified as benign lymphoid hyperplasia by histopathologic and immunologic criteria exhibited clonal immunoglobulin heavy and/or light chain gene rearrangements. Among the 13 cases in which we investigated the organization of both the immunoglobulin heavy and light chain genes, ten cases exhibited clonal heavy and light chain rearrangements, two cases exhibited heavy but no light chain gene rearrangements, and one case exhibited light chain but no heavy chain gene rearrangements. These findings demonstrate that morphologically benign and immunophenotypically polyclonal extranodal, noncutaneous lymphoid hyperplasias frequently contain clonal B-cell populations.

One possible conclusion that can be drawn from these results is that the extranodal lymphoid neoplasms we studied had been misinterpreted histopathologically, that their monoclonal B-cell nature was not detected immunophenotypically owing to technical shortcomings, and that all of these neoplasms actually represent malignant B-cell lymphomas. However, none of the pathologic specimens obtained from these patients contained dense sheets of monotonous small lymphocytes or atypical lymphocytes suggestive of a malignant lymphoma. The pathologic specimens obtained from patients 11 through 13 and 16 exhibited the classical histopathologic features of benign lymphoepithelial lesion of the salivary gland and chronic lymphocytic thyroiditis, respectively. The ocular adnexal and breast lymphoid neoplasms were always composed of a variable admixture of small, mature and benign-appearing lymphocytes, plasmacytoid lymphocytes, immunoblasts, and mature plasma cells. Moreover, only two of the 16 patients developed overt malignant lymphoma, and in both instances the lymphoma was limited to a solitary extranodal site. Patient 1 developed a lymphoma of the right upper eyelid 43 months after undergoing exci-
sional biopsy of a lymphoid neoplasm of the left upper eyelid. Patient 3 developed follicular lymphomas of the right and left breasts 25 and 31 months, respectively, after undergoing subtotal excision of a lymphoid neoplasm of the left lower eyelid. The original ocular adnexal lymphoid neoplasms occurring in patients 1 and 3 exhibited solitary and multiple faint, barely perceptible clonal rearrangement bands, respectively, and not a high-intensity rearrangement band as one might expect to find in a B-cell lymphoma. Therefore, we cannot exclude the possibility that the lymphomas occurring in these two patients represent new, primary extranodal lymphomas unrelated to the patients’ original extranodal lymphoid neoplasms. Unfortunately, tissue from these follow-up biopsies is not available for study. None of the remaining 14 patients developed overt lymphoma during a mean follow-up period of 30 months, although they were treated only by local excision or by incisional biopsy combined with local irradiation. In summary, these extranodal lymphoid neoplasms lacked obvious clinical and histopathologic evidence of malignancy, despite the frequent presence of clonal B-cell populations detectable by antigen receptor gene rearrangement analysis. Therefore, these extranodal noncutaneous lymphoid neoplasms probably should not be reclassified as malignant lymphomas on the basis of molecular genetic criteria.

We also may have failed to identify these small clonal B-cell populations immunophenotypically because they were selectively lost during extraction from the pathologic specimens. However, our cell isolation techniques are carefully monitored to avoid cell loss and have been used successfully in the immunophenotypic identification of clonal B-cell populations for many years. Cell viability was consistently >90%, and appreciable cell loss was not noted in any of the cases included in this study. Furthermore, 11 of these 16 extranodal noncutaneous lymphoid neoplasms were studied by an immunoperoxidase tissue section method that avoids the deficiencies inherent in cell suspension techniques. The results obtained by cell suspension and tissue section methods were essentially identical. Therefore, the discrepancy between morphologic examination, immunophenotypic analysis, and molecular genetic determination of clonality does not appear to reside at the technical level.

The alternative conclusion is that the extranodal lymphoid neoplasms we studied represent a heterogeneous group of lymphoid hyperplasias containing morphologically and immunophenotypically occult monoclonal and oligoclonal B-cell expansions and therefore represent variable stages of neoplastic progression up to and including malignant lymphoma. Several lines of evidence suggest that this is the more likely conclusion.

First, the patterns of immunoglobulin gene rearrangement in these lymphoid neoplasms are themselves heterogeneous, suggesting the variable nature of the clonal B-cell populations they represent. Three cases exhibited solitary and two cases exhibited multiple faint, often barely perceptible, rearrangement bands, suggesting the presence of one or more minor monoclonal or oligoclonal B-cell expansions. Ten cases exhibited one or two clear and definite rearrangement bands, suggesting the presence of a sizable clonal B-cell population. Finally, one case exhibited multiple rearrangement bands of variable intensity, suggesting the presence of a sizable B-cell clone superimposed on a background of B-cell oligoclonality.

Second, clinically and histopathologically overt malignant lymphomas are often associated with karyotypic abnormalities, such as chromosomal translocations. Although cytogenetic analysis of these cases could not be performed, c-myc, bcl-1, and bcl-2 gene rearrangements, which are associated with certain chromosomal translocations, were investigated. None of the 12 cases studied exhibited c-myc gene rearrangements. This is understandable since c-myc gene rearrangements are preferentially associated with Burkitt’s lymphoma, which does not occur frequently in these anatomic sites. However, only three ocular adnexal of 12
extranodal lymphoid neoplasms that we investigated exhibited \textit{bcl}-1 or \textit{bcl}-2 gene rearrangements. This represents a lower percentage of cases with \textit{bcl}-1 and \textit{bcl}-2 gene rearrangements than we would expect to find among a group of diffuse and follicular small lymphocytic lymphomas, the most common histopathologic types of lymphoma to occur in the extranodal sites included in this study. On the other hand, \textit{bcl}-1 and \textit{bcl}-2 gene rearrangements are not known to occur in benign lymph node hyperplasias. Therefore, their presence in occasional extranodal lymphoid neoplasms containing clonal B-cell populations strongly suggests that these occult clonal B-cell populations may represent the earliest identifiable stages of malignant lymphoma.

Third, clonal immunoglobulin and/or T_\beta gene rearrangements have been frequently demonstrated in lymphoproliferative disorders that do not represent overt malignant lymphoma and are not associated with the inevitable future development of malignant lymphoma; eg, we reported the frequent occurrence of clonal immunoglobulin gene rearrangements in the hyperplastic lymph nodes of patients with AIDS\textsuperscript{11} and suggested that these clonal B-cell proliferations may be related to the increased incidence of malignant lymphoma in these patients. However, none of these patients developed overt malignant lymphoma during 18 months of follow-up. Hanson et al\textsuperscript{14} reported the frequent presence of clonal immunoglobulin gene rearrangements in multicentric Castleman’s disease, which is generally believed to represent a benign lymphoproliferative disorder only occasionally associated with the development of malignant lymphoma. Fishleder et al\textsuperscript{43} consistently found clonal immunoglobulin heavy and light chain gene rearrangements in benign lymphoproliferative lesional the salivary gland without overt evidence of malignant lymphoma. Therefore, while the finding of clonal immunoglobulin gene rearrangements generally indicates the presence of a clonal B-cell population, it does not necessarily indicate that the clonal B-cell population is malignant.

EBV-infected and immortalized B-cell clones are long-lived and replicate in vivo and in vitro where they can be established as long-term cell lines.\textsuperscript{46} We hypothesized that EBV-immortalized B-cell clones may account for the oligoclonal B-cell proliferations present in AIDS-associated hyperplastic lymphadenopathy.\textsuperscript{11} Hanson et al\textsuperscript{14} found EBV DNA sequences in their cases of Castleman’s disease exhibiting clonal immunoglobulin gene rearrangements, suggesting that EBV infection also accounts for the clonal B-cell proliferations in these lesions. EBV-induced B-cell clones may account for the minor clonal B-cell populations found in some cases of Hodgkin’s disease as well.\textsuperscript{45} However, the monoclonal and oligoclonal B-cell proliferations consistently present in the extranodal lymphoid neoplasms that we studied do not appear to represent EBV-infected and immortalized B-cell clones. We found no evidence of EBV DNA sequences on hybridization of \textit{Bam}HI- and \textit{Hind}III-digested DNAs to DNA probes that detect the EBNA-1 and EBNA-2 genes, respectively.

Several clinical and scientific observations suggest that benign lymphoid hyperplasias and malignant B cell lymphomas occurring in extranodal noncutaneous sites are pathogenetically related. Patients with Sjogren’s syndrome and benign lymphoepithelial lesion of the salivary gland have an increased incidence of malignant lymphoma.\textsuperscript{46,47} Patients with chronic lymphocytic thyroiditis also have an increased incidence of malignant lymphoma,\textsuperscript{48} and the thyroid glands of patients with thyroid lymphomas nearly always exhibit the histopathologic features of chronic lymphocytic thyroiditis.\textsuperscript{49} Monotypic B-cell populations have been demonstrated in pseudolymphomas,\textsuperscript{50,51} and Schmid et al\textsuperscript{52} described monoclonal B-cell proliferation centers in some benign lymphoepithelial lesions in patients who later developed malignant lymphoma. Fishleder et al\textsuperscript{53} recently reported the uniform occurrence of clonal immunoglobulin heavy chain and \kappa light chain gene rearrangements in nine benign salivary gland lymphoepithelial lesions, including those associated and those unassociated with malignant lymphoma. Moreover, they demonstrated identical patterns of immunoglobulin heavy and light chain gene rearrangements in separate pathologic specimens obtained from the same patient, eg, lymphoma involving a cervical lymph node and lymphoma arising in a benign lymphoepithelial lesion of the salivary gland.\textsuperscript{43}

The results of our studies further support the contention that a pathogenetic relationship exits between extranodal noncutaneous lymphoid hyperplasia and malignant B-cell lymphoma. First, we confirmed the report of Fishleder et al\textsuperscript{43} that clonal immunoglobulin gene rearrangements are uniformly present in benign lymphoepithelial lesions of the salivary glands. Second, we extended their observations by demonstrating that lymphoid hyperplasias occurring in the ocular adnexa, breast, and thyroid gland also frequently exhibit clonal immunoglobulin gene rearrangements, even in the absence of clinically and histopathologically detectable lymphoma. Third, we characterized these clonal B-cell populations more completely. We demonstrated that they (a) are not related to EBV infection; (b) are heterogeneous, ranging from minor monoclonal and oligoclonal B-cell populations to significant clonal B-cell populations; and (c) exhibit evidence of malignant transformation (ie, \textit{bcl}-1 or \textit{bcl}-2 gene rearrangement) in some instances. These results strongly suggest that the benign and malignant lymphoid neoplasms that occur in the various extranodal noncutaneous sites represent a continuous and progressive spectrum of B-cell neoplasia. Initially, these extranodal clonal B-cell populations may result as a consequence of local or general defects in immune surveillance and/or the mechanisms that normally regulate B-cell proliferation. Ultimately, these clonal B-cell expansions may become susceptible to additional genetic alterations (ie, \textit{bcl}-1, \textit{bcl}-2 or as yet undetermined) and undergo malignant transformation. Obviously, careful longitudinal studies are necessary to substantiate these hypotheses. However, this explanation accounts for certain documented but previously unexplainable clinical situations such as patients with concurrent extranodal lymphoid hyperplasia and systemic lymphoma\textsuperscript{53} and patients who develop an extranodal malignant lymphoma after the occurrence of a pseudolymphoma in the same anatomic site.\textsuperscript{53} In this respect, these extranodal noncutaneous B-cell proliferations may be analogous to the diverse group of cutaneous T-cell lymphoprolifer-
ative disorders (ie, lymphomatoid papulosis, Mucha-Haberman disease, and pagetoid reticulosis). Many of these lesions exhibit clonal Tγ gene rearrangements, although some patients with these disorders have a benign, indolent, and nonprogressive clinical course and others eventually develop cutaneous T-cell lymphoma.54,55

Finally, we must consider the significance of these results in light of the expanding application of antigen receptor gene rearrangement analysis in the differential diagnosis between benign lymphoid hyperplasia and malignant lymphoma. Clearly, very few of the patients we studied whose lesions contained clonal immunoglobulin heavy and light chain gene rearrangements developed overt malignant lymphoma, despite the conservative therapeutic approach generally used. Therefore, designating these extranodal lymphoid neoplasms as malignant lymphomas based on antigen receptor gene rearrangement analysis does not appear warranted at this time. It appears more appropriate to continue to designate them benign lymphoid hyperplasia, according to their histopathologic and immunophenotypic characteristics, but to comprehend that such lesions frequently contain occult clonal B-cell populations, are probably related pathogenetically to development of extranodal B-cell lymphoma, and therefore may represent prelymphomatous states. Therefore, abdicating the traditional therapeutic approaches for benign lymphoepithelial lesion of the salivary gland and chronic lymphocytic thyroiditis and treating the patients aggressively (eg, with systemic chemotherapy) also appears unwarranted at this time. The best therapeutic approach for patients with an extranodal lymphoid hyperplasia (eg, of the ocular adnexa) may be to establish that the patient truly belongs to clinical stage IE, irradiate the local disease site, and then carefully monitor the patient for development of additional evidence of lymphoid neoplasia. In our experience, only a few patients treated in this manner develop overt extranodal or systemic malignant lymphoma.56 The description and documentation of additional markers of malignant transformation should eventually allow us to distinguish more reliably between lymphoid hyperplasia and malignant lymphoma occurring in extranodal sites and provide additional insight into the pathogenesis and natural history of extranodal lymphoid neoplasms.

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Extranodal noncutaneous lymphoid hyperplasias represent a continuous spectrum of B-cell neoplasia: demonstration by molecular genetic analysis

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