Utility of Microdissection and Polymerase Chain Reaction for the Detection of Immunoglobulin Gene Rearrangement and Translocation in Primary Intraocular Lymphoma

De Fen Shen, PhD,[1] Zhengping Zhuang, MD, PhD,[2] Phuc LeHoang, MD,[3] Roland Böni, MD,[2] Sherman Zheng, MD, PhD,[4] Robert B. Nussenblatt, MD,[1] Chi-Chao Chan, MD[1]

Objective: Primary intraocular lymphoma, a non-Hodgkin's lymphoma, is a primary central nervous system lymphoma (PCNSL). Diagnosis is usually made by identifying malignant, large B lymphocytes in the vitreous, eye, brain, and cerebral spinal fluid; however, these cells are few, friable, and difficult to recognize. Recently, clonal heavy chain immunoglobulin (IgH) gene rearrangement and bcl-2 gene translocation have been reported in systemic B-cell lymphoma and are used for the detection of malignant cells and in making a diagnosis. The authors investigated the molecular changes in three eyes and a chorioretinal biopsy specimen of four patients with PCNSL.

Design: Human tissue study.

Materials: Five ocular specimens of PCNSL were collected.

Intervention: The first patient had a diagnostic enucleation of the left eye. The second patient underwent diagnostic chorioretinal biopsy. In the third case, a pair of autopsied eyes with reactive lymphoplasmacytic infiltrates of a patient with acquired immune deficiency syndrome (AIDS) were studied. In the fourth case, an enucleated eye of a patient with AIDS-associated lymphoma was sampled.

Main Outcome Measures: The bcl-2 and IgH genes of the lymphoma cells from routine, paraffin-embedded, formaldehyde-fixed, or frozen histologic tissue sections were analyzed using microdissection and polymerase chain reaction (PCR) technique.

Results: Lymphoma cells obtained from the above four cases showed IgH rearrangement gene in the third framework of the V_H region. Bcl-2-associated translocation also was detected in three cases (cases 1, 2, and 4).

Conclusion: Rearrangement of the IgH gene can serve as a molecular marker for PCNSL. Microdissection allows for procurement and analysis of specific, selected, minute cell populations that are obtained from histologic sections of the complex, heterogeneous tissue. Translocation of IgH and bcl-2, the apoptotic "survival" signal and proto-oncogene, could contribute to the pathogenesis of PCNSL. The combination of microdissection and PCR is a powerful tool for studies of small lesions and cell populations and for understanding disease mechanisms. *Ophthalmology 1998;105:1664–1669*

Primary intraocular lymphoma is a component of primary central nervous system lymphoma (PCNSL).[1–3] PCNSL is a large B-cell, non-Hodgkin's lymphoma that arises within the brain, spinal cord, leptomeninges, and/or the eye.[3,4] PCNSL with ocular involvement, which typically masquerades as a chronic uveitis, has been increasingly recognized. The disease is aggressive, and the 5-year survival rate is less than 33%.[5,6] An early correct diagnosis will allow prompt, appropriate treatment for patients with PCNSL and offer them the optimum chance of visual maintenance and long-term survival.

Because appropriate treatment of PCNSL involves an aggressive approach of radiation therapy or chemotherapy or both,[1,7,8] a definitive pathologic diagnosis is always needed. This must be based on the identification of lymphoma cells in the eye. However, the malignant cells may be rare and difficult to recognize, especially in patients with only ocular involvement without central nervous system lesions.[2,8] In a series of 12 cases of ocular PCNSL at the

Originally received: August 22, 1997.
Revision accepted: March 17, 1998. Manuscript no. 97485.

[1] Laboratory of Immunology, National Eye Institute, Bethesda, Maryland.

[2] Laboratory of Pathology, National Cancer Institute, Bethesda, Maryland.

[3] Department of Ophthalmology, Pitie-Salpetriere Hospital, Paris, France.

[4] Department of Pathology, New York University Medical Center, New York, New York.

Financial support: none.

The authors have no financial interest in any product described in this article.

Reprint requests to Chi-Chao Chan, MD, Building 10, Room 10N103, NIH/NEI, 10 Center Drive, MSC 1858, Bethesda, MD 20892-1858.

National Eye Institute, 30% had a previous false-negative biopsy specimen.[2] We have reported the association of interleukin-10 and the presence of PCNSL in the vitreous may be a useful clue in making a diagnosis; however, pathologic confirmation is still mandatory.[9,10]

The development of rapid polymerase chain reaction (PCR) protocols for the amplification of rearranged heavy chain immunoglobulin (IgH) gene sequence has facilitated the identification of clonal IgH rearrangements in systemic non-Hodgkin's lymphoma and leukemia of B-cell lineage.[11–15] The aberrant third framework region (FR3) of the IgH variable (V) region gene has been detected or rearranged in the majority of systemic lymphoma cases, particularly in nonfollicular, non-Hodgkin's lymphoma.[11,16] Successful amplification of the rearranged IgH gene on material extracted from frozen or paraffin-embedded formaldehyde-fixed samples has also been reported using the FR3 primer.[11,15,16]

The bcl-2 protein is expressed at highest levels in progenitor and long-lived cells in tissues characterized by apoptotic cell death.[17,18] In non-Hodgkin's lymphoma and possibly other malignancies, pathologic expression of bcl-2 protein is believed to be a fundamental event in oncogenesis.[19] The bcl-2 gene, located on chromosome 18, is involved in the t(14;18) chromosomal translocation.[20] The reciprocal t(14;18) chromosomal translocation brings the bcl-2 gene into juxtaposition with the IgH promoter located on chromosome 14. The resultant fusion gene causes bcl-2 gene deregulation and expression of the bcl-2 protein.[20] This chromosomal abnormality, resulting in production of high levels of bcl-2 protein, is observed in approximately 85% of follicular non-Hodgkin's lymphomas and in approximately 20% of diffuse large B-cell lymphomas.[21–23] Despite a significant correlation between bcl-2 protein expression and high relapse rate, there is a discrepancy between the frequency of high expression of the protein and the bcl-2 gene rearrangement in non-Hodgkin's lymphoma.[24,25]

The current study was designed to evaluate the molecular genetics of PCNSL in the eye. With microdissection and the PCR technique, we were able to detect gene rearrangement in the IgH variable region and bcl-2 major breakpoint region in the lesion of PCNSL.

Materials and Methods

Five ocular specimens were collected for the study under research protocols at the National Eye Institute and approved by the institutional review board. The first was an enucleated left eye obtained from a 48-year-old woman with PCNSL. Her detailed clinical history and histopathology of the eye were reported previously.[4] Briefly, the patient presented with bilateral progressive vitritis and multiple retinal infiltrates involving the optic nerve head. Later, she also had brain lesions develop that were detected by computed tomography. Her left eye became blind and was enucleated. Histopathologic analysis confirmed monoclonal B-cell lymphoma in the eye (Fig 1A).

The second specimen was a vitreoretinochoroidal biopsy specimen obtained from a 67-year-old woman. This patient had bilateral vitritis and chorioretinitis of unknown etiology develop. After inconclusive, noninvasive evaluation and significant deterioration of vision in her left eye, the patient's vitreoretinochoroidal biopsy was performed 6 weeks after initial presentation. Vitrectomy and cerebrospinal fluid specimens failed to identify neoplastic cells but showed an elevation of interleukin-10 levels. The frozen biopsy specimen sent to the National Eye Institute showed monoclonal B-lymphocytic infiltration without definite malignant cells in the retina (Fig 2). The diagnosis of PCNSL was made. Two months after the initial presentation, a right frontal mass was found by magnetic resonance imaging.

The third and forth specimens were a pair of autopsy eyes from a 29-year-old man with AIDS. Six months before his death, the patient's ocular examination disclosed bilateral typical cytomegalovirus retinitis and choroiditis compatible with mycobacterial infection. The patient died of multiple systemic opportunistic infection without manifestation of malignancy. Autopsy results of the eyes showed old cytomegalovirus retinitis with chorioretinal scar and retinal detachment. *Mycobacterium avium* was also found in the choroid. Aggregations of plasmacytic lymphoid proliferation were observed in the vitreous and beneath the detached retina (Figs 3A, B). Immunohistochemical staining of these lymphoid cells was inconclusive for lymphoma.

The fifth specimen was obtained from a patient with AIDS who lost vision in one eye secondary to a rapid growth of PCNSL involving the orbit. Later he had severe, uncontrollable pain develop, and the eye had to be enucleated. Histologic examination confirmed the diagnosis. The AIDS-associated lymphoma was characterized by many foci of large immunoblastic monoclonal B cells, mainly in the uveal tissue, and a necrotic retina (Fig 4A).

Microdissection was performed as described.[26,27] Briefly, either 5-μm frozen or 10% buffered formaldehyde fixed-paraffin sections were stained with hematoxylin and eosin. The paraffin sections required deparaffinization. Cells of interest were selected by visualization under the light microscope and microdissected using a 30-gauge needle. The histologic area of interest on the slide was gently scraped until the selected cells became detached from the tissue section. These loose cells were picked up carefully by the needle and immediately placed in a single-step extraction buffer containing 0.2 mg/ml proteinase K, which provides the starting point for PCR amplification.

DNA was extracted from the microdissected cells within 1.5 μl of protease K-enriched, single-step extraction buffer.[26,27] The PCR-amplifiable mixture contained the microdissected DNA; 400 nmol/l of ^{32}P-labeled sense primer; 400 nmol/l of antisense primer; 200 nmol/l of deoxyadenosine triphosphate (dATP), deoxycytidine triphosphate (dCTP), deoxyguanosine triphosphate (dGTP), and deoxythymidine triphosphate (dTTP); and 0.5 unit of Taq polymerase in a final volume of 10 μl of 1X Taq polymerase buffer containing 1.5 mmol/l of magnesium chloride (Perkin Elmer, Branchburg, NJ). The PCR reaction was performed for 35 cycles at 94° C for 45 seconds, 56° C for 1 minute, and 72° C for 1 minute. Two primers were used: FR3A, the third framework of the V_H region from the IgH gene: upstream, 5-ACA CGG CYS TGT ATT ACT GT-3 and downstream of the consensus J_H region, 5-GGA TGG TAT CAA GCT TTG AGG AGA CGG TGA CCA-3; and bcl-2, the bcl-2 major breakpoint region: upstream, 5-TTA GAG AGT TGC TTT ACG TGG CCT-3 and downstream of the consensus J_H region, 5-ACC TGA GGA GAC GGT GAC C-3.[11] The FR3A primers were synthesized by New York University Medical Center. Bcl-2 primers were purchased from Bio Synthesis, Lemisville, Texas. The expected sizes of PCR products for FR3A and bcl-2 are 100 to 120 base pairs and 100 to 300 base pairs, respectively.

The ^{32}P-labeled amplified DNA was analyzed on 16% polyacrylamide gel for IgH gene rearrangement product and on 3% agarose gel for bcl-2 gene product. The resulting autoradiogram was developed using Kodak X-Omat film (Eastman Kodak, Rochester, NY).

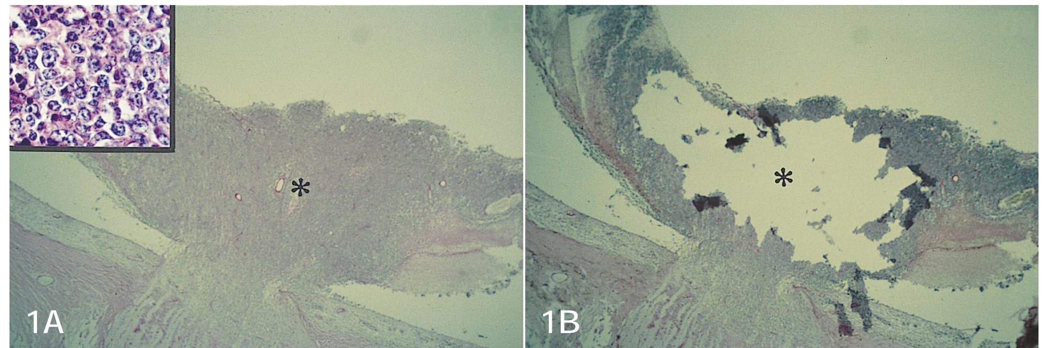

Figure 1. (Case 1) Photomicrographs showing a large lymphoma (asterisk) at the optic nerve head. **A,** premicrodissection, **B,** postmicrodissection. (Inset) Higher magnification showing the lymphoma cells (stain, hematoxylin–eosin; magnification, ×25; inset, magnification, ×400).

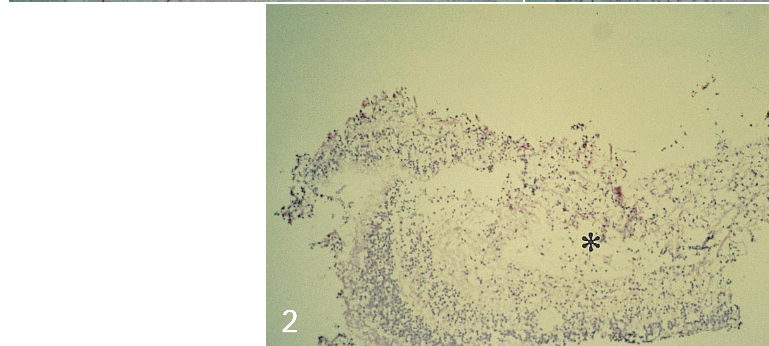

Figure 2. (Case 2) Photomicrograph showing many abnormal cells (asterisk) without characteristic neoplastic morphology in the retina (stain, hematoxylin–eosin; magnification, ×100).

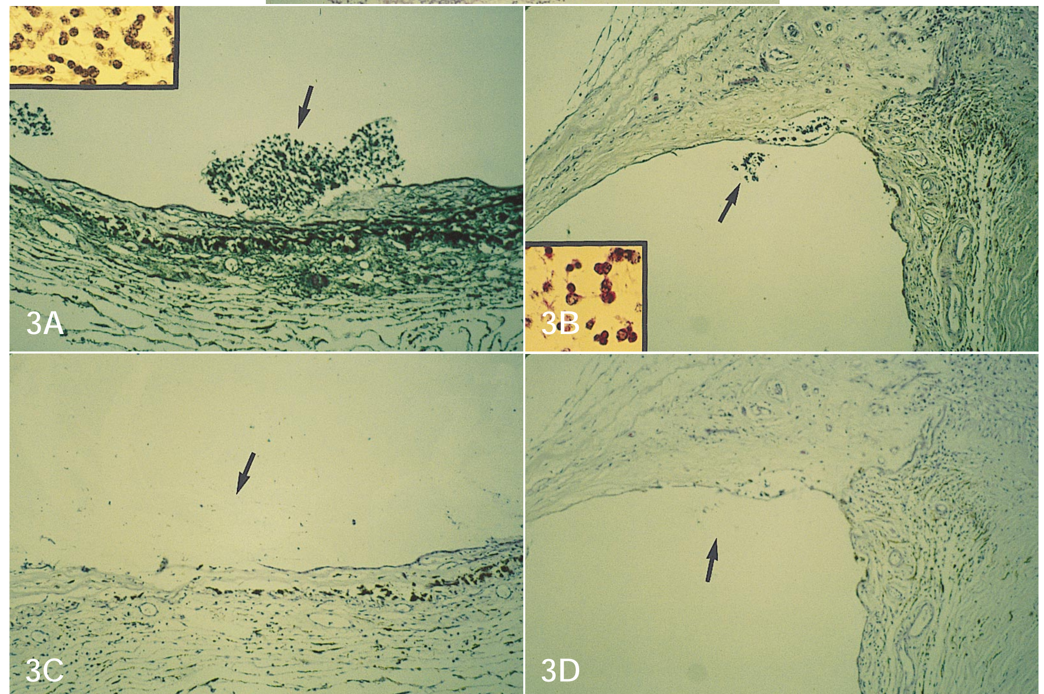

Figure 3. (Case 3) Photomicrographs showing lymphoid proliferative cells (arrow) in the subretinal spaces of both eyes. **A,B,** premicrodissection in the right and left eyes, respectively, **C,D,** postmicrodissection in the right and left eyes, respectively. (Inset) Higher magnification showing the lymphoma cells (stain, hematoxylin–eosin; magnification, ×100; inset, magnification, ×250).

Positive control DNA was obtained from a monoclonal B-lymphoma cell line 453. The negative control DNAs included specimens from the microdissected normal retina and a case with reactive lymphoid hyperplasia,[28] as well as a polyclonal B-cell line 472.

Results

Microdissection obtains the selected group of malignant (cases 1 and 4) and suspicious neoplastic (cases 2 and 3) cells presented on each histologic slide of each case (Figs 1B, 3C, 3D, and 4B). Therefore, the PCR product of microdissection represents the relatively homogeneous cell population obtained from a heterogeneous ocular tissue section. With these approaches, the appearance of one discrete band (monorearrangement) on the film is the expected result of the amplification of a DNA from a monoclonal B-cell population.

All selected DNAs from four cases were amplified successfully using FR3A primer (Fig 5). These data included the single tissue case in case 2, and the two eyes in specimens 3 and 4 did not have definite histologic conformation of the systemic diagnosis. One assumes the diagnosis was appropriate, although the first and last

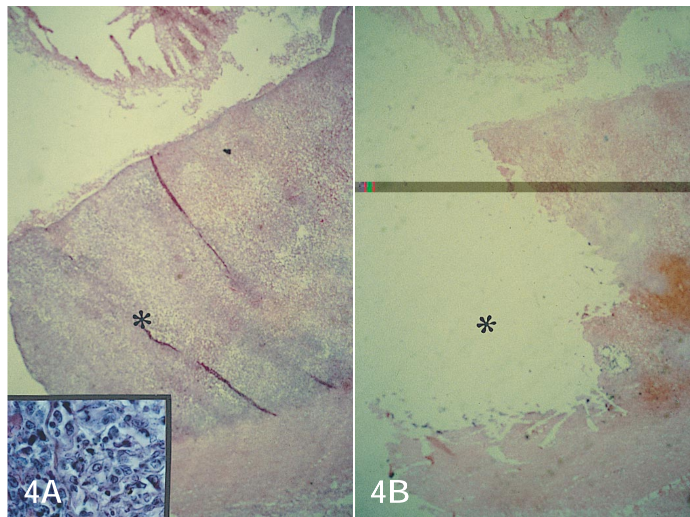

Figure 4. (Case 4) Photomicrographs showing multiple lymphoma nodules (asterisk) in the choroid. **A,** premicrodissection, **B,** postmicrodissection. (Inset) Higher magnification showing the lymphoma cells (stain, hematoxylin–eosin; magnification, ×25; inset, magnification, ×400).

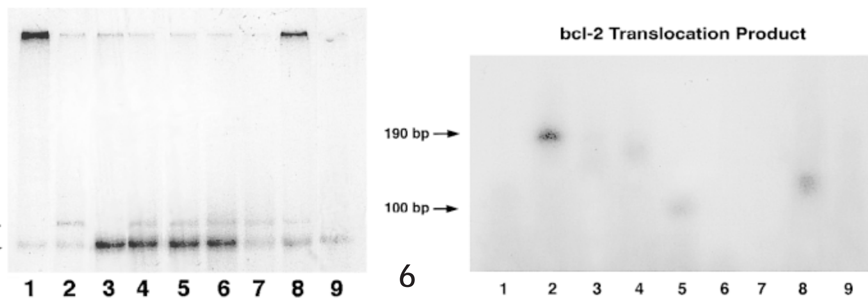

Figure 5. Polymerase chain reaction (PCR) amplification showing rearrangement of third framework region in the V_H region of heavy chain immunoglobulin gene in all five specimens. Lane 1, normal retina; lane 2, monoclonal B-cell line 453; lane 3, case 1 without microdissection; lanes 4 through 8, cases 1, 2, 3 (right and left eyes), and 4, respectively; lane 9, the case of choroidal reactive lymphoid hyperplasia. The PCR products are located in lanes 2, 4, 5, 6, 7, and 8. The primer–dimer pair is an artifactual product of a 3 end complementary.

Figure 6. Polymerase chain reaction amplification showing translocation of bcl-2 in two nonacquired immune deficiency syndrome cases (cases 1 and 2) and one AIDS case (case 4). Lane 1, polyclonal B-cell line 472; lane 2, monoclonal B-cell line 453; lane 3, case 1 without microdissection; lanes 4 through 8, cases 1, 2, 3 (right and left eyes), and 4, respectively; lane 9, the case of choroidal reactive lymphoid hyperplasia.

cases had further histologic evidence that PCNSL was the diagnosis and confirmed the results of IgH gene rearrangement: a molecular nonhistologic verification using microdissection and PCR.

DNAs from cases 1, 2, and 4 also showed amplification with bcl-2 primer (Fig 6). The positive DNA control specimen showed a sharp band accordingly and the negative control specimens were without the band (other ocular tumor or normal retina), multiple bands seen on the polyacrylamide gel, or a smear on the agarose gel (polyclonal B-cell line). No positive signals were detected on the collected cells without microdissection (e.g., from the whole eye section, lane 3; Figs 5 and 6).

Discussion

Using microdissection and PCR amplification, we have successfully detected monoclonal IgH gene rearrangements in all four cases of PCNSL, including the two cases without a decisive diagnosis (cases 2 and 3). The combination of IgH gene rearrangement and bcl-2/J_H is considered to be the most reliable diagnostic indication for systemic B-cell lymphoma.[11,15,16,20,21,25] These molecular changes have not been reported in PCNSL involving the eye. Clonal analyses by PCR-based IgH gene rearrangements have been applied to both frozen and paraffin-fixed archival tissue sections and can be useful in tissue diagnosis, particularly for those patients who present a diagnostic dilemma. Single-cell analysis of IgH gene rearrangement for diagnosing B-cell lymphoma can even be performed on paraffin-embedded archival sections that were stained previously by immunohistochemistry or nonisotopic in situ hybridization.

Even the most sophisticated genetic testing methods will be of limited value if the input DNA, RNA, or proteins are not derived from pure populations of cells exhibiting the characteristic disease morphology. This is the main reason that we have failed to detect clonal abnormalities from the whole eye section. Microdissection allows us to identify the lesion and select for the isolated group of neoplastic cells under microscopic visualization. This concept and technique are leading a rapid revolution in cancer molecular diagnostics.[29,30] The current study also proves microdissection to be a valuable tool for evaluation of a specific single-cell population.

Immunoglobulins manufactured by B lymphocytes consist of two identical pairs of light (or) and heavy (IgH) chains. IgH chain is formed by a variable and a constant domain. The genes for the variable (antigen-binding) domains are generated by recombination between separate

types of gene segments, termed V_H (variable), D_H (diversity), and J_H (joining). The variability of the IgH gene is linked to three regions designated as complementary determining regions (CDR), which are separated by four relatively conserved framework regions (FR).[31,32] CDR I and II are encoded by V_H genes. CDR III, the most variable region of immunoglobulin, is the result of both the random assortment of V_H, D_H, and J_H regions and the occurrence of deletions or insertions of nucleotides at the junction sites between V_H and D_H and between D_H and J_H.[15,33] To date, there is no proof that V gene rearrangement occurs at any significant frequency during normal B-cell differentiation.[34]

Clonal expansions of B cells carry identical copies of unique V_H–D_H–J_H junctional region sequences. Therefore, rearranged IgH genes are used as markers for lineage and clonality in human lymphoid neoplasm, and DNA sequences at the junction of V_H, D_H, and J_H segments can be used as clone-specific markers in individual patients with systemic lymphoma.[12,15,35–38] FR3A, the primer chosen to detect aberrant IgH gene in the current study, is based on a consensus sequence for codons 89 through 95 from 17 human V regions. This primer would be expected to generate a fragment of approximately 100 to 120 bases in length.[36]

Rearrangements of IgH genes, particularly in the V_H segment, have also been reported in AIDS-associated lymphoma.[39,40] PCR amplifies the presence and precise location of a neoplastic mutation: the aberrant IgH gene rearrangement in lymphoma. This highly sensitive molecular technique requires only small quantities of DNA that may be partially degraded, such as that extracted from paraffin-embedded tissues. In the current study, monoclonality of FR3 rearrangement (a single band on the gel) is detected in all five specimens obtained from four patients with and without AIDS. This is consistent with that of monoclonal B-cell line (positive control). In contrast, there is absence of single band in different negative control specimens including normal retina (no band), a case of choroidal lymphoid hyperplasia (no band), and polyclonal B-cell line (multiple bands).

In the t(14;18) chromosomal translocation, the bcl-2 gene is brought into close proximity to the J_H region of the IgH locus (the "major breakpoint cluster region" and "minor breakpoint cluster region" map within or flanking bcl-2, respectively), but leaves the coding sequence intact.[41,42] The association of the bcl-2 gene with the heavy chain locus results in high levels of bcl-2 expression. Although bcl-2 by itself is not a strong oncogene, its overexpression in hematopoietic cell lines leads to increased survival times and complements the oncogene, c-*myc,* in cell transformation.[18] Insertion of bcl-2 protein into the pre-B-cell line has been shown to prolong survival of these cells independent of growth factors. In transgenic mice, the presence of bcl-2 with immunoglobulin enhancer is associated with follicular hyperplasia.[43]

The t(14;18) chromosomal translocation creates a unique segment of DNA in the lymphoma cells composed of bcl-2/J_H sequence. Because the specific size of the generated fragment is derived from the junction between the bcl-2 and J_H gene, the size of the amplified sequence may vary among lymphoma samples obtained from different patients.[44,45] We have detected an amplified product of bcl-2/J_H in three of four cases, including an AIDS-associated lymphoblastic PCNSL (case 4). The other case of AIDS-related PCNSL (case 3), which has a reactive lymphoplasmacytic feature, is negative for bcl-2 rearrangement.

Instead of the bcl-2 gene, breakpoint involving the IgH locus and c-*myc* has been reported in AIDS-related lymphoma.[46,47] In the current study, the negative bcl-2 translocation at the major breakpoint cluster region may correspond to the relatively benign and early stage of B-cell neoplasm occurring in case 3, a patient with AIDS. In contrast, positive bcl-2 signals in the neoplastic DNA may result in rapid progression of the high-grade lymphoma developing in another patient with AIDS (case 4).

Although several morphologic criteria have been made as guidelines for histologic diagnosis of PCNSL, certain cases remain difficult to confidently classify. Characterization of the malignancy at the molecular level will aid in developing more sensitive detection methods for earlier and more accurate diagnosis. Specifically, the utilization of tissue microdissection and PCR amplification can be a practical and reliable method for diagnosing PCNSL. Early precise diagnosis for this devastating disease should have significant implications with respect to patient prognosis and recommendations for therapy. The increased understanding of the molecular events (IgH, bcl-2, and *myc* systems in PCNSL), including the individual steps of oncogenesis and their regulation, will provide a potential target for therapeutic intervention.

Acknowledgments. The authors thank Drs. Uday Desai and Grangadhara Sundar, Henry Ford Health Science Center, Detroit, for their contribution of the specimen (case 2).

References

1. Freeman LN, Schachat AP, Knox DL, et al. Clinical features, laboratory investigations, and survival in ocular reticulum cell sarcoma. Ophthalmology 1987;94:1631–9.
2. Whitcup SM, de Smet MD, Rubin BI, et al. Intraocular lymphoma. Clinical and histopathologic diagnosis. Ophthalmology 1993;100:1399–406.
3. Rockwood EJ, Zakov ZN, Bay JW. Combined malignant lymphoma of the eye and CNS (reticulum-cell sarcoma). Report of three cases. J Neurosurg 1984;61:369–74.
4. Lopez JS, Chan C-C, Burnier M, et al. Immunohistochemistry findings in primary intraocular lymphoma. Am J Ophthalmol 1991;112:472–4.
5. Rouwen AJP, Wijermans PW, Boen–Tan TN, Stilma JS. Intraocular non-Hodgkin's lymphoma treated with systemic and intrathecal chemotherapy and radiotherapy. A case report and review of the literature. Graefes Arch Clin Exp Ophthalmol 1989;227:355–9.
6. Peterson K, Gordon KB, Heinemann MH, DeAngelis LM. The clinical spectrum of ocular lymphoma. Cancer 1993; 72:843–9.
7. DeAngelis LM, Yahalom J, Thaler HT, Kher U. Combined modality therapy for primary CNS lymphoma. J Clin Oncol 1992;10:635–43.

8. Char DH, Ljung BM, Miller T, Phillips T. Primary intraocular lymphoma (ocular reticulum cell sarcoma) diagnosis and management. Ophthalmology 1988;95:625–30.
9. Chan C-C, Whitcup SM, Solomon D, Nussenblatt RB. Interleukin-10 in the vitreous of patients with primary intraocular lymphoma. Am J Ophthalmol 1995;120:671–3.
10. Whitcup SM, Stark–Vancs W, Wittes RE, et al. Association of interleukin 10 in the vitreous and cerebrospinal fluid and primary central nervous system lymphoma. Arch Ophthalmol 1997;115:1157–60.
11. Chen Y-T, Whitney KD, Chen Y. Clonality analysis of B-cell lymphoma in fresh-frozen and paraffin-embedded tissues: the effects of variable polymerase chain reaction parameters. Mod Pathol 1994;7:429–34.
12. Linke B, Bolz I, Fayyazi A, et al. Automated high resolution PCR fragment analysis for identification of clonally rearranged immunoglobulin heavy chain genes. Leukemia 1997; 11:1055–62.
13. Rockman SP. Determination of clonality in patients who present with diagnostic dilemmas: a laboratory experience and review of the literature. Leukemia 1997;11:852–62.
14. Jeffers MD, McCorriston J, Farquharson MA, et al. Analysis of clonality in cytologic material using the polymerase chain reaction (PCR). Cytopathology 1997;8:114–21.
15. Achille A, Scarpa A, Montresor M, et al. Routine application of polymerase chain reaction in the diagnosis of monoclonality of B-cell lymphoid proliferations. Diagn Mol Pathol 1995; 4:14–24.
16. Abdel–Rheheim FA, Edwards E, Arber DA. Utility of a rapid polymerase chain reaction panel for the detection of molecular changes in B-cell lymphoma. Arch Pathol Lab Med 1996;120: 357–63.
17. Hockenbery DM, Zutter M, Hickey W, et al. BCL2 protein is topographically restricted in tissues characterized by apoptotic cell death. Proc Natl Acad Sci U S A. 1991;88:6961–5.
18. Vaux DL, Cory S, Adams JM. Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. Nature 1988;335:440–2.
19. Nunez G, Seto M, Seremetis S, et al. Growth-and tumor-promoting effects of deregulated BCL2 in human B-lymphoblastoid cells. Proc Natl Acad Sci U S A 1989;86:4589–93.
20. Weiss LM, Warnke RA, Sklar J, Cleary ML. Molecular analysis of the t(14;18) chromosomal translocation in malignant lymphomas. N Engl J Med 1987;317:1185–9.
21. Jacobson JO, Wilkes BM, Kwiatkowski DJ, et al. bcl-2 rearrangements in de novo diffuse large cell lymphoma. Association with distinctive clinical features. Cancer 1993;72:231–6.
22. Pezzella F, Ralfkiaer E, Gatter KC, Mason DY. The 14;18 translocation in European cases of follicular lymphoma: comparison of Southern blotting and the polymerase chain reaction. Br J Haematol 1990;76:58–64.
23. Yunis JJ, Mayer MG, Arnesen MA, et al. bcl-2 and other genomic alterations in the prognosis of large-cell lymphoma. N Engl J Med 1989;320:1047–54.
24. Hermine O, Haioun C, Lepage E, et al. Prognostic significance of bcl-2 protein expression in aggressive non-Hodgkin's lymphoma. Blood 1996;87:265–72.
25. Hill ME, MacLennan KA, Cunningham DC, et al. Prognostic significance of BCL-2 expression and bcl-2 major breakpoint region rearrangement in diffuse large cell non-Hodgkin's lymphoma: a British National Lymphoma Investigation Study. Blood 1996;88:1046–51.
26. Zhuang Z, Bertheau P, Emmert–Buck MR, et al. A microdissection technique for archival DNA analysis of specific cell populations in lesions $<$ 1 mm in size. Am J Pathol 1995; 146:620–5.
27. Zhuang Z, Merino MJ, Chuaqui R, et al. Identical allelic loss on chromosome 11q13 in microdissected in situ and invasive human breast cancer. Cancer Res 1995;55:467–71.
28. Cheung MK, Martin DF, Chan CC, et al. Diagnosis of reactive lymphoid hyperplasia by choroidal biopsy. Am J Ophthalmol 1994;118:457–62.
29. Caldas C. Molecular staging of cancer: is it time? Lancet 1997;350:231.
30. Emmert–Buck MR, Bonner RF, Smith PD, et al. Laser capture microdissection. Science 1996;274:998–1001.
31. Tonegawa S. Somatic generation of antibody diversity. Nature 1983;302:575–81.
32. Early P, Huang H, Davis M, et al. An immunoglobulin heavy chain variable region gene is generated from three segments of DNA: V_H, D and J_H. Cell 1980;19:981–92.
33. Lewis SM, Agard E, Suh S, Czyzyk L. Cryptic signals and the fidelity of V(D)J joining. Mol Cell Biol 1997;17:3125–36.
34. Lewis SM. The mechanism of V(D)J joining: lessons from molecular, immunological, and comparative analyses. Adv Immunol 1994;56:27–150.
35. Ramasamy I, Brisco M, Morley A. Improved PCR method for detecting monoclonal immunoglobulin heavy chain rearrangement in B cell neoplasms. J Clin Pathol 1992;45:770–5.
36. Trainor KJ, Brisco MJ, Story CJ, Morley AA. Monoclonality in B-lymphoproliferative disorders detected at the DNA level. Blood 1990;75:2220–2.
37. Calvert RJ, Evans PA, Randerson JA, et al. The significance of B-cell clonality in gastric lymphoid infiltrates. J Pathol 1996; 180:26–32.
38. Armes JE, Southey M, Eades S, et al. Molecular analysis in the diagnosis of pediatric lymphomas. Pediatr Pathol Lab Med 1996;16:435–49.
39. Bessudo A, Cherepakhin V, Johnson TA, et al. Favored use of immunoglobulin V_H4 genes in AIDS-associated B-cell lymphoma. Blood 1996;88:252–60.
40. Eclache V, Magnac C, Pritsch O, et al. Complete nucleotide sequence of Ig V genes in three cases of Burkitt lymphoma associated with AIDS. Leuk Lymphoma 1996;20:281–90.
41. Brada M. Impact of molecular biology on our understanding of non-Hodgkin lymphoma. Eur J Cancer 1991;27:315–20.
42. Ratech H. The uses of molecular biology in hematopathology. Oncogenes as diagnostic markers in non-Hodgkin's malignant lymphoma. Am J Clin Pathol 1993;99:381–4.
43. McDonnell TJ, Deane N, Platt FM, et al. bcl-2-immunoglobulin transgenic mice demonstrate extended B cell survival and follicular lymphoproliferation. Cell 1989;57:79–88.
44. Stetler–Stevenson M, Raffeld M, Cohen P, Cossman J. Detection of occult follicular lymphoma by specific DNA amplification. Blood 1988;72:1822–5.
45. Lambrechts AC, Hupkes PE, Dorssers LCJ, van't Veer MB. Translocation (14;18)-positive cells are present in the circulation of the majority of patients with localized (stage I and II) follicular non-Hodgkin's lymphoma. Blood 1993;82:2510–6.
46. Groopman JE, Sullivan JL, Mulder C, et al. Pathogenesis of B cell lymphoma in a patient with AIDS. Blood 1986;67:612–5.
47. Werner CA, Döhner H, Joos S, et al. High-level DNA amplifications are common genetic aberrations in B-cell neoplasms. Am J Pathol 1997;151:335–42.