Lymphocytosis in the cerebrospinal fluid of a patient with chronic lymphocytic leukemia: the value of immunologic analysis[1]

Andrew J. Fishleder, M.D.
Fred V. Lucas, M.D.

The distinction of lymphomatous or leukemic lymphocytes from reactive lymphocytes in the cerebrospinal fluid (CSF) is of great clinical significance and is usually readily accomplished on morphologic grounds. However, the "malignant" lymphocytes of chronic lymphocytic leukemia (CLL) are mature and therefore closely resemble reactive lymphocytes in cytocentrifuged CSF preparation. We recently studied a patient with CLL [white blood cell (WBC) count of 30,700/μL with 72% lymphocytes] who developed a prominent lymphocytosis in the CSF (WBC, 1,700 μL with 98% lymphocytes). The morphology of the CSF lymphocytes suggested leukemic involvement of the central nervous system (CNS); however, immunologic analysis was not confirmatory. The peripheral blood lymphocytes were demonstrated to be monoclonal for kappa light chains consistent with a B-cell lymphoproliferative disorder, whereas 95% of the lymphocytes in the CSF were positive for T_{11} antigen, a marker for mature T-cells. This report demonstrates the utility of immunophenotyping studies as an adjunct to the differential diagnosis of neoplastic versus nonneoplastic involvement of the CNS in disorders such as CLL in which morphologic differentiation may be difficult.

Index terms: Leukemia, lymphocytic, chronic · Lymphocytosis

Cleve Clin Q 53: 213–216, Summer 1986

When lymphocytosis occurs in the cerebrospinal fluid (CSF) of patients with lymphoma or leukemia, it is of therapeutic importance that a reactive process be distinguished from lymphomatous infiltration of the central nervous system (CNS). In most cases, the distinction can be made on morphologic grounds; however, the malignant lymphocytes of chronic lymphocytic leukemia (CLL) may closely resemble reactive lymphocytes in cytocentrifuged CSF preparations.

We recently studied a patient with CLL who developed a prominent lymphocytosis in the CSF that consisted predominantly of mature lymphocytes, making the differentiation between viral meningitis and leukemic involvement of the CNS difficult. However, comparison of the immunologic phenotype of the lymphocytes in the peripheral blood and CSF indicated the reactive nature of the CSF population. This report demonstrates the value of lymphocyte subtyping in differentiating neoplastic from reactive lymphocytoses.

Case report

An 82-year-old white man with a three-year history of CLL presented with a severe bifrontal headache. Physical examination revealed a confused man with splenomegaly and skin lesions on his right upper extremity consistent with herpes zoster infection. The latter diagnosis was confirmed by a Tzanck test smear. A complete blood count (CBC) included a white blood cell (WBC) count of 30,700/μL with 72% lymphocytes. A lumbar puncture was performed and the fluid contained WBC, 1,700/μL (98% lymphocytes); red blood cell (RBC) count, 600/μL; protein, 255 mg/dL; and

[1] Department of Laboratory Hematology, The Cleveland Clinic Foundation. Submitted for publication Dec 1985; accepted Feb 1986. lp

0009/8787/86/02/0213/04/$2.00/0

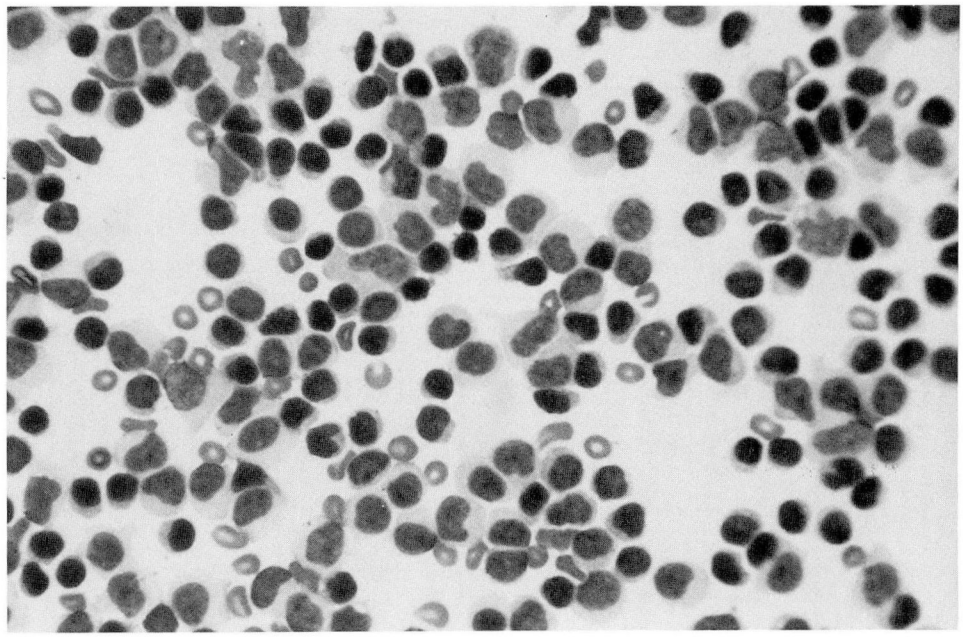

Fig. 1. Cerebrospinal fluid (Wright stain, ×400).

glucose, 33 mg /dL. The morphology of the lymphocytes was interpreted as being consistent with leukemic involvement of the CNS. Immunocytochemical analysis was then performed in an attempt to confirm this impression.

Materials and methods

Preparation of cerebrospinal fluid

Two milliliters of cerebrospinal fluid was obtained by lumbar puncture. Air-dried, cytospin preparations of undiluted CSF were made using a Shandon Cytospin 2 cytocentrifuge at 1,500 rpm for four minutes and a differential cell count was then performed on a Wright-stained specimen. Air-dried cytospin preparations kept at room temperature for 24 hours were immunostained by a direct immunoperoxidase technique[1] using antibodies for kappa and lambda light chains (Tago) and by an avidin-biotin-complex technique[2] using an antibody for T_{11} antigen (Coulter), which identifies mature T-cells. The substrate color-reaction product was developed using 3-amino-9-ethylcarbazole (AEC) for all immunostained procedures.

Preparation of peripheral blood

A 10-mL blood sample anticoagulated with heparin was passed through a Ficoll-Hypaque density gradient to allow separation of the lymphocyte population. The lymphocytes were resuspended in RPMI medium to a cell concentration of 10×10^6/mL and cytospin preparations were prepared from a 1:20 dilution of the cell suspension. A differential count was performed using a Wright-stained preparation and immunoperoxidase stains for kappa and lambda light chains, and T_{11} antigen were examined as for the CSF.

The cell suspension from the peripheral blood was also analyzed by flow cytometry using a FACS 440 (Becton-Dickinson) for kappa and lambda light chains as well as T_{11} antigen. The presence of surface immunoglobulin (SIg) was detected by standard techniques.

Results

The CSF obtained was clear and contained RBC, 600/μL and WBC, 1,700/μL. The differential WBC count included 98% lymphocytes, 1% reactive lymphocytes, and 1% neutrophils (*Fig. 1*). Immunostaining of cytocentrifuge preparations from the CSF revealed that the lymphocytes were mainly T-cell in origin (T_{11} Ag positive) (*Fig. 2*), with negative staining for kappa and lambda light chains indicating only rare B-cells to be present.

A CBC performed on the same day as the

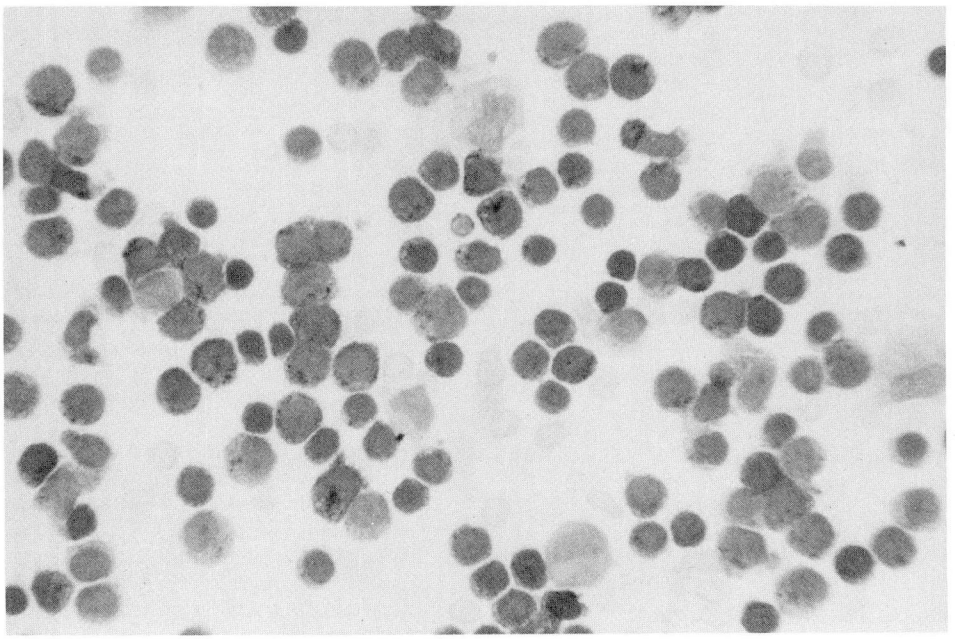

Fig. 2. Cerebrospinal fluid (immunoperoxidase stain, T_{11} antigen, ×400).

lumbar puncture revealed WBC, 30,700/μL with a differential including 72% lymphocytes. Flow cytometry and immunoperoxidase staining of the peripheral WBCs revealed a B-cell lymphoproliferative disorder that was monoclonal for kappa light chains as well as a smaller percentage of residual normal T-cells (*Table*). These results suggested that the lineage of the lymphocytes in the CSF (T-cells) was distinct from that in the peripheral blood (B-cells). Therefore, they were felt to be reactive, presumably to a viral meningitis associated with the herpes zoster infection.

The diagnosis of reactive lymphocytosis is further supported by subsequent progressively decreasing CSF WBC counts (1,700 to 105/μL) during the two-week hospitalization. A follow-up physical examination five weeks after discharge revealed the patient's encephalopathy to be essentially cleared.

Discussion

The differential diagnosis of lymphocytosis within the CSF includes a wide variety of infectious, noninfectious, and neoplastic processes. Viral meningitides are probably the most common cause of infectious lymphocytosis, but resolving bacterial meningitis may also demonstrate a predominantly lymphocytic population.[3] A number of demyelinating disorders, most notably multiple sclerosis, have also been associated with CSF lymphocytosis. In contrast to the mature or reactive lymphocytes that are identified in these two broad groups of disorders, the lymphoid cells present in lymphomatous leptomeningitis more commonly demonstrate a markedly atypical morphology in Wright-stained preparations. Overall, nonneoplastic lymphocytoses tend to demonstrate a morphologic continuum that extends from mature through reactive lymphocytes, whereas lymphomatous leptomeningitides demonstrate an atypical lymphoid population that is distinct from the mature lymphocyte population. Although the morphologic characterization of lymphomatous leptomeningitis is usually straightforward, in patients with chronic lymphocytic leukemia, the neoplastic population is composed of mature lymphocytes and the differentiation between mature or reactive lymphocytosis and involvement of the CNS by the lymphocytic leukemia can be difficult.[4]

With the development of techniques for immunologic typing of lymphocytic populations, it has become well recognized that most cases of chronic lymphocytic leukemia are of B-cell origin and are therefore monoclonal, composed of lymphocytes all possessing identical immunoglobulin

Table. Immunophenotypic studies

	Flow cytometry peripheral blood (%)	Immunocytochemistry peripheral blood	Immunocytochemistry CSF
Kappa light chains	49.5	Positive (72%)	Negative
Lambda light chains	6.9	Negative	Negative
T_{11} antigen	33.9	Positive (20%)	Positive (95%)

light chains on their surface.[5] In contrast, reactive B-cell proliferations are polyclonal and demonstrate different surface immunoglobulins.[6] It has been further demonstrated that the CSF lymphocytosis associated with most cases of viral meningitis[7–9] and multiple sclerosis[10–12] or other demyelinating disorders is T-cell in origin. In patients with known B-cell lymphoproliferative disorders, therefore, the immunologic subtype of CSF lymphocytes can readily distinguish a reactive from neoplastic lymphoid process. As illustrated, this can be particularly helpful in CLL where the Wright-stained morphology may be equivocal.

Andrew J. Fishleder, M.D.
Department of Laboratory Hematology
The Cleveland Clinic Foundation
9500 Euclid Ave.
Cleveland, OH 44106

References

1. Tubbs RR, Sheibani KA, Weiss RA, Sebek BA, Deodhar SD. Tissue immunomicroscopic evaluation of monoclonality of B-cell lymphomas. Comparison with cell suspension studies. Am J Clin Pathol 1981; **76:**24–28.
2. Hsu SM, Raine L, Fanger H. The use of antiavidin antibody and avidin-biotin-peroxidase complex in immunoperoxidase technics. Am J Clin Pathol 1981; **75:**816–821.
3. Kjeldsberg CR, Knight JA. Body Fluids. Laboratory Examination of Cerebrospinal, Synovial, and Serous Fluids: A Textbook Atlas. Chicago, American Society of Clinical Pathologists, 1982.
4. Poltorak M, Czlonkowska A, Nowicka K. Chronic lymphocytic leukemia: study of cell subsets in cerebrospinal fluid and peripheral blood. Eur Neurol 1983; **22:**289–292.
5. Tubbs RR, Fishleder A, Weiss RA, Savage RA, Sebek BA, Weick JK. Immunohistologic cellular phenotypes of lymphoproliferative disorders. Am J Pathol 1983; **113:**207–221.
6. Gale RP, Foon KA. Chronic lymphocytic leukemia. Recent advances in biology and treatment. Ann Intern Med 1985; **103:**101–120.
7. Frydén A, Kam-Hansen S, Maller R, Link H. Active and total T-cells in blood and cerebrospinal fluid during the course of aseptic meningitis. Acta Neurol Scand 1980; **61:**306–312.
8. Członkowska A, Półtorak M, Vainiene M, Czachorowska M, Korlak J. Differences between lymphocyte subsets of the cerebrospinal fluid in subacute sclerosing panencephalitis and acute aseptic meningitis. J Neuroimmunol 1981; **1:**173–181.
9. Pelc S, De Maertelaere D. CSF cells in tuberculous meningitis. Humoral and cellular response. J Neurol Sci 1981; **49:**223–228.
10. Booss J, Esiri MM, Tourtellotte WW, Mason DY. Immunohistological analysis of T lymphocyte subsets in the central nervous system in chronic progressive multiple sclerosis. J Neurol Sci 1983; **62:**219–232.
11. Hommes OR, Brinkman CJJ. T-cell subsets in spinal fluid of multiple sclerosis patients. J Neuroimmunol 1984; **6:**123–130.
12. Hafler DA, Fox DA, Manning ME, Schlossmann SF, Reinherz EL, Weiner HL. *In vivo* activated T lymphocytes in the peripheral blood and cerebrospinal fluid of patients with multiple sclerosis. N Engl J Med 1985; **312:**1405–1411.