Familial Chronic Lymphocytic Leukemia

JOSEPH F. FRAUMENI, JR., M.D., F.A.C.P., CHARLES L. VOGEL, M.D., and VINCENT T. DEVITA, M.D.

Bethesda, Maryland

SUMMARY **A battery of laboratory tests was applied in the study of a family with chronic lymphocytic leukemia in three sibs, the progeny of a consanguineous mating. Two survivors with leukemia and a sib showed impaired in vitro lymphocyte transformation in response to phytohemagglutinin and selective deficiency of serum immunoglobulin levels. Two other sibs showed only selective immunoglobulin deficiency. The array of findings suggests that a genetically controlled immune mechanism is involved in familial clusters of chronic lymphocytic leukemia.**

FAMILY AGGREGATION of leukemia offers an opportunity to study its cause. Laboratory determinations in the family studied suggested a heritable predisposition to immune abnormalities and chronic lymphocytic leukemia.

CASE REPORTS

Figure 1 shows the pedigree over three generations. The parents (I-1, 2) were second-cousins of Welsh ancestry and always lived in West Virginia. The proband (II-6) and six of the seven surviving sibs (II-7, 8, 10–13) were seen at the Outpatient Clinic of the National Institutes of Health (NIH) in July 1967 for detailed personal and family histories, physical examinations, and laboratory studies. Supplementary data were obtained from private physicians, hospital records, and death certificates. The sibs did not have an occupation in common nor were they exposed to known or suspected leukemogens. They lived apart from one another after childhood. None was unduly susceptible to infections, although each had recurrent boils as children and young adults. All had osteoarthritis, and those seen at NIH showed Heberden's nodes on examination and degenerative changes on bone X rays. Case histories of four sibs (three with leukemia and one with multiple immunologic abnormalities) are summarized below.

CASE 1

The proband (II-6), a 64-year-old white man, was seen in 1967. In 1950 he had complained of pain in both shoulders and had had slight lymphadenopathy. The total leukocyte count was normal, but relative lymphocytosis to 70% was observed on repeated tests over the next 2 years. A lymph node biopsy showed lymphadenitis. Symptoms then subsided. However, in 1958 he developed marked generalized lymphadenopathy and a leukocyte count of 140,000/mm³ with 93% lymphocytes. Bone marrow aspiration and cervical lymph node biopsy were diagnostic of chronic lymphocytic leukemia, which was treated with adrenal cortical steroids and a variety of antileukemia drugs. On physical examination in July 1967 the patient was chronically ill and cushingoid in appearance but had no lymphadenopathy or visceromegaly.

CASE 2

A brother (II-1) of the proband developed fatigue, cervical lymphadenopathy, and splenomegaly in 1960, when he was 65 years old. The leukocyte count was 58,000/mm³ and was mostly lymphocytes. Lymph node biopsy and bone marrow aspiration were consistent with chronic lymphocytic leukemia (a diagnosis confirmed on review of pathologic specimens at NIH). He received chemotherapy, splenic irradiation, adrenal cortical steroids, and transfusions. The course was complicated by progressive anemia and thrombocytopenia, with negative Coombs' tests. The patient died from sepsis at age 70. Autopsy showed bronchopneumonia, cholelithiasis with cholecystitis, and marked leukemic involvement of the liver, spleen, and mediastinal lymph nodes.

Received March 7, 1969; accepted April 30, 1969.

From the Epidemiology Branch and Solid Tumor Service of the Medicine Branch, National Cancer Institute, National Institutes of Health, Bethesda, Md.

Requests for reprints should be addressed to Joseph F. Fraumeni, Jr., M.D., 410 Wiscon Bldg., National Institutes of Health, Bethesda, Md. 20014

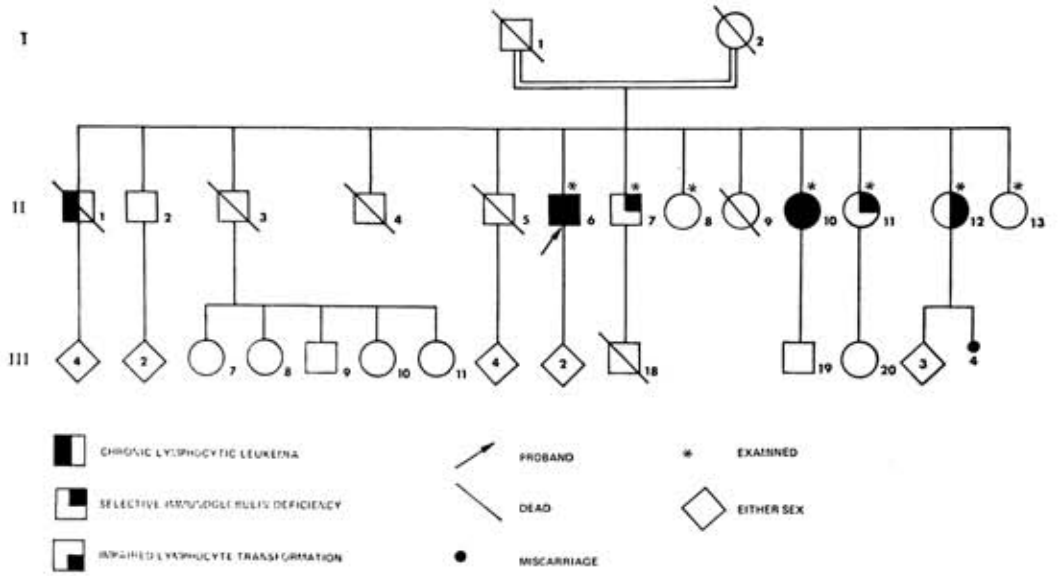

FIGURE 1. Pedigree of study family over three generations.

CASE 3

In 1956, during surgery for a cystocele, a 48-year-old sister (II-10) of the proband was found to be anemic. Further study of the peripheral blood and bone marrow aspirate was diagnostic of chronic lymphocytic leukemia. Adrenal cortical steroids were administered for 2 months and discontinued. The patient remained asymptomatic over the years and received no other treatment for the leukemia. Lymphocytosis and normochromic anemia were present on repeated laboratory studies. Physical examination in 1967 showed no lymphadenopathy, visceromegaly, or other abnormalities. X-ray study showed the spleen to be enlarged 4 cm below the left costal margin.

CASE 4

A sister (II-12), 53 years old in 1967, is reported here because of the striking immune defects discovered on laboratory study (*see* below). A throat infection and polyarthritis at age 25 were attributed to rheumatic fever and treated successfully with salicylates. At age 30 she developed increasing pain and stiffness in the hands and neck. Degenerative changes in the cervical vertebrae required surgical intervention in 1966, with removal of exostotic bone and a herniated disk and fusion of the cervical bodies. She had had ureterolithiasis on two occasions with spontaneous passage of calculi and had had four miscarriages. There was no history of repeated infections. Physical examination showed severe deformity of the proximal and distal interphalangeal joints of the hands, compatible with osteoarthritis. However, mixed involvement with rheumatoid arthritis or an autoimmune process was considered a possibility, especially in view of the early onset of symptoms, the positive bentonite flocculation test, and normochromic anemia on laboratory study (*see* below).

FAMILY HISTORY

Other sibs of the proband included a 64-year-old brother (II-3), who died with carcinoma of the prostate, and a 50-year-old brother (II-5), who died of far-advanced pulmonary tuberculosis. Two sibs (II-4, 9) died in infancy of undetermined causes. Five sibs (II-2, 7, 8, 11, 13) are in good health, and the four seen at NIH had no history of unusual findings and a normal physical examination. The father (I-1) of the proband died of Bright's disease at age 66. The mother (I-2) died at age 89 of high blood pressure with congestive heart failure.

Extensive inquiry into the remainder of the family history showed a few individuals with cancer or allied disorders. An 87-year-old paternal uncle and a 92-year-old maternal aunt died with carcinoma of the bladder. Of the 22 living members of the third generation, the oldest is 47 years old (III-1). The only death occurred in an infant with congenital pyloric stenosis and Rh incompatibility (III-18). One of the 22 living members (III-8) had a masculin-

TABLE 1. Hematologic Findings in Peripheral Blood and Bone Marrow of Seven Sibs

	II-6*	II-7	II-8	II-10*	II-11	II-12	II-13
Hemoglobin, *g/100 ml*	13.8†	13.2	12.3	11.0	12.4	10.8	12.6
Leukocytes, */mm³*	43.6	7.5	7.3	43.8	5.6	5.7	4.7
Platelets, */mm³*	175.0³	263.0³	231.0³	np‡	173.0³	294.0³	147.0³
Percent lymphocytes							
In peripheral blood, %	96	29	31	90	25	np‡	42
In bone marrow, %	90	10–15	30§	90	<10	5	<10
Blood group	A^+	A^+	A^+	O^+	A^+	O^+	A^+

* Patients with chronic lymphocytic leukemia.
† Patient recently transfused.
‡ np = not performed.
§ On repeat study, value was within normal range.

izing ovarian tumor removed at surgery, while III-11 had a colectomy for ulcerative colitis. A son of III-9 died in infancy with mongolism.

Laboratory Studies

HEMATOLOGY

The peripheral blood hemograms and bone marrow examinations of two surviving patients (II-6, 10) were diagnostic of chronic lymphocytic leukemia (Table 1). In addition, an asymptomatic sib (II-8) had 30% lymphocytes on bone marrow aspiration without other laboratory abnormalities, but a repeat examination performed elsewhere was within normal limits. Other sibs had isolated abnormalities—a low hemoglobin (II-12) and relative lymphocytosis in peripheral blood (II-13). Red blood cell indexes, distribution of blood groups, and a full battery of blood chemistries showed no deviations.

CYTOGENETIC STUDIES

Chromosomal analysis of bone marrow aspirates by the method of Tijo and Whang (1) showed no abnormalities in either the leukemic or normal sibs.

VIRUS-LIKE PARTICLES

Plasma obtained from each subject was examined by electron microscopy (2) and showed no C-type particles.

IMMUNOGLOBULIN STUDIES

Paper electrophoresis of serum proteins showed no abnormalities other than a low fraction of gamma globulin in the two leukemic patients and in one sib (II-12). Quantitative immunoglobulin determinations (3) by the Immunology Branch of the National Cancer Institute are shown in Table 2. There were decreased levels of IgG and IgM in the proband (II-6) and of IgA and IgM in the leukemic sib (II-10). A diminution of the IgM and IgG components occurred in II-12. In two other sibs selective deficiencies of IgM (II-7) and IgG (II-11) were found.

A qualitative serum electrophoresis was unremarkable. The bentonite flocculation test was

TABLE 2. Serum Immunoglobulin Levels and Percentage of Transformed Lymphocytes (in Leukocyte Cultures) Among Seven Sibs

Immunoglobulins, *mg/ml*	Normal Range	II-6*	II-7	II-8	II-10*	II-11	II-12	II-13
IgG	10.2–14.6	6.4	12.0	13.6	11.2	9.2	8.1	13.6
IgA	2.1– 3.5	2.15	3.03	4.2	1.22	3.53	2.13	4.9
IgM	0.8– 1.6	0.24	0.69	1.49	0.54	1.7	0.40	2.16
IgD	0.0– 0.3	0.041	0.063	0.017	0.018	0.050	0.026	0.181
Type K	6.0–10.0	2.6	9.6	9.2	8.5	6.8	7.2	13.3
Type L	3.3– 5.7	1.48	5.4	6.3	4.4	3.46	3.5	6.9
Transformed lymphocytes, %†	44.9–69.1	2	55	65	9	88	5	65

* Patients with chronic lymphocytic leukemia.
† In vitro response to phytohemagglutinin.

positive (1:128) in II-12, but it was negative in the other subjects. Lupus erythematosus preparations and Coombs' tests (direct and indirect) were negative in all sibs.

SKIN TESTS

A series of five antigens were administered as 0.1-ml intradermal injections in the forearm. The antigens were *Candida albicans* extract, 1:100; mumps antigen; intermediate strength purified protein derivative of tuberculin; *Trichophyton*, 1:30; and histoplasmin, 1:100. The tests were read from 48 to 72 hr, and induration equal to or greater than 5 mm in diameter was recorded as a positive reaction. There was no evidence of anergy among any sibs since each showed skin sensitivity to at least one antigen.

LYMPHOCYTE TRANSFORMATION

Lymphocytes were cultured at 37 C for 96 hr in 15-ml sterile, capped culture tubes using media TC 199 (with penicillin and streptomycin, NIH media unit) supplemented to 25% by fetal calf serum, with a final cell concentration of 1×10^6 cells/ml and a culture volume of 4 ml. Duplicate cultures were stimulated with 0.1 ml of phytohemagglutinin M.

Tritiated thymidine (0.1 μc/ml) was added to each culture 24 hr before harvesting. After harvesting, slides were prepared and covered with Kodak AR10 stripping film. They were exposed for 1 week at 4 C, developed, and stained with Giemsa stain.

The normal value for lymphocyte transformation harvested at 96 hr in this laboratory is 57% ($\pm$12.1). A clear impairment of transformation occurred in the two sibs with leukemia (II-6, 10) and in II-12 (Table 2).

Discussion

Features of this kindred not previously reported in familial chronic lymphocytic leukemia are the parental consanguinity and the immune defects detected in the nonleukemic sibs.

The compilation of case reports (4–11) strongly suggests a tendency for chronic lymphocytic leukemia to aggregate in certain kinships, especially among sibs. Most workers believe, partly because of the lack of evidence (as in the present study) for environmental leukemogens or for consistent chromosomal defects (5), that inherited factors are crucial in these family aggregates. In this country parental consanguinity has been reported infrequently in familial leukemia, but its occurrence in two sibships with striking aggregations of acute childhood leukemia (12, 13) suggests the influence of recessive genes. Recessive inheritance is implicated also in leukemia-prone disorders, such as Bloom's syndrome, Fanconi's aplastic anemia, and ataxia-telangiectasia (14). Furthermore, a recent study from Japan, where 4 to 5% of marriages are between first-cousins (15), showed parental consanguinity in four of seven sib-pairs with leukemia at various ages (16). None had chronic lymphocytic leukemia, a rare disease in Japan. (A similarly low frequency among Japanese migrants to the United States (17) suggests a genetically determined resistance rather than lack of exposure to environmental leukemogens.) Together with these observations, the parental consanguinity in the family under study suggests that recessive genes may play a role in the heritable predisposition to certain forms of leukemia. Environmental influences should not be discounted, however, since familial aggregates may reflect the interaction of genetic susceptibility and leukemogenic agents.

Chronic lymphocytic leukemia is characteristically accompanied by a variety of immunologic aberrations, including immunoglobulin deficiency (18) and impaired transformation of lymphocytes in vitro (19). Our study showed these immunologic abnormalities in apparently normal sibs of familial cases. The familial constellation of immune defects and leukemia may have etiologic significance in view of the predisposition of certain immune-deficiency disorders to lymphoma and lymphocytic leukemia (20, 21). In Dameshek's unifying scheme of the "immunoproliferative disorders," cellular growth within the immunocyte complex may be accelerated or "accumulative," as in chronic lymphocytic leukemia, or

otherwise disturbed, as in the autoimmune or immune-deficiency syndromes (22). The presence of immunologic incompetence in the leukemic and "normal" sibs of the family under study is consistent with [1] the overlapping of immunologic dysfunction and lymphocytic neoplasia within individuals and families, and [2] the notion that an inherited defect of immune mechanisms is causally related to familial aggregates of chronic lymphocytic leukemia (22).

Furthermore, our study suggests that in various members of the same family the presumed genetic defect may be expressed, clinically or subclinically, as different forms of the immunoproliferative process. Arthritis occurs excessively in hypogammaglobulinemic patients (21), and the rheumatic process in a nonleukemic sister (II-12) may have been related, in part, to her immune-deficiency state. The familial array of diseases brings to mind a report of three brothers with chronic lymphocytic leukemia and one sister with "severe rheumatoid arthritis and atopic dermatitis" (9). Familial immune defects may characterize the immunoproliferative disorders generally, since immunologic abnormalities have been described in asymptomatic relatives of patients with Waldenström's macroglobulinemia (23, 24), other monoclonal gammopathies (24), and acquired agammaglobulinemia (25). The possibility that immune defects underlie other forms of familial leukemia is suggested by a recent report of immunoglobulin deficiency among members of a family in which a father and son had "acute undifferentiated" leukemia (26). Longitudinal (follow-up) investigation of the immunologically deficient relatives in family aggregates of leukemia should clarify the role of immune mechanisms in leukemogenesis and may present an opportunity for identifying causal agents. It seems important, when leukemia occurs in unusual circumstances or patterns, to utilize etiologic studies to the fullest extent possible.

Acknowledgments

The authors are grateful to Dr. Jacqueline Whang-Peng for the cytogenetic studies, Dr. Albert J. Dalton for the electron microscopy, Dr. William Terry for immunoglobulin determinations, and Miss Charlene Denham for assistance with lymphocyte transformation studies. The skin-test antigens were provided by the Hollister-Stier Laboratories, Spokane, Wash. (*Trichophyton, Candida albicans* extract in the form of Dermatophytin O); Eli Lilly and Co., Indianapolis, Ind. (mumps antigen, purified protein derivative of tuberculin); and Parke, Davis and Co., Detroit, Mich. (histoplasmin). The tritiated thymidine was provided by Schwarz Bioresearch Inc., Orangeburg, N. Y.; and phytohemagglutinin M, by General Biochemicals, Chagrin Falls, Ohio.

References

1. Tjio, J. H., Whang, J.: Chromosome preparations of bone marrow cells without prior *in vitro* culture or *in vivo* colchicine administration. *Stain Techn.* 37: 17, 1962.
2. Porter, G. H., III, Dalton, A. J., Moloney, J. B., Mitchell, E. Z.: Association of electron-dense particles with human acute leukemia. *J. Nat. Cancer Inst.* 33: 547, 1964.
3. Fahey, J. L., McKelvey, E. M.: Quantitative determination of serum immunoglobulins in antibody-agar plates. *J. Immun.* 94: 84, 1965.
4. Gunz, F. W.: Leukaemia in New Zealand and Australia. *Path. Microbiol. (Basel)* 27: 697, 1964.
5. Fitzgerald, P. H., Crossen, P. E., Adams, A. C., Sharman, C. V., Gunz, F. W.: Chromosome studies in familial leukaemia. *J. Med. Genet.* 3: 96, 1966.
6. Portmann, U. V., Robinson, W. H.: Lymphatic leukemia occurring simultaneously in Negro brother and sister. *Cleveland Clin. Quart.* 18: 33, 1951.
7. Rigby, P. G., Pratt, P. T., Rosenlof, R. C., Lemon, H. M.: Genetic relationships in familial leukemia and lymphoma. *Arch. Intern. Med. (Chicago)* 121: 67, 1968.
8. Gunz, F. W., Dameshek, W.: Chronic lymphocytic leukemia in a family, including twin brothers and a son. *JAMA* 164: 1323, 1957.
9. Reilly, E. B., Rapaport, S. I., Karr, N. W., Mills, H., Carpenter, G. E.: Familial chronic lymphatic leukemia. *Arch. Intern. Med. (Chicago)* 90: 87, 1952.
10. Wisniewski, D., Weinreich, J.: Lymphatische Leukämie bei Vater und Sohn. *Blut* 12: 241, 1966.
11. Furbetta, D., Solinas, P.: Hereditary chronic lymphatic leukemia? *Proceedings of the Second International Congress of Human Genetics* 2: 1078, 1963.

12. JOHNSON, M. J. E., PETERS, C. H.: Lymphomas in four siblings. *JAMA* 163, 20, 1957.
13. STEINBERG, A. G.: The genetics of acute leukemia in children. *Cancer* 13: 985, 1960.
14. MILLER, R. W.: Persons with exceptionally high risk of leukemia. *Cancer Res.* 27: 2420, 1967.
15. SCHULL, W. J., NEEL, J. V.: *The Effects of Inbreeding in Japanese Children.* Harper and Row, Publishers, New York, 1965, p. 11.
16. KURITA, S., KAMEI, Y., TAKEI, T.: Clinical and genetic study on leukemia. *Jap. J. Clin. Hemat.* 8: 560, 1967.
17. HAENSZEL, W., KURIHARA, M.: Studies of Japanese migrants. I. Mortality from cancer and other diseases among Japanese in the United States. *J. Nat. Cancer Inst.* 40: 43, 1968.
18. FAHEY, J. L.: Antibodies and immunoglobulins. II. Normal development and changes in disease. *JAMA* 194: 255, 1965.
19. SCHREK, R.: Effect of phytohemagglutinin on lymphocytes from patients with chronic lymphocytic leukemia. *Arch. Path. (Chicago)* 83: 58, 1967.
20. FRAUMENI, J. F., JR., MILLER, R. W.: Epidemiology of human leukemia: recent observations. *J. Nat. Cancer Inst.* 38: 593, 1967.
21. MEDICAL RESEARCH COUNCIL: Hypogammaglobulinaemia in the United Kingdom. *Lancet* 1: 163, 1969.
22. DAMESHEK, W.: Chronic lymphocytic leukemia—an accumulative disease of immunologically incompetent lymphocytes. *Blood* 29 (suppl.): 566, 1967.
23. SELIGMANN, M., DANON, F., MIHAESCO, C., FUDENBERG, H. H.: Immunoglobulin abnormalities in families of patients with Waldenström's macroglobulinemia. *Amer. J. Med.* 43: 66, 1967.
24. WILLIAMS, R. C., JR., ERICKSON, J. L., POLESKY, H. F., SWAIM, W. R.: Studies of monoclonal immunoglobulins (M-components) in various kindreds. *Ann. Intern. Med.* 67: 309, 1967.
25. KAMIN, R. M., FUDENBERG, H. H., DOUGLAS, S. D.: A genetic defect in "acquired" agammaglobulinemia. *Proc. Nat. Acad. Sci. USA* 60: 881, 1968.
26. TWOMEY, J. J., LEVIN, W. C., MELNICK, M. B., TROBAUGH, F. E., ALLGOOD, J. W.: Laboratory studies on a family with a father and son affected by acute leukemia. *Blood* 29: 920, 1967.