The Control of Leukemia in Relation to Its Etiology and Pathogenesis[1]

HENRY S. KAPLAN

Department of Radiology, Stanford University School of Medicine, Palo Alto, California

SUMMARY

A general discussion of approaches to the prevention and cure of human leukemia is presented, based upon contemporary investigations on the etiology and pathogenesis of the murine leukemias. Many of these are known to be caused by viral agents, in some instances potentiated by physical or chemical agents, which act upon a specific cell population, the susceptibility of which may be significantly modified by constitutional and environmental factors. It is, therefore, conceivable that leukemia in mouse or man, if successfully eradicated, might be induced again by persistent virus. It is suggested that conventional treatment, which is presently aimed at the destruction of the leukemic cell population and at supportive treatment, should be supplemented by measures directed toward the inhibition of viral replication and the diminution of target-cell susceptibility.

This symposium is focused on obstacles to the control of leukemia in man. The term "control" generally refers both to the prevention of a disease and to its cure. Although I anticipate that most of our attention will be directed toward the latter problem, any discussion of the implications for leukemia control of our current knowledge of the process of leukemia development may properly address itself to both facets of the problem of control.

Such a discussion must, of necessity, extrapolate from the body of experimental evidence derived from studies of mouse leukemia. The relationship of leukemia in the mouse to leukemia in man has been extensively discussed (5, 8, 25). The thymic lymphomas of the mouse evolve in a manner that rather closely simulates the clinical pattern of lymphosarcoma and chronic lymphatic leukemia in man, and chronic myeloid leukemia in the mouse is probably also the counterpart of the corresponding variant in man. However, there is controversy about whether any murine counterpart of acute human leukemia exists, and monocytic leukemia is exceedingly rare in the mouse, if it occurs at all. Under these circumstances, it would seem appropriate to confine our extrapolations to general principles, rather than to specific details of leukemogenesis.

PREVENTION OF LEUKEMIA

Prevention by avoiding exposure to or diminishing the activity of leukemogenic agents.—Avoidance of exposure obviously requires that we know the identity of the agents that are leukemogenic for man. At present, convincing evidence for leukemogenic activity has been presented only for ionizing radiation (3); there is also much circumstantial evidence to implicate benzol as a human leukemogen (19). Recently, because of the discovery of several viruses that are leukemogenic in the mouse, there has been mounting interest in the possibility that some forms of human leukemia may also be due to viruses. However, no candidate virus has yet been identified, and the problem of fulfilling Koch's postulates for the identification of a human leukemogenic virus remains a formidable one.

A number of leukemogenic agents have been positively identified in the mouse: the carcinogenic hydrocarbons (42, 43), ionizing radiations (9, 23), estrogens (32), urethan (4, 6, 30), and several viruses (7, 11, 12, 41, 44). Moreover, a link between the external carcinogens and viral induction of mouse leukemia has now been established in the case of ionizing radiation (13, 22, 33, 36) and perhaps also with the carcinogenic hydrocarbons (48).

Exposure to ionizing radiation or to benzol clearly cannot account for more than a small fraction of human leukemias. Until agents that account for the great bulk of cases have been positively identified, there is little that can be done in the way of prevention. If these major leukemogens should prove to be viruses, the possibility of vaccination may be entertained. However, our optimism about this possibility should be tempered by the fact that several of the mouse leukemia viruses have proved to be exceedingly weak antigens, although they do induce tissue-specific antigenic responses (31, 46).

Prevention by regulation of host susceptibility factors.—The susceptibility of the host to the leukemogenic action of any agent has been shown in the mouse to be determined by a number of constitutional and environmental factors, which collectively have a decisive influence on the process of leukemia development (25, 34, 49). The factors of importance include age, sex, nutrition, hormonal

[1] Much of the experimental work cited herein was supported by Grant CA-03352 from the National Cancer Institute, NIH, USPHS.

balance (29), and, in the case of the lymphatic leukemias, the presence of an intact thymus (24, 27, 35, 38, 39). Although the thymus has recently been shown to play an important role in the evolution of immunologic competence (40), there is as yet no evidence to link this newly discovered function of the thymus to its important role in murine leukemogenesis. Constitutional factors that tend to stimulate thymic growth augment the leukemogenic response, whereas those which involute the thymus are inhibitory (29). Moreover, there appears to be a good general correlation between the presence in the thymus of an abundant population of highly immature lymphoid cells and susceptibility to lymphatic leukemia development (1, 26).

Virtually nothing is known about the role of constitutional factors in human leukemogenesis. Although a number of retrospective epidemiologic studies have been conducted, they have shed little light on this problem. In terms of experimental design, the prospective type of study appears more promising. In such a study, a defined population of persons would be subjected to an extensive battery of clinical and laboratory studies to characterize them as thoroughly as possible with respect to constitutional factors that might contribute to leukemia susceptibility, such as the presence of disturbances in the hematopoietic system, chromosomal abnormalities, hormonal imbalances, abnormal immunologic reactions, and serologic or other evidence of latent or active viral infection. Such an intensively studied population would then be followed for a number of years to determine whether those persons in the population who had exhibited 1 or more of these kinds of abnormality were, in fact, the persons destined to develop leukemia or whether incidence of leukemia was distributed randomly with respect to such constitutional factors. However, the very low incidence of leukemia in the general population would make a study in unselected persons logistically prohibitive. Such prospective studies could be undertaken only in selected populations of persons in whom a very significantly elevated incidence of leukemia could be anticipated. Recently, 2 candidate groups have emerged: (*a*) mongols and (*b*) identical twins of leukemic persons. There is now enough evidence on the association of mongolism and leukemia to suggest that incidence of leukemia is concentrated perhaps 30–50-fold in persons with mongolism (18, 47). The fact that such persons are often institutionalized would also tend to diminish the difficulty of conducting a prospective study. The observation of pairs of leukemic twins in retrospective studies has recently led to a more specific analysis, which suggests that there may indeed be a remarkably high incidence of concordance in identical twins (37). Although identical twins are not appreciably more susceptible to leukemia than persons of corresponding age in the general population, if 1 twin should get leukemia, the probability that the other twin will also become leukemic may be as high as 1 in 9. It would therefore seem that serious consideration should be given to prospective studies on 1 or both of these candidate populations.

CURE

The principal goal of conventional treatment in leukemia has been the eradication of the leukemic cell population by anti-metabolites or cytotoxic agents. The great toxicity of many of these agents has, in many instances, caused such severe injury to the normal tissues as to require discontinuation of treatment. Such experiences have gradually led to the explicit recognition of a second major goal of treatment: supportive measures aimed at the maintenance or restitution of homeostasis in the patient. If we now recognize the increasing probability that a substantial fraction of cases of human leukemia will ultimately prove to be due to viral agents, then due consideration must be given to the possibility that the successful achievement of the 1st 2 objectives might be frustrated by the persistence of the leukemogenic virus and the induction of a 2nd round of leukemia. A truly comprehensive program for the treatment of leukemia must take this potential hazard into account and should therefore include measures aimed at *prevention* of the reinduction of leukemia.

Elimination of the leukemic cell population.—The successful transfer of leukemia from 1 mouse to another by the inoculation of a single cell (10, 16) indicates that the persistence of even a single viable leukemic cell in a patient with leukemia might frustrate therapeutic attempts at cure by permitting renewed proliferation of the leukemic cell population. This point of view is supported by the results of attempts to eradicate autochthonous leukemias and lymphomas in the mouse by the use of large doses of steroids or X-rays (28); recrudescence could be traced in many instances to the persistence of viable tumor cell foci. It has been demonstrated that the transplantation in mice of leukemic cells bearing a recognizable karyotypic abnormality leads to the proliferation and dissemination of a leukemic cell population that exhibits the same karyotypic abnormality. The recent quantitative studies of Skipper and his associates, which will be presented here, lend additional support to the view that the cure of leukemia requires the eradication of every leukemic cell. Recent quantitative studies suggest that the killing of leukemia cells by irradiation (17) and perhaps also by chemotherapeutic agents follows an exponential dose-response relationship (after an initial "shoulder"). This would make it possible to estimate tumoricidal dose levels of such agents. For any one class of agent, such tumoricidal dose levels are beyond the limits of tolerance of the normal tissues. Nonetheless, this quantitative approach may make it possible to predict the effects of multiple agents used in tandem in such a way as to maximize the destruction of leukemic cells and to minimize normal tissue injury. We should keep in mind the possibility that immunologic approaches also hold some promise of effective and much more highly selective cytotoxic attack upon the leukemic cell population. The potentialities of this approach will be discussed by Dr. Day.

Another approach to the elimination of the leukemic cell population deserves mention. There is increasing evidence that sustained replication of either the complete virus or at least a major part of its deoxyribonucleic acid

(DNA) or ribonucleic acid (RNA) may be essential for the maintenance of neoplastic behavior in virus-induced cancers and leukemias (14, 21). In other words, if we could rid a virus-induced cancer or leukemia cell of its oncogenic virus, it might revert to normal, nonneoplastic behavior. We have now begun to learn how viruses appropriate the metabolic machinery of their host cells for the replication of the viral genome and protein components. It appears that the RNA viruses are capable of inducing in their host cells the formation of a completely new enzyme protein: an RNA-dependent RNA polymerase (2), which synthesizes new viral RNA on the old viral RNA template. Since such enzymes apparently do not exist and have no recognizable normal function in the host cell, agents capable of selectively inhibiting the viral RNA polymerases might conceivably disengage the cell from vira lontrol and thus enable the tumor cell population to lose its neoplastic attributes. The highly selective inhibition of RNA polymerase by actinomycin D, which competes for the enzyme's binding site on DNA (15, 45), encourages the hope that equally selective and potent anti-viral chemotherapeutic agents may exist.

Suppression of the leukemogenic virus.—Latarjet (Institut Curie, Paris, personal communication) has demonstrated that radiation-inactivated preparations of Rous virus can strongly inhibit the production of Rous virus in cell cultures *in vitro*; he has now obtained evidence suggesting that irradiated cell-free extracts from strain AK mouse leukemias, when injected into young AK mice, can significantly reduce the incidence of leukemia. On the basis of these encouraging results, Latarjet has now proposed that patients with malignant lymphomas and leukemias might receive, in addition to conventional therapy, experimental adjunct treatment with irradiated cell-free extracts of their own leukemic cells, which might contribute to the control of their disease through interference with the replication of residual virus.

The capacity to produce interferon (20) appears to vary widely from one virus to another and even among mutants of the same virus. However, interferon produced by one virus can effectively inhibit the replication of other viruses. Little is known about the capacity of the leukemia viruses to induce interferon production; however, advantage could be taken of the nonspecificity of its anti-viral action by deliberately infecting patients with innocuous viruses that are known to be good interferon producers. Finally, selective anti-viral chemotherapeutic agents, if they become available, should help to eliminate residual leukemogenic virus from successfully treated patients.

Reduction of the substrate cell population.—Suppression of the constitutional susceptibility of the treated leukemic individual should be considered. Perhaps it could be suppressed by such measures as maintenance doses of adrenal steroid hormones, which by their lympholytic action might reduce the population of susceptible cells on which residual virus could act to induce a 2nd round of leukemia.

Supportive measures: maintenance or restitution of homeostasis.—Measures aimed at the control of hemorrhage by platelet transfusion and at the control of infection by granulocyte transfusion will be discussed in detail in this symposium and need not be considered further here.

In summary, contemporary investigations on the etiology and pathogenesis of the murine leukemias indicate that many, and perhaps all, of these are caused by viral agents, in some instances potentiated by physical or chemical agents, which act upon a specific cell population, whose susceptibility may be significantly modified by constitutional and environmental factors. It is therefore conceivable that leukemia in mouse or man, if successfully eradicated, might be induced again in the same host by persistent virus. It is suggested that conventional treatment, which is presently aimed at the destruction of the leukemic cell population and at supportive treatment, should be supplemented by measures directed toward the inhibition of viral replication and the diminution of target-cell susceptibility.

REFERENCES

1. Axelrad, A. A., and Van der Gaag, H. C. Susceptibility to Lymphoma Induction by Gross' Passage A Virus in C3Hf/Bl Mice of Different Ages: Relation to Thymic Cell Multiplication and Differentiation. J. Natl. Cancer Inst., *28:* 1065–93, 1962.
2. Baltimore, D., and Franklin, R. M. Preliminary Data on a Virus-specific Enzyme System Responsible for the Synthesis of Viral RNA. Biochem. Biophys. Res. Commun., *9:* 388–92, 1962.
3. Cronkite, E. P., Moloney, W., and Bond, V. P. Radiation Leukemogenesis, an Analysis of the Problem. Am. J. Med., *28:* 673–82, 1960.
4. Doell, R. G., and Carnes, W. H. Urethan Induction of Thymic Lymphoma in C57BL Mice. Nature, *194:* 588, 1962.
5. Dunn, T. B. Normal and Pathologic Anatomy of the Reticular Tissue in Laboratory Mice, with a Classification and Discussion of Neoplasms. J. Natl. Cancer Inst., *14:* 1281–1433, 1954.
6. Fiore-Donati, L., Chieco-Bianchi, L., De Benedictis, G., and Maiorano, G. Leukaemogenesis by Urethan in New-born Swiss Mice. Nature, *190:* 278–79, 1961.
7. Friend, C. Cell-free Transmission in Adult Swiss Mice of a Disease Having the Character of a Leukemia. J. Exptl. Med., *105:* 307–18, 1957.
8. Furth, J., Ferris, H. W., and Reznikoff, P. Relation of Leukemia of Animals to Leukemia of Man. J. Am. Med. Assoc., *105:* 1824–30, 1935.
9. Furth, J., and Furth, G. B. Neoplastic Disease Produced in Mice by General Irradiation with X-rays. Am. J. Cancer, *28:* 54, 1936.
10. Furth, J., and Kahn, M. C. The Transmission of Leukemia of Mice with a Single Cell. *Ibid.*, *31:* 276–82, 1937.
11. Graffi, A. Chloroleukemia of Mice. Ann. N. Y. Acad. Sci., *68:* 540–58, 1957.
12. Gross, L. "Spontaneous" Leukemia Developing in C_3H Mice Following Inoculation, in Infancy, with AK-leukemic Extracts, or AK-embryos. Proc. Soc. Exptl. Biol. Med., *76:* 27–32, 1951.
13. ———. Attempt To Recover Filterable Agent from X-ray-induced Leukemia. Acta Haematol., *19:* 353–61, 1958.
14. Habel, K. Antigenic Properties of Cells Transformed by Polyoma Virus. Cold Spring Harbor Symp. Quant. Biol., *27:* 433–38, 1962.
15. Haselkorn, R. Actinomycin D as a Probe for Nucleic Acid Secondary Structure. Science, *143:* 662–84, 1964.
16. Hewitt, H. B. Studies of the Dissemination and Quantitative Transplantation of a Lymphocytic Leukaemia of CBA Mice. Brit. J. Cancer, *12:* 378–401, 1958.
17. Hewitt, H. B., and Wilson, C. W. Further Studies Relating to the Implications of Radiation Survival Curve Data for

Treatment of CBA Mouse Leukemia by Whole-Body Irradiation. *Ibid.*, *14:* 186–94, 1960.
18. Holland, W. W., Doll, R., and Carter, C. O. The Mortality from Leukaemia and Other Cancers among Patients with Down's Syndrome (Mongols) and among Their Parents. *Ibid.*, *16:* 177–86, 1962.
19. Hunter, F. T. Chronic Exposure to Benzene (Benzol). II. The Clinical Effects. J. Ind. Hyg. Toxicol., *21:* 331–54, 1939.
20. Isaacs, A. Antiviral Action of Interferon. Brit. Med. J., *ii:* 353–55, 1962.
21. Ito, Y., and Evans, C. A. Induction of Tumors in Domestic Rabbits with Nucleic Acid Preparations from Partially Purified Shope Papilloma Virus and from Extracts of the Papillomas of Domestic and Cottontail Rabbits. J. Exptl. Med., *114:* 485–500, 1961.
22. Jenkins, V. K., and Upton, A. C. Cell-free Transmission of Radiogenic Myeloid Leukemia in the Mouse. Cancer Res., *23:* 1748–55, 1963.
23. Kaplan, H. S. Observations on Radiation-induced Lymphoid Tumors of Mice. *Ibid.*, *7:* 141–47, 1947.
24. ———. Influence of Thymectomy, Splenectomy, and Gonadectomy on Incidence of Radiation-induced Lymphoid Tumors in Strain C_{57} Black Mice. J. Natl. Cancer Inst., *11:* 83–90, 1950.
25. ———. On the Etiology and Pathogenesis of the Leukemias: *A Review*. Cancer Res., *14:* 535–48, 1954.
26. ———. The Role of Cell Differentiation as a Determinant of Susceptibility to Virus Carcinogenesis. *Ibid.*, *21:* 981–83, 1961.
27. Kaplan, H. S., Carnes, W. H., Brown, M. B., and Hirsch, B. B. Indirect Induction of Lymphomas in Irradiated Mice. I. Tumor Incidence and Morphology in Mice Bearing Non-irradiated Thymic Grafts. *Ibid.*, *16:* 422–25, 1956.
28. Kaplan, H. S., and Nagareda, C. S. On the Possibility of Cure of Malignant Lymphoid Tumors. I. Treatment of Autochthonous Lymphoid Tumors in C_{57}BL Mice with Massive Doses of Lymphocytolytic Agents. Blood, *18:* 166–75, 1961.
29. Kaplan, H. S., Nagareda, C. S., and Brown, M. B. Endocrine Factors and Radiation-induced Lymphoid Tumors of Mice. Recent Progr. Hormone Res., *10:* 293–338, 1954.
30. Kawamoto, S., Ida, N., Kirschbaum, A., and Taylor, G. Urethan and Leukemogenesis in Mice. Cancer Res., *18:* 725–29, 1958.
31. Klein, G., Sjögren, H. O., and Klein, E. Demonstration of Host Resistance against Isotransplantation of Lymphomas Induced by the Gross Agent. *Ibid.*, *22:* 955–61, 1962.
32. Lacassagne, A. Sarcomes lymphoïdes apparus chez des souris longuement traitées par des hormones oestrogènes. Compt. Rend. Soc. Biol., *126:* 193, 1937.
33. Latarjet, R., and Duplan, J.-F. Experiment and Discussion on Leukaemogenesis by Cell-free Extracts of Radiation-induced Leukaemia in Mice. Intern. J. Radiation. Biol., *5:* 339–44, 1962.
34. Law, L. W. Present Status of Nonviral Factors in the Etiology of Reticular Neoplasms of the Mouse. Ann. N. Y. Acad. Sci., *68:* 616–35, 1957.
35. Law, L. W., and Miller, J. H. The Influence of Thymectomy on the Incidence of Carcinogen-induced Leukemia in Strain DBA Mice. J. Natl. Cancer Inst., *11:* 425–38, 1950.
36. Lieberman, M., and Kaplan, H. S. Leukemogenic Activity of Filtrates from Radiation-induced Lymphoid Tumors of Mice. Science, *130:* 387–88, 1959.
37. MacMahon, B., and Levy, M. A. Prenatal Origin of Childhood Leukemia: Evidence from Twins. New Engl. J. Med. (in press).
38. McEndy, D. P., Boon, M. C., and Furth, J. On the Role of Thymus, Spleen, and Gonads in the Development of Leukemia in a High-Leukemia Stock of Mice. Cancer Res., *4:* 377–83, 1944.
39. Miller, J. F. A. P. Analysis of the Thymus Influence in Leukaemogenesis. Nature, *191:* 248–49, 1961.
40. ———. Immunological Function of the Thymus. Lancet, pp. 748–49, 1961.
41. Moloney, J. B. Biological Studies on a Lymphoid-leukemia Virus Extracted from Sarcoma 37. I. Origin and Introductory Investigations. J. Natl. Cancer Inst., *24:* 933–51, 1960.
42. Morton, J. J., and Mider, G. B. The Production of Lymphomatosis in Mice of Known Genetic Constitution. Science, *87:* 327, 1938.
43. Rask-Nielsen, R. On the Development of Leukemia in Four Low-leukemic Strains of Mice Following Injection of a Small Dose of 9,10-Dimethyl-1,2-benzanthracene into the Thymus. J. Natl. Cancer Inst., *16:* 1129–36, 1956.
44. Rauscher, F. J. A Virus-induced Disease of Mice Characterized by Erythrocytopoiesis and Lymphoid Leukemia. *Ibid.*, *29:* 515–43, 1962.
45. Reich, E. Actinomycin: Correlation of Structure and Function of Its Complexes with Purines and DNA. Science, *143:* 684–89, 1964.
46. Sachs, L. Transplantability of an X-ray-induced and a Virus-induced Leukemia in Isologous Mice Inoculated with a Leukemia Virus. J. Natl. Cancer Inst., *29:* 759–64, 1962.
47. Sutow, W. W., and Welsh, V. C. Acute Leukemia and Mongolism. J. Pediat., *52:* 176–81, 1958.
48. Toth, B. Development of Malignant Lymphomas by Cell-free Filtrates Prepared from a Chemically Induced Mouse Lymphoma. Proc. Soc. Exptl. Biol. Med., *112:* 873–75, 1963.
49. Upton, A. C., and Furth, J. Host Factors in the Pathogenesis of Leukemia in Animals and in Man. *In*: Proc. Third Natl. Cancer Conf., pp. 312–24. Philadelphia: J. B. Lippincott Company, 1957.