Leukemia: Much Is Known, but the Picture Is Still Confused

If any type of human cancer is caused by a virus, it is probably leukemia. The virus may not be an infectious virus in the same sense as those that cause polio and measles, but strong evidence is accumulating that some form of virus is involved in the etiology of leukemia. There is, however, a welter of evidence indicating that many other factors, including environmental influences and genetic predisposition, are also involved. The scenario presented by this evidence is complex, confusing, even contradictory, but this may be simply a reflection of the fact that more is known about leukemia than about any other type of cancer. The problems encountered in understanding and treating leukemia typify those found in other types of cancer and are illustrative of the general directions in which cancer research is headed.

For many years, it was assumed that the symptoms of leukemia arose because leukemic leukocytes proliferated much more rapidly than healthy ones. In 1953, however, the Italian investigators G. Astaldi and C. Mauri demonstrated that leukemic cells actually proliferate more slowly than their healthy counterparts (although there are occasional periods of rapid growth). Subsequent work by many investigators has shown that the primary lesion in leukemia is a block in the differentiation of leukocytes. Most leukemic cells never mature into functional entities. Not only is the body thus deprived of vital components of its immune system (see box), but also the cells accumulate in the blood and in certain organs, forcing out healthy cells and interfering with organ function.

The proximate cause of the block in maturation is still unknown, but many scientists think it results from the loss of a specific factor necessary for maturation. In 1966, Leo Sachs of the Weizmann Institute in Rehovot, Israel, developed a system for growing leukemic cells in culture. Using this system, he and Michael Perrin, now at the National Cancer Institute (NCI) in Bethesda, demonstrated that cultured leukocytes from patients with acute myelocytic leukemia could apparently be induced to mature in the presence of a particular glycoprotein from blood serum. This substance is called colony-stimulating factor (CSF) because the mature cells form colonies in the culture.

CSF is present in the blood of leukemia patients, but it is not yet clear whether its concentration is lower in these patients than in healthy individuals or whether a membrane defect prevents CSF from exerting its normal control over differentiation. The former possibility is supported somewhat by observations indicating that systemic factors are important. Alvin M. Mauer of the University of Cincinnati, for example, has shown that the proliferative activity of leukemic cells is the same at different bone marrow sites, indicating that some substance in the blood regulates the leukemic process.

Is the Maturation Functional?

But there is no firm evidence, argue some scientists, such as Fred Stohlman of St. Elizabeth's Hospital, Boston, that the CSF-induced changes are a functional maturation, and a great deal more work will be necessary before any conclusions can be drawn about the role of CSF. In any case, work on CSF is proceeding very slowly: there is no rapid assay for CSF, and this makes its isolation very tedious. Sachs and others have argued that it may be very useful in treating human leukemia, but a demonstration of this possibility is probably far in the future.

If the proximate cause of blocked maturation is a mystery, so too is the ultimate cause. But perhaps the greatest excitement in leukemia research today surrounds the possibility that human leukemia may be caused by a virus. It has long been recognized that leukemia (and other cancers arising in mesenchymal cells) can be triggered in several species of animals—rodents, cats, cows, birds, and subhuman primates—by agents known as type C RNA tumor viruses or oncornaviruses. Many virologists think that human leukemias should not differ grossly from these animal leukemias.

Oncornaviruses (*Science*, 22 March, p. 1181) replicate in infected cells through the mediation of an enzyme known as RNA-directed DNA polymerase or reverse transcriptase. Reverse transcriptase directs the production of a DNA copy of the virus's RNA genome. This copy, called the provirus, then serves as a template for production by the host cell of more RNA viruses. More important, the provirus can be inserted into the DNA genome of the host cell where, if the virus contains oncogenic (tumor initiating) information, it can assume control of the host cell's proliferation and transform it into a malignant cell. To date, all oncogenic RNA viruses have been shown to contain a reverse transcriptase and virtually all RNA viruses that contain a reverse transcriptase have been shown to be oncogenic. But despite a great deal of effort by many investigators, a reverse transcriptase has never been found in any nonmalignant cell not infected by an oncornavirus.

In 1970, Robert C. Gallo and his associates at NCI demonstrated that granulocytes (see box) from humans with acute myelocytic leukemia contain a reverse transcriptase analogous to those found in oncornaviruses. Subsequently, Sol Spiegelman and his associates at the Institute for Cancer Research at Columbia University, New York City, and Gallo have independently shown that the reverse transcriptase is found in a cytoplasmic particle which has the same density as oncornaviruses and which also contains RNA of a size characteristic of oncornaviruses. Both groups have shown by molecular hybridization experiments that DNA produced by the RNA–reverse transcriptase complex contains base sequences homologous to those in mouse leukemia and sarcoma viruses, but does not contain sequences homologous to those of avian oncornaviruses or of a mouse type B RNA virus (mouse mammary tumor virus). Gallo and David Gillespie of Litton Bionetics, Bethesda, have also shown that this DNA contains even more sequences homologous to those in simian sarcoma virus, an oncornavirus that produces a leukemia-like disease in primates.

Gallo and his associates have purified the reverse transcriptase from leukemic granulocytes and have shown that it accepts the same types of nucleic

acid templates as the enzymes from animal oncornaviruses. And finally, he and George J. Todaro of NCI have shown that the purified enzyme is immunologically very closely related to the reverse transcriptases from simian sarcoma virus and from gibbon ape leukemia virus, less closely related to those from mouse and cat oncornaviruses, and unrelated to that from a chicken oncornavirus. Since many scientists now accept the postulate that reverse transcriptase is unequivocally associated with cell transformation by RNA viruses, the presence in leukemic human cells of a reverse transcriptase closely related to those of viruses that cause leukemia in other primates is highly suggestive of the possibility that human leukemia is caused by an RNA virus.

Spiegelman, meanwhile, has demonstrated that DNA produced by the RNA–reverse transcriptase complex from human granulocytes contains sequences that will not hybridize with DNA from healthy tissues (muscle, for example) from the same patient. This finding suggests that extra—presumably oncogenic—genetic information has been added to the genome of bone marrow cells sometime after birth. This hypothesis is supported by experiments with identical human twins, one of whom has leukemia. By hybridization experiments, Spiegelman has shown for two sets of twins that the leukocyte genome of the leukemic twin contains DNA sequences not present in leukocytes of the healthy twin, again suggesting that oncogenic information has been added after birth.

Gallo's and Spiegelman's results are by far the strongest evidence supporting viral involvement in leukemia, but a recent observation by E. Donnall Thomas of the University of Washington School of Medicine, Seattle, adds further support. Thomas has been treating terminal myelocytic leukemia patients by destroying their bone marrow with drugs or radiation and transplanting healthy bone marrow from a donor. In this manner, all the leukemic cells can be killed and the healthy graft cells can take their place; the primary problems with this approach are obtaining bone marrow that is compatible with the patient and preventing infections during the period before the grafted cells begin functioning. Most investigators have been largely unsuccessful in their attempts at grafting bone marrow.

By improving the immunological match between the patient and the donor, Thomas has had better success with the grafts than most investigators have, but he has recently observed two interesting cases in which the patient had a relapse of leukemia. Subsequent examination of chromosomes from the leukemic cells indicated that they were from the male donor rather than from the female patient, indicating that the patient harbored some agent—possibly viral—capable of transforming the transplanted cells.

The ultimate proof of the viral hypothesis would be the isolation of a leukemia virus from humans, and that possibility is beginning to appear more likely. Two years ago, for example, Boris Lapin of the Institute of Pathology and Therapy at Sukhumia in the U.S.S.R. revealed that he and his associates had succeeded in inducing leukemia in primates by inoculating them with blood from human leukemia patients and had isolated what they believed to be a human type C RNA leukemia virus. U.S. scientists have not been able to duplicate Lapin's experiments and have obtained only equivocal results with materials provided by him. Late last year, however, NCI received one of Lapin's leukemic baboons, and preliminary experiments with this animal have been described by NCI officials as promising. One U.S. scientist is also on the verge of announcing the isolation of a putative human leukemia

What Is Leukemia?

Leukemia, cancer of the blood, is characterized by the uncontrolled proliferation and accumulation of leukocytes (white blood cells). Just as there are many different types of leukocytes, there are many different types of leukemia, but the four most important forms are derived from only two types of cells.

Acute and chronic lymphocytic leukemias (also known as lymphoblastic leukemias) are malignancies of lymphocytes, cells produced in the lymphoid organs—the spleen, lymph nodes, and thymus—and in the bone marrow. Lymphocytes can be divided into two morphologically indistinguishable subgroups, depending on their function. One subgroup, called thymus dependent or T cells, is involved in the phenomenon known as cellular immunity, the process by which the body distinguishes between self and nonself. The second subgroup, thymus independent or B cells, controls the production of circulating antibodies, substances that attack infectious microorganisms. Recent research by Jun Minowada and his associates at Roswell Park Memorial Institute, Buffalo, and by others suggests that acute lymphocytic leukemia is a disorder of T cells, while chronic lymphocytic leukemia is a disorder of B cells.

Acute and chronic myelocytic leukemias (also known as granulocytic or myelogenous leukemias) are disorders of granulocytes. Granulocytes, produced by bone marrow, engulf and digest bacteria and other small particles. There is as yet no evidence to support a biochemical distinction between the acute and chronic forms of myelocytic leukemia.

Acute leukemias generally appear suddenly, with symptoms like those of a cold, and progress rapidly. The lymph nodes, spleen, and liver may become infiltrated with leukocytes and enlarged; there is often bone pain, paleness, a tendency to bleed easily, and a high susceptibility to infections. The most common causes of death, which occurs at a median of 3 months without treatment, are hemorrhaging and uncontrolled infections. The chronic leukemias begin much more slowly; many cases are discovered during routine blood examinations, and several years may pass before significant symptoms appear. The symptoms are similar to those of the acute leukemias, but the life expectancy without treatment is about 3 years after onset.

Acute lymphocytic leukemia is the most common cancer of childhood (about 3000 cases per year), but it is more common in adults. Acute myelocytic leukemia occurs much less frequently in children, and the chronic forms occur almost exclusively in adults. Leukemias strike about 19,000 individuals in the United States each year and take the lives of approximately 14,000.—T.H.M.

virus from cultured human leukemic cells treated with dexamethasone, a chemical that stimulates the expression of viruses in infected cells.

Despite all the evidence implicating viruses in human leukemia, it is clear that a number of other factors are also involved. The most important of these are chromosomal aberrations. Evidence is increasing that environmentally induced and genetically determined chromosome breakage and rearrangement increase the incidence of leukemia.

A well-established environmental cause of leukemia is radiation, as is deduced from the much higher incidence among radiologists (before protective precautions began to be taken routinely) and among survivors of the nuclear blasts that ended World War II. Radiation increases the incidence of all types of leukemia except chronic lymphocytic leukemia. The principal leukemogenic effect of irradiation is believed to be breakage of chromosomes in bone marrow cells (but radiation is also known to activate latent viruses). Similarly, benzene, the only chemical thought to be leukemogenic, also induces breakage in bone marrow chromosomes. But genetic factors apparently play a more important role in leukemogenesis, even when radiation is present. Studies of the Hiroshima and Nagasaki survivors by the Atomic Bomb Casualty Commission indicate that only 1 of every 100 individuals exposed to the highest levels of radiation contracted leukemia.

In 1960, Peter Nowell and David Hungerford of the University of Pennsylvania, Philadelphia, observed that more than 90 percent of patients with chronic myelocytic leukemia (and a small fraction of patients with the acute form) have a specific chromosomal abnormality: the long arm of chromosome number 21 from their granulocytes—now known as the Philadelphia chromosome—is shorter than its counterpart in cells from healthy tissues. Last year, Janet D. Rowley of the University of Chicago found that these patients have additional DNA in their chromosome number 9 and that the mass of the added material is about equal to that missing from number 21. It thus seems likely that translocation of genetic information from chromosome 21 to chromosome 9 may cause or increase the incidence of leukemia.

Certain other types of genetic disorders also predispose toward a greater incidence of leukemia. For example, children with Down's syndrome (mongolism), a genetic defect characterized by mental retardation and certain other abnormalities, have a 15-fold higher incidence of acute leukemia as compared to normal children. The specific genetic lesion in Down's syndrome, interestingly, is the presence of an extra chromosome 21. Similarly, about 10 percent of children with Fanconi's aplastic anemia, a genetic defect in which all blood components show a reduced proliferation, develop acute monomyelocytic leukemia, a rare form that accounts for less than 4 percent of all leukemias.

There are, according to Robert Miller of NCI, several other genetic defects that are associated with a higher incidence of leukemia. The common feature that links these defects, he says, is chromosomal fragility; that is, each of the disorders makes the afflicted individual's chromosomes more susceptible to breakage and rearrangement. It may be, Miller says, that breakage makes cells more susceptible to viral infection or to expression of latent viruses, but it is also possible that rearrangement and defective repair of DNA may lead to inappropriate expression of genes, a condition that is also thought to be oncogenic in some instances (*Science*, 12 April, p. 147).

Breakage at Specific Sites

There is some evidence that induction of leukemia may be involved with breakage at specific chromosome sites. Fred Hecht of the University of Oregon Medical School, Portland, has presented evidence that in one genetic disorder, ataxia telangiectasia (characterized by failure of muscular coordination, pulmonary disease, and abnormal eye movements), the occurrence of preleukemic granulocyte proliferation in several patients and of chronic myelocytic leukemia in at least one patient is associated with breakage of chromosome number 14. This finding is consistent, Hecht says, with the observation by other investigators that breakages in chromosome 14 are found in patients with Burkitt's lymphoma, a leukemia-like tumor of the lymph gland.

Still other genetic factors not associated with fragility are also linked to leukemia. Among white children, for example, there is a marked peak in mortality from acute lymphocytic leukemia at the age of 4 years. This peak occurs at the age of 1 in children with Down's syndrome, and does not occur at all in black children. Identical twins have a much greater risk of contracting leukemia if one has already done so; for the second twin, this risk declines from about 100 percent if the first contracted leukemia before age 1 to about 20 percent if it was contracted at age 6. It is thus clear that a wide variety of effects are implicated in the induction of leukemia. There may eventually be some hypothesis that will reconcile all the seemingly contradictory data, but for now, the etiology of leukemia remains an enigma.

The accumulation of knowledge about leukemia has contributed to the success of therapy for leukemia patients. Perhaps the most important of these contributions was obtained from a knowledge of the kinetics of leukemic cell proliferation, which has allowed chemotherapists to design much more effective drug schedules. Knowledge of some of the biochemical pathways of leukemic cells has provided guidance in the use of antimetabolites, drugs that interfere with the cell's metabolism. And the discovery of reverse transcriptase in leukemic cells has sparked a great deal of research for inhibitors of the enzyme and of viral replication. Nevertheless, most of the therapeutic advances in leukemia have resulted from the same sort of sophisticated trial-and-error techniques necessary in other types of cancer. The task of devising therapies has been made all the harder by the grossly different susceptibilities of the various types of leukemia and by age-dependent differences within any one type.

The greatest success in leukemia therapy has been achieved in treating children with acute lymphocytic leukemia. Twenty years ago, the median survival for children with this disease was about 3 months, and even as recently as 12 years ago, chemotherapy was expected to be no more than palliative. Today, the median survival is 5 years, and many afflicted children have been alive for 10 years or longer.

It is difficult to identify individuals who have made the greatest contributions to this achievement because the effort has been largely a group one. Leukemia patients are encountered so infrequently that significant results from clinical studies can be obtained only by pooling results from patients treated at many institutions. Changes or improvements in drug regimens are thus frequently a matter of group consensus rather than individual choice.

Leukemia chemotherapy began in 1947 when the late Sidney Farber of

the Children's Cancer Research Foundation in Boston started obtaining brief remissions with the antimetabolites aminopterin and methotrexate. Many of the concepts now used in leukemia therapy were later developed at NCI by Emil J. Freireich, who is now at the M. D. Anderson Hospital and Tumor Institute, Houston. Some of the best results are now being obtained by Donald Pinkel and Joseph Simone at St. Jude Children's Research Hospital in Memphis, one of the few institutions with enough patients to conduct clinical trials independently.

Pinkel and Simone's regimen, typical of those used at other institutions, includes induction of a remission with vincristine and steroid hormones, x-irradiation to destroy leukemic lymphocytes harbored in the central nervous system, and a 3-year course of chemotherapy consisting of daily doses of 6-mercaptopurine and weekly doses of methotrexate. An initial remission is obtained in about 90 percent of children under the age of 10. In their first series of studies, which lasted from 1962 to 1965, Pinkel and Simone used what is now recognized to be an insufficient dose of radiation, but 7 of 41 children in those studies are still alive. With a larger radiation dose in subsequent studies, 19 of 38 children are still alive after six or more years. And only 1 of 40 children in current studies has had a relapse after 2½ years. Similar results have been obtained by the Acute Leukemia Group B, a consortium of clinical groups in the northeastern United States, whose chairman is James F. Holland of the Mt. Sinai School of Medicine, New York City.

A major contribution to the success of these antileukemic regimens has been the development of supportive therapy to counteract the side effects of leukemia. The most important of these are hemorrhaging, caused by loss of platelets from the blood, and a high susceptibility to infectious diseases resulting from suppression of the immune system by both the leukemia and the chemotherapeutic agents.

The hemorrhaging can be controlled by transfusing the patient with platelets, but obtaining platelets was once a major problem. In the late 1950's, however, Isaac Djerassi, now at the Mercy Catholic Medical Center, Darby, Pennsylvania, and Edmund Klein, now at Roswell Park Memorial Institute, Buffalo, and, independently, Freireich developed methods for removing platelets from blood and returning the red blood cells to the donor. With this technique, now one donor can provide a continuing supply of platelets for a leukemia victim. More recently, Freireich and Djerassi have independently developed a similar plasma pheresis technique for collecting functional granulocytes from patients with chronic myelocytic leukemia; these patients produce much greater quantities of granulocytes than do healthy persons, and as many as ten leukemia patients can be helped by one donor. Transfused into compatible patients with acute lymphocytic leukemia, these granulocytes help fight off infections while the patient's own immune system is recovering.

A remission of acute lymphocytic leukemia that persists for five or more years is frequently considered to be a cure—or the next best thing to one. Some recent evidence, however, suggests that the potential for a relapse still exists within these patients. By molecular hybridization experiments with the probe described previously, Spiegelman has shown that lymphocytes from many of these long-term survivors still contain (presumably) oncogenic DNA sequences not found in corresponding cells from healthy individuals. This finding, he says, indicates that the therapeutic regimen has removed the symptoms of the malignancy but not the underlying cause, so that the symptoms may recur at a later date.

Less Success in Adults

The success in treating acute lymphocytic leukemia in children is not even approached in adults with the same disease. The median survival of the patient varies inversely with his age at the onset of the disease; the older the patient, the less susceptible he is to therapy. Drugs such as vincristine and prednisone, which produce a high rate of remissions in young children, are only minimally effective in adults. Treatment with newer drugs such as adriamycin and arabinosylcytosine, has been somewhat successful, but the median survival for adults is still less than 1 year.

The present status of therapy for acute myelocytic leukemia, according to Myron Karon of the Los Angeles Children's Hospital, is similar to that for acute lymphocytic leukemia about 4 years ago. An initial remission can now be obtained in about 80 percent of patients with acute myelocytic leukemia, compared to only 30 percent a few years ago. The median survival, similarly, has been extended from 1 month to about 18 months.

Because acute myelocytic leukemia is more resistant to therapy than is acute lymphocytic leukemia, one approach to its control, pioneered by Freireich, involves an intensified course of chemotherapy—higher doses given more frequently. But this regimen greatly depresses the patient's immune system, making him much more susceptible to infections. Freireich thus isolates the patients in laminar air-flow bedrooms in a sterile environment. Of 20 patients treated in this fashion, 15 are still in remission with a median disease-free period of about 2 years. Some scientists, however, are skeptical that the isolated environment is a necessary facet of the protocol and Freireich is beginning a clinical study to determine whether it is.

The outlook for both chronic lymphocytic leukemia and chronic myelocytic leukemia is much less promising, according to Paul Carbone of NCI. The median survival in both types is less than 3 years and has not been improved by any form of therapy, probably because the chronic types have generally received only minimal treatment. There is some prospect for improvement, however, as clinicians are beginning to treat the chronic diseases much more aggressively. For instance, Bayerd Clarkson of Memorial Sloan-Kettering Cancer Center, New York City, has found that an aggressive protocol that includes removal of the spleen, radiation, and intensive chemotherapy produces a high rate of remissions in adults with chronic myelocytic leukemia. This approach seems particularly promising because in 4 of 25 patients, it had led to the disappearance of the Philadelphia chromosome, the genetic marker associated with the disease, but the protocol has been in use for too short a time for a full assessment.

Therapy of leukemia thus encompasses the full range of possibilities, from a high percentage of potential cures in children with acute lymphocytic leukemia to almost none in adults with chronic lymphocytic leukemia. It is clear, however, that progress is being made in treatment of each type of disease and especially in obtaining a greater knowledge of its molecular biology. The knowledge of leukemia that is being rapidly obtained opens many new areas of potential therapy and even prevention. And in the end, that is what cancer research is all about.—THOMAS H. MAUGH II