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Studies on the Mechanisms of Chemically Induced Leukemia/Lymphoma

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Leukemias associated with potential exposure to chemicals or viruses are a major concern in modern society. The mechanism of leukemogenesis in rodents is poorly understood, making risk assessment of human exposure difficult. Studies at CIIT are attempting to elucidate the respective roles of chemical exposure and retroviruses in leukemogenesis in order to evaluate the appropriateness of animal models for extrapolating risk to man and to provide information necessary to identify human populations at risk.

Few diseases command the respect that is associated with leukemia or lymphoma. Despite therapeutic advances in recent years, most notably with Hodgkin's disease, many leukemias and lymphomas remain among the most aggressive cancers known, with survival after diagnosis often measured in months rather than years. Secondary leukemias, those occurring as a result of radiation, chemotherapy or exposure to benzene, carry a particularly dismal prognosis. Leukemias are neoplasms of the hematopoietic system (blood and blood-forming organs) that usually are disseminated via the blood. Lymphomas are neoplasms of the immune system that may behave like solid tumors or alternatively spread via the blood or lymph. Because a significant number of leukemias or lymphomas share a common origin in the blood-forming cells of the bone marrow and also share other biological characteristics, they are often classified together. On occasion, determining whether a given disease should be classified as a lymphoma or a leukemia is difficult or even arbitrary.

Stem Cells and Blood Formation

All the cells of the blood, including erythrocytes (red blood cells), leukocytes (white blood cells) and platelets, share a common heritage in a single type of cell that normally resides in the bone marrow. This "pluripotential stem cell" (PSC) is capable of either replicating itself or giving rise to any of the various cell types found in the peripheral blood and immune system. To appreciate this feat, one should consider that in the normal adult the frequency of PSC in the bone marrow is between 1 in 1000 and 1 in 10,000 cells, yet the bone marrow replaces over 10,000,000,000 (10^{11}) blood cells every day and is capable of increasing this output as much as sixfold in order to meet increasing demands. The process through which this occurs involves a complex interaction of growth factors, accessory cells and feedback mechanisms that regulate the differentiation and replication of blood precursor cells and in turn the production of mature blood cells (Fig. 1).

Bone Marrow Damage, Myelodysplastic Syndrome, and Leukemogenesis

A predominant feature linking leukemia in man to experimental models of the disease is the association between leukemia and damage to the bone marrow. Although great efforts have been made in recent years to refine therapeutic modalities, leukemias remain the most frequent neoplasms arising secondary to chemotherapy and/or radiation employed in the treatment of cancers, transplantation rejection or immune complex diseases. Similarly, experimental models of leukemogenesis—be they spontaneous or induced through exposure to drugs, chemicals, radiation, or viruses—invariably involve the hematopoietic (blood producing) stem cells that reside in the bone marrow and ultimately give rise to the cells of the blood and immune system. It is now recognized clinically that acute leukemia is often preceded months or even years by functional abnormalities of the blood and bone marrow, and that bone marrow suppression, aplastic anemia and myeloid leukemia represent a continuum or spectrum of blood dyscrasias (abnormalities) rather than separate or unrelated entities. Since these functional changes involve abnormalities in the growth of blood precursor cells, they have been collectively termed ***myelodysplastic syndrome*** (MDS) (Galton, 1986).

MDS describes a variable but well-

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recognized set of blood disorders that have high propensity for terminating in acute myeloid leukemia (AML). Previously defined diseases that are included within MDS are aplastic or refractory anemia and preleukemia (Galton, 1986). Frequent signs include: anemia, leukopenia (a decrease in white blood cells), and thrombocytopenia (decrease in platelets), accompanied by increased cellularity of the bone marrow. The rate of transformation of MDS to AML varies from study to study but is about 30% in "spontaneous" MDS and between 50 and 70% in MDS occurring secondary to chemotherapy or benzene exposure. The relationship between MDS and benzene leukemogenesis appears to be exceptionally strong, with MDS preceding the onset of leukemia in virtually every case for which data is available (Bagby, 1986). However, determining whether MDS precedes every case of benzene-induced leukemia remains an impossibility. It should be pointed out that secondary MDS itself is considered a neoplastic process and carries a grave prognosis, with a mortality of between 30 and 40% for those cases that do not progress on to AML.

Evidence exists to indicate the great majority of cases of MDS and most leukemias are the result of abnormalities originating within a single stem cell, *i.e.*, they are clonal in origin (McCulloch *et al.*, 1982). Studies utilizing cytogenetic or biochemical markers to identify the origins of abnormal cell populations have revealed that such clonal abnormalities may be present for years prior to the clinical recognition of leukemia. Thus a paradigm has emerged that implicates the stem cell in a multistep process that ultimately leads to the presentation of leukemia. Elucidation of the molecular events that precede leukemogenesis is a goal yet to be achieved. However, it seems reasonable to assume that at least one fundamental lesion must involve structural changes at the level of the genome (*i.e.*, a mutation) of the stem cell. Certainly, the propensity of alkylating agents to cause leukemia, the prevalence of chromosomal abnormalities in leukemias and their clonal origin provide a rational basis for this concept. However, since many leukemias are characterized by what appear to be normal blood cells that are abnormally regulated with respect to growth and maturation, it has been postulated that the genetic information may be normal but regulation defective. In this regard it is probably not insignificant that certain normal cells of the hematoporetic and immune systems (*e.g.*, lymphocytes) have characteristics usually ascribed to cancer cells, such as the ability to replicate, invade other

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Schematic Diagram of Hematopoiesis

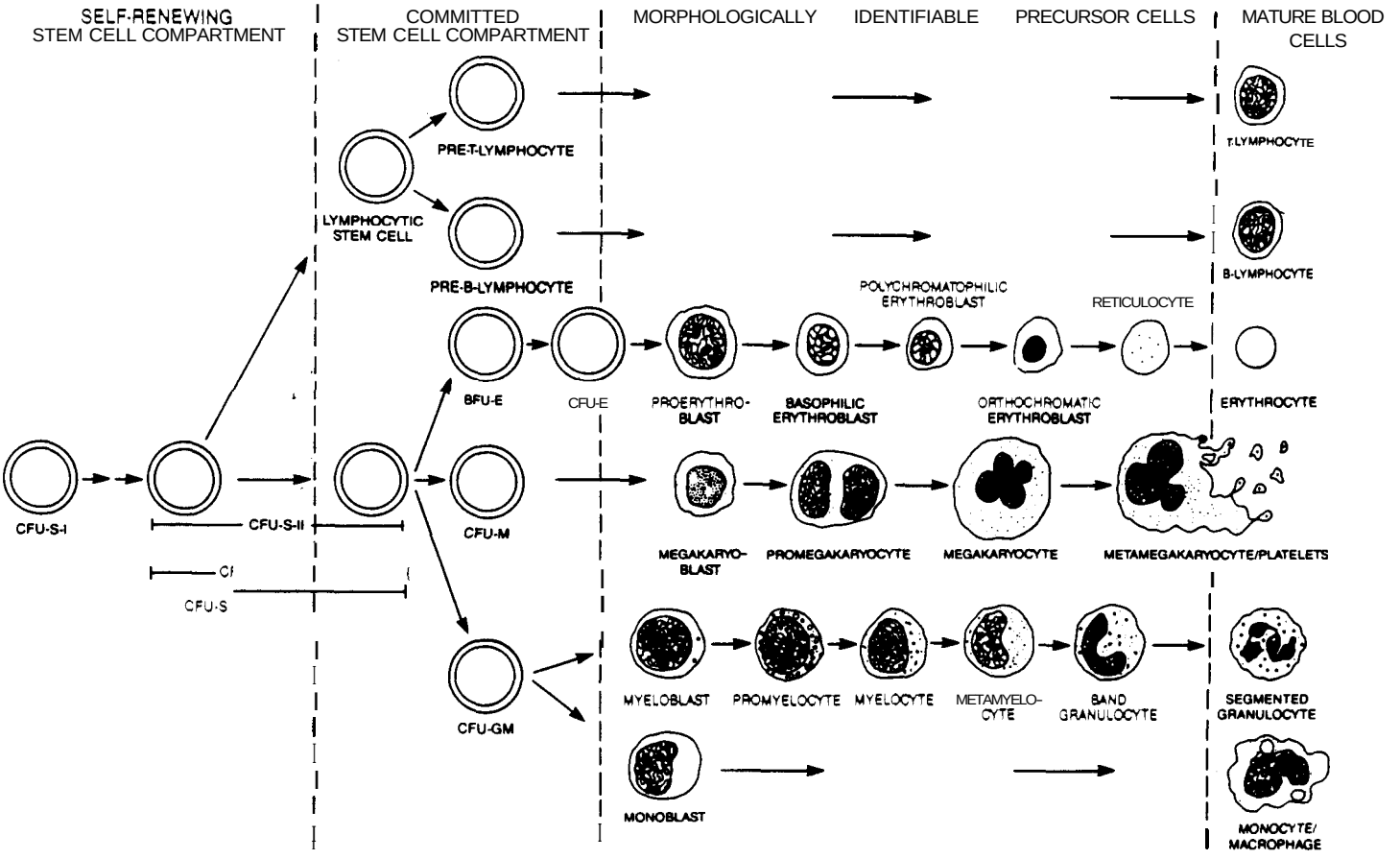

Fig. 1. The production of blood cells involves the progressive maturation and replication of precursor cells in the bone marrow. This process begins with the pluripotential stem cell (PSC), which has limited replicative abillty but is capable of self-renewal. Alternatively, it can differentiate to produce cells committed to any of the different blood cell pathways. Differentiation along a committed pathway is accompanied by a progressive increase in replication such that the initial division of a PSC is amplified a billion-fold prior to the appearance of mature blood cells.

Various assays have been described that measure stem cells by determining their ability to form colonies (*i.e.*, colony-forming units—CFU). Several of these have been found to measure stem cells at overlapping stages in the commitment process. Assays that measure stem cells with the capability of self-renewal include CFU-S (spleen) and CFU-mix (or granulocyte, erythrocyte, megakaryocyte, macrophage). Assays that measure multipotential and committed stem-cell populations Include: BFU-E (burst forming unit-erythrocyte), CFU-E (erythrocyte), CFU-M (megakaryocyte), and CFU-GM (granulocyte-macrophage).

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tissues, kill cells, etc., but that the number and activity of these cells are normally held in check by highly effective and redundant control mechanisms.

Since clonal abnormalities can be present for long periods of time prior to the onset of frank leukemia, there is considerable likelihood that both hypotheses are correct. It may be that structural damage to the DNA results in a heritable defect that conveys a proliferative advantage on a target stem cell, presumably via altered regulation of any number of potential cellular "oncogenes," but ultimate expression of the malignant phenotype requires intervening influences that favor or select for abnormal gene expression but are not necessarily themselves genetic events.

Viruses and Leukemia

In John Steinbeck's tragedy Of Mice and Men, the mouse stands as a symbol of man's inability to understand and control his destiny. To almost everyone involved in the study of leukemogenesis, the analogy is an undeniably apt one. Murine models of leukemogenesis present perhaps the best opportunity to unlock the mysteries of this disease. Nevertheless, the investigator attempting to use these animal models is confronted with a puzzle of Gordian proportion. One of the culprits implicated in this dilemma is a family of retroviruses, commonly referred to as murine leukemia viruses (MuLV). Retroviruses are RNA viruses that commandeer the normal machinery of a cell to produce a complementary piece of DNA which is then inserted into the nuclear DNA of the cell. Once this pro-viral DNA sequence is integrated into the cell, it becomes a permanent part of its genome, encoding for the production of retrovirus (Fig. 2). In a great number of species, including man, retroviruses have been implicated in many diseases of the blood and immune system, including leukemia and acquired immune deficiency syndrome (AIDS) (Table 1). The first human retrovirus, T-cell lymphotrophic virus (HTLV-I) was discovered in 1978 (Poiesz et *al.*, 1980; Hinuma et *al.*, 1981; Popovic et *al.*, 1982). It causes adult T-cell leukemia and to date appears to be restricted to southeastern Japan, the Caribbean, certain regions in Italy, the southern United States, South America and Africa.

HTLV-I is an infectious retrovirus that is passed horizontally, *i.e.*, from one person to another. As such it is an exogenous retrovirus, capable of being transmitted from one blood cell to another, but is not transmitted vertically from one generation to another through the germ cells. If the target of a retrovirus happens to be an ovarian or testicular germ cell, the encoded proviral sequence will be inherited as a "normal" gene by all further progeny. Retroviruses inherited in such a manner are called "endogenous" to distinguish them from exogenous or infectious retroviruses. None of the known infectious human retroviruses (*e.g.*, HTLV-I, -II, HIV) are known to target germ cells and thus to be transmitted vertically. Nevertheless, virtually all mammals, including man, possess some DNA sequences encoding for retroviral elements. In some species, including man, endogenous sequences are rare and there is yet no evidence to indicate that they are expressed. However, it is a legacy of the great majority of strains of laboratory mouse to possess numerous genes encoding for retroviruses. This includes the CD1, C57BL/6 and C3H strains as well as the B6C3F1 hybrid frequently used in carcinogenicity studies. Certain of these endogenous retroviruses are known to produce a high frequency of "spontaneous" leukemias in specific strains of mice, *e.g.* AKR and C58 (Hartley et al., 1977; Chattopadhyay et al., 1982), are implicated in certain strain-specific models of radiation-induced leukemogenesis (C57BL/6, C3H and RF) (Gross, 1959; Leiberman and Kaplan: 1959; Kaplan, 1967; Upton et *al.*, 1958: Haran-Ghera, 1976) and appear to influence the incidence of 1,3-butadiene-induced leukemia in the B6C3F1 mouse (Irons *et al.*, 1988). In spontaneous leukemias of the mouse, the role of retrovirus is definitive (Cloyd *et al.*, 1980), yet it must be said that the specific mechanisms or role played by virus in any of the other models of leukemogenesis remains obscure. And in at least one model, 3-methylcholanthrene-induced leukemias in the RF mouse, no convincing role for retrovirus has yet been established (Goodenow and Lilly, 1984; Chinsky *et al.*, 1985). A characteristic common to all naturally occurring mammalian retroviruses, both exogenous (*e.g.*, HTLV-I) or endogenous (*e.g.*, MuLV), is that they do not contain a directly transforming gene or "oncogene" in their coding sequences. Therefore, any proposals to account for the role of these viruses in leukemogenesis must invoke alternative explanations.

Infection of a Target Cell by a Retrovirus

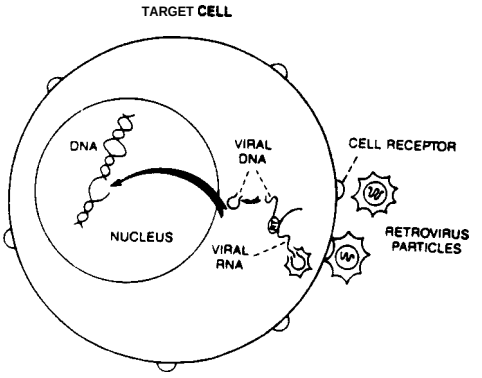

Fig. 2 A retrovirus targets and enters cells by interacting with receptors on the cell plasma membrane. Upon entering the cell, the retrovirus releases its RNA into the cytoplasm. A viral enzyme, reverse transcriptase, catalyzes the synthesis of DNA complementary to the viral RNA, which is then integrated into the cell's nuclear DNA. Thus the information encoded in the viral RNA is passed on to any daughter cells produced through subsequent division of the cell.

Chemicals and Leukemogenesis

A number of chemicals and drugs are known to cause bone marrow toxicity in man or experimental animals, and it is therefore not surprising that they have been implicated in experimental leukemogenesis. These include benzene, butadiene, ethylene oxide, and a wide variety of alkylating agents. To date, benzene is the only occupational hazard definitely linked to acute leukemia in man. Although seemingly unrelated, all of these agents share one or more characteristics in common: They all either distribute to the bone marrow in appreciable concentrations or give rise to reactive metabolites with relatively long half-lives in biological systems. These metabolic intermediates, in turn, are able to survive transit to the bone marrow and are capable of further bioactivation or direct interaction with cells in the target tissue. From the perspective of

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TABLE 1
RETROVIRUSES CAUSING PATHOLOGY IN NATURAL HOSTS

	Virus		Diseases
1.	Feline Leukemia Virus (FeLV)		Leukemia, anemia, immunosuppression
2.	Bovine Leukemia Virus (BLV)		Lymphoma
3.	Mouse Leukemia Virus (MuLV)		Leukemia, lymphoma
4.	Simian Lymphoma Virus (SLV)	-	Lymphoma
5.	Human T-Cell Leukemia Virus I (HTLV-I)	-	Adult T-cell leukemia
6.	Human T-cell Leukemia Virus II (HTLV-II)	-	Histiocytic/reticulum cell leukemia
7.	Equine Infectious Anemia Virus (EIAV)	-	Anemia
8.	Visna Virus		Neurological and pulmonary disease in sheep
9.	Human Immunodeficiency Virus (LAV/HTLV-III/HIV)		AIDS

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understanding the role of chemical exposure in experimental leukemogenesis and its significance with respect to human health, two of these compounds, benzene and 1,3-butadiene, represent an interesting dichotomy. Benzene is one of few industrial or environmental agents for which there is sufficient data to establish its leukemogenicity in man. Nevertheless, the history of research into its mechanism of action has been one repeatedly hampered by the lack of an appropriate animal model for benzene-induced leukemogenesis. Butadiene, on the other hand, is a potent leukemogen in the B6C3F1 mouse; however, understanding the significance of this model with respect to occupational exposure to 1,3-butadiene poses a formidable challenge.

Butadiene

Although not acutely toxic to humans or animals, 1,3-butadiene has been demonstrated to be a carcinogen in mice and rats (Huff et *al.*, 1985; Owen, 1981). Previous studies have revealed an extremely high incidence of thymic lymphoma/leukemia in the B6C3F1 mouse but not in rats chronically exposed to butadiene, and in 1983 a research program was inaugurated at CIIT to study the mechanisms of 1,3-butadiene-induced lymphoma/leukemia (Irons, 1985). At that time, the major goal was to ascertain the relevance of using the B6C3F1 mouse as a model for the extrapolation of risk of leukemia to man. Although this is still a major objective, the results of studies in our laboratory and others have led us to realize that it is also important that we determine if the mouse can be used as a model for virus activation, and if chemicals can interact with viruses in producing neoplasia either in the mouse or man.

Because of the frequent association of endogenous retrovirus with spontaneous lymphomas in the mouse, the finding of increased thymic lymphoma in the B6C3F1 mouse at least suggested the possibility of mouse retroviral involvement. Understanding the particular role or roles that a retrovirus might play in this process is a daunting task; nevertheless, in practical terms, the number of possibilities can be reduced to a relatively small number. Several alternative scenarios were initially evaluated (Table 2). Our initial approach was to (1) characterize the pathogenesis of BD-leukemogenesis in the B6C3F1 mouse, including the molecular alterations associated with BD toxicity and tumor development; and (2) compare the incidence of leukemia in B6C3F1 and NIH Swiss mice chronically exposed to BD (1250ppm for one year). A particular class of MuLV, ecotropic MuLV, has been implicated in spontaneous and radiation-induced models of leukemogenesis. Ecotropic MuLV proviral sequences are incomplete in the NIH Swiss and no viruses are found following BD exposure (Irons et *al.*, 1987b). We therefore felt that a comparison of the B6C3F1 and NIH Swiss strains represented a reasonable first approach to determining the influence of this class of retroviruses on leukemogenesis associated with BD exposure.

We were able to confirm that thymic lymphoma is the major cause of death in B6C3F1 mice chronically exposed to BD for one year and that the specific tumor type is a T-cell lymphoma (Irons et *al.*, 1986c). We also determined that the bone marrow is a major target of BD toxicity (Irons et *al.*, 1986a,b; Leiderman et *al.*, 1986). The bone marrow has consistently been found to be a target in animal and human leukemias and the megaloblastic anemia observed in the B6C3F1 mouse following BD exposure has features similar to MDS. In the B6C3F1 mouse, traditionally used in carcinogenesis bioassays, the incidence of spontaneous leukemia is low and endogenous retrovirus (MuLV) expression is suppressed. Nevertheless, results of our studies suggest that MuLV plays a role in BD-induced leukemia. First, we discovered an unusual selective activation of a class of MuLV (ecotropic) that occurs during the preleukemic phase of BD-exposure (Irons, et *al.*, 1987a,b). Preliminary studies indicate that the mechanism of viral activation probably involves de *novo* increases in virus production in individual cells rather than alterations in the ability of the mice to restrict virus movement. In addition, we observed a marked difference between the incidence of lymphoma observed in B6C3F1 and NIH Swiss mice exposed to BD (**50%** and **10%**, respectively) (Irons et *al.*, 1988). Significantly, toxicity to the bone marrow was qualitatively and quantitatively identical between the two strains despite the difference in leukemia incidence. In fact, of all the parameters (both cytogenetic and hematologic) evaluated in these studies, activation of retrovirus is the only endpoint that correlates with leukemogenesis (Irons et *al.*, 1986a,b; 1987a,b,c). Although its role in leukemogenesis is presently unknown, retrovirus activation has been shown to precede spontaneous leukemia development in other mouse models involving virus (*e.g.*, AKR mice). Furthermore, treatment of B6C3F1 mice with 5-azacytidine, a prototype activator of mouse retroviruses (Niwa and Sugahara, 1981), also results in leukemia/lymphoma. Thus, current evidence suggests that the measurement of retrovirus activation may be a useful screening tool for identifying potential leukemogens in the B6C3F1 mouse model.

Preliminary characterization of BD-induced thymic lymphomas reveals them to be uniformly of T-cell origin, to express varying amounts of retroviral gene products, and to be clonally derived (Irons et *al.*, 1987a). Tentatively, these tumors appear to be arrested at a particularly late stage in the lymphocyte differentiation pathway and to proliferate via growth factor-dependent pathways employed by normal T lymphocytes. In all of these aspects, they are curiously analogous to HTLV-I associated T-cell leukemias in man. Further analysis of these tumors may enable us to evaluate the potential interactions of virus and chemical with cellular macromolecules involved in the transformation process. Using this model system, it may be possible to simultaneously evaluate the respective contributions of genetic structural alterations in proviral sequences and proto-oncogenes as well as structural or functional alterations of surface molecules that regulate cell growth and behavior at the plasma membrane.

While these findings are provocative and provide a presumptive indication that MuLV are involved in BD-induced lymphoma/leukemia, it presently cannot be ruled out that other genes may contribute to differences in susceptibility between these two strains. Nevertheless, it appears likely that the number of viable scenarios for the role of retrovirus can, for

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TABLE 2

Accounting for Mechanisms of BD-Induced Leukemogenesis in the Mouse: Alternative Scenarios

1. BD exposure resulted in altered mouse retroviruses that were by themselves leukemogenic but that BD alone was not.
2. **Both activated retroviruses and BD were independently leukemogenic.**
3. **Mouse retroviruses were necessary but not leukemogenic by themselves (Le., cocarcinogenic).**
4. Mouse retroviruses were activated by BD but played no role in leukemogenesis.
5. Mouse retroviruses were not activated by BD and played no role in leukemogenesis.

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all practical purposes, be reduced to two: either activated retroviruses and BD are independently leukemogenic, or MuLV is co-carcinogenic, *i.e.*, a significant influence but not by itself leukemogenic in this chemical leukemogenesis model (Table 2). These alternatives can be tested experimentally. In addition, it becomes necessary to determine how valid are mouse models of retrovirus activation as predictors of the potential of chemicals to alter the biology of retroviruses, such as HTLV-I or HIV, in human cells. Although little is known about the mechanism of HTLV-I transformation, evidence strongly suggests that environmental factors influence latency in HTLV-I-associated neoplasms, and chemically induced alterations in HTLV-I antigen expression has been reported in human cells *in vitro.* By examining the potential of butadiene or its metabolites to alter the biology of HTLV-I in human blood cells in culture, we hope to clarify the significance of the mouse retrovirus model to man and epidemiologic findings that may suggest a population at potential risk. In addition to qualitative analyses of the effects of chemicals on viruses in human and mouse cells, it may be possible to quantitate relative differences in species sensitivity at a cellular level. This would be of considerable value in extrapolating *in vivo* animal data to man.

Benzene

Since late in the last century, occupational exposure to benzene has been associated with bone marrow toxicity (Santesson, 1897). The most frequently reported effects have been cytopenias (a decrease in one or more of the different types of circulating blood leukocytes or white cells), anemia and pancytopenia or aplastic anemia (a severe and usually irreversible bone marrow suppression culminating in the inability to effectively replace any of the cellular elements of the blood) (Goldwater, 1941). The first report of leukemia associated with benzene exposure appeared in 1928 (Delore and Borgomano, 1928), yet benzene was not universally recognized as a human leukemogen until the mid-70's. Recognition appears to have awaited two independent developments: (1) Although scattered individual cases appeared in the literature, it remained for Aksoy et a/. and Vigliani and Saita to provide the first organized studies of benzene exposure in the workplace; and (2) this has coincided with relatively recent recognition that MDS and AML represent a continuum or spectrum of blood dyscrasias rather than separate or unrelated entities.

The principal neoplastic disease now associated with benzene exposure is acute myelogenous leukemia (AML) (Vigliani and Forni, 1975; Infante et *al.*, 1977). Benzene-induced AML's are predominantly myeloblastic (FAB classification-M1), although a particularly strong association has been established between benzene exposure and the erythroleukemic variant of AML (FAB classification-M6) (Galavotti and Roisi. 1950; Aksoy et *al.,* 1976). Further studies have suggested an association with lymphoma as well (Aksoy *et al.*, 1974; Vianna and Polan, 1979; Bernard, 1942). From the results of these and other studies one can safely conclude that occupational exposure to benzene is associated with an increased risk of hematologic neoplasms.

Despite the weight of evidence implicating benzene as a leukemogen in man, there is no clear understanding of the pathogenesis of the disease or the quantitative relationship between benzene exposure and leukemogenesis. Although chronic exposure to 100 ppm benzene is known to result in bone marrow damage and an increased incidence of leukemia in man, the significance of exposure to low concentrations of benzene is controversial. A prevailing sentiment among clinical hematologists is that blood dyscrasias indicative of bone marrow damage frequently, if not invariably, precede the development of secondary AML. However, it presently cannot be concluded that frank bone marrow damage is an absolute prerequisite in every case. Thus, the evidence linking blood dyscrasias or leukemia with exposure to benzene at lower concentrations in man is controversial, and there is little agreement among clinicians, epidemiologists, and researchers as to the significance of exposure to benzene at low concentrations (Infante et *al.,* 1977; Harris, 1977). It is not known, for example, whether bone marrow suppression, frequently seen at higher exposure concentrations, is a requirement for the evolution of AML, implying a threshold below which benzene is not leukemogenic, or alternatively, whether there is no effective threshold for benzene. This debate is not likely to be resolved without an increased understanding of the mechanisms of leukemogenesis.

Historically, such studies have been hampered by the lack of an appropriate animal model for benzene-induced leukemogenesis. To varying degrees, investigators have been successful in demonstrating temporary or reversible bone marrow toxicity following benzene exposure in rats and mice (Snyder et *al.,* 1978; Snyder et *al.,* 1982a,b; Goldstein *et al.,* 1982; Cronkite *et al.,* 1984; Cronkiteet *al.,* 1985; Green et *al.,* 1981). Nevertheless, previous attempts to produce a useful leukemia model have been disappointing. However, as a consequence of recent studies conducted by Dr. Eugene Cronkite at Brookhaven National Laboratories and results obtained in experiments at CIIT, it would now appear that these difficulties have been largely overcome.

Early studies at CIIT revealed that toxicity of benzene or its metabolites to bone marrow cells followed a Pattern exhibited by certain cancer chemotherapeutic agents that are toxic to cells only at certain stages in their growth cycle (Irons et *al.,* 1979). The proportion of cells affected following exposure to such a cycle-specific agent is governed by the number of cells in a sensitive phase regardless of the dose of the compound administered (Irons and Horan, 1978). For such agents, the regimen or temporal pattern of exposure may be equally as significant as the actual exposure concentration.

While investigating the effects of concentration and duration of exposure on benzene-induced bone marrow suppression in mice, Dr. Cronkite and his co-workers adopted an exposure regimen that deviated significantly from the traditional lifetime exposure protocols previously used to study the leukemogenicity of benzene. They exposed mice for 16 weeks to 300 ppm benzene and held them for lifetime observation. Two models of benzene-induced leukemia have been described by Cronkite using this regimen: thymic lymphoma/leukemia in the C57BL/6 mouse, a mouse known to carry MuLV and found to have a high incidence of this tumor type following radiation exposure; and myelogenous leukemia in the CBA/Ca mouse, which has a very low spontaneous incidence of AML but is known to develop a high incidence of AML after exposure to radiation (Cronkite, 1987).

Most recently, in experiments again intended to characterize the regimen-dose dependence of benzene bone marrow toxicity, we have obtained preliminary results that confirm and extend the use of the C57BL/6 mouse as a model for benzene leukemia. In these experiments, mice were exposed to 100 or 300 ppm, either 3 or 6 days/week for 12 weeks and held for observation. At approximately 6 months, MDS was encountered in animals in each of the exposure groups. Included in the spectrum of hematopoietic lesions encountered were not only T-cell lymphoma/leukemia but myelogenous leukemia as well (Cathro et *al.,* 1988). With these models we are now afforded an opportunity to investigate systematically potential mechanisms of benzene toxicity to the bone marrow and leukemogenesis.

The history of research in chemical leukemogenesis suggests that numerous

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factors, including retrovirus background, the regimen of chemical exposure, and its duration may be important considerations in carcinogenesis that have so far received little attention. Understanding the relationship between chemical toxicity, retroviral genes and leukemogenesis will aid in providing an informed evaluation of both the appropriateness of the mouseasamodel for extrapolating risk of leukemogenesis to man and the relative importance of chronic high versus low level or transient chemical exposures with respect to human health. Such information is critically important for the development of sound regulatory and industrial exposure policies.

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Service Awards Presented

Twenty-six CIIT empioyees were recognized for five and ten years' service at the institute's Christmas party on December 12.

Reaching the ten year mark were (seated, left to right): Dr. Richard J. Levine, Epidemiology; Charles A. Howard, Building Services; Dr. Henry d'A. Heck, Biochemical Toxicology and Pathobiology; Dr. Byron E. Butteworth, Genetic Toxicology; Betsy Gross Bermudez, Experimental Pathology and Toxicology; and Diane J. Abemethy, Genetic Toxicology.

Standing, left to right: Edna A. Mangum, Administration; Delorise H. Williams, Experimental Pathology and Toxicology; Corrie N. Smith, Experimental Pathology and Toxicology; Charles M. Overton, Purchasing; and Dr. Douglas E. Rickert, Biochemical Toxicology and Pathobiology. Not pictured is Donald F. Deyo, Biochemical Toxicology and Pathobiology.

Recognized for five years' service were (seated, left to rlght): Gary Trent Jones, Experimental Pathology and Toxicology; Betty A. Honeycutt, Genetic Toxicology; Linda K. Garvey, Experimental Pathology and Toxicology; Karen M. Dold, Cellular and Molecular Toxicology; Dr. Jack H. Dean, Cellular and Molecular Toxicology; and Kaye S. Nagy, Purchasing.

Standing: R. Dwight McFarland, Building Services; Carl U. Parkinson, Jr., Experimental Pathology and Toxicology; Dr. Thomas R. Skopek, Genetic Toxicology; Dr. Frank Welsch, Cellular and Molecular Toxicology; and Donald B. Stedman, Cellular and Molecular Toxicology. Five-year employees not pictured are Dr. William F. Greenlee, Cellular and Molecular Toxicology; Lloyd D. Lauer, Cellular and Molecular Toxicology: and Mary S. Morris, Experimental Pathology and Toxicology.