Advances in the Viral Etiology of Leukemia and Lymphoma

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PREVIOUS EXPOSURE to certain chemicals or radiation has been implicated as an etiologic factor in the initiation of leukemia of man in a minor fraction of cases.[21] Certain predisposing hereditary conditions have also been identified,[43] and clearly there are unusual families with many members with leukemia suggestive of complex hereditary factors. However, the factors contributing to the development of most cases of human leukemia are still unknown.

Studies of many animal leukemias clearly implicate certain viruses. RNA tumor viruses (oncornaviruses) have been frequently isolated from or associated with the leukemic cells of animals as diverse as fish,[68] snakes,[94] birds,[87] rodents,[42] cats,[23] cattle,[14] and nonhuman primates.[49-52,89] The study of these isolates from different animals has led to defining two major evolutionary classes of oncornaviruses[1,12,40,56,91] (Table 1). Class 1 viruses are true endogenous viruses. The genes for these viruses are found in normal, nontransformed cells of the natural host of the virus and appear to be under host control. Usually they are not oncogenic for the homologous host. A role for these endogenous viruses in normal development, including the normal differentiation of hematopoietic cells, has been proposed but not proved.[1,39] In contrast, class 2 viruses contain at least some genetic information distinct from or completely unrelated to genes found in normal cells of the virus's natural host.

The proposal has been made that class 2 viruses evolve from class 1 viruses via direct genetic change of the class 1 "virogene" by an environmental event (such as radiation, carcinogens, or spontaneous change). The class 2 RNA tumor viruses then become self-infectious or oncogenic for the original species and sometimes for other species.[12,38,91]

Interspecies transmission of RNA tumor viruses has been shown clearly in recent years (see the recent review by Todaro[91]). The transmission of viral information between species may be especially pertinent to human leukemia, since "footprints" related to different groups of type-C oncornaviruses have been identified in human leukemic tissue (reviewed in Refs. 29, 32, and 35). Thus molecular probes derived from the murine leukemia virus, strain Rauscher (MuLV$_R$), the simian sarcoma virus (SiSV)/gibbon ape leukemia virus (GaLV) group, baboon endogenous virus (BaEV), and feline leukemia virus (FeLV) have been detected in different cases of human leukemia. This finding does *not*

Abbreviations: RT, reverse transcriptase [RNA-directed (dependent) DNA polymerase]; cDNA, DNA complementary to a viral RNA; SD, simultaneous detection test (see Fig. 1); MuLV, murine leukemia virus; MuLV$_R$, MuLV strain Rauscher; SiSV, simian sarcoma virus; GaLV, gibbon ape leukemia virus; BaEV, baboon endogenous virus; FeLV, feline leukemia virus.

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Table 1. Evolutionary Classes of Type-C RNA Tumor Viruses

Property	Class 1 (Endogenous)	Class 2 (Exogenous)
Source	Normal tissue (usually) Normal cell genes $\xrightarrow{\;*\;}$	Malignant tissue (usually) RNA tumor virus
Transmission	Vertical, "proviral"	Horizontal, infectious, can infect germ line and become vertically transmitted
Nucleotide sequences	Present in uninfected cell	At least same sequences not found in uninfected cells
Oncogenic	Usually not in same species, may be oncogenic in heterologous host	Usually found in natural host; also identified as well in other select species.
Role (usual)	? Embryonic development ? Differentiation	? Genetic information transfer
Examples	Baboon endogenous virus Feline endogenous virus	Simian sarcoma virus Gibbon ape leukemia virus Murine leukemia virus Feline leukemia virus

*Genetic perturbation, radiation, chemicals, spontaneous mutation, recombination with host cellular sequences.

mean that the "viral marker" found in human cells is necessarily or even likely derived from one of the viruses used to generate the test probe, but this information does suggest the detection of a viral marker from a related virus.

Numerous laboratory isolates of different strains of MuLV are known,[42] recently some even from wild mice.[71] The only animal isolate of SiSV was obtained from a fibrosarcoma of a pet woolly monkey.[89] It is a highly infectious virus with the capacity to transform efficiently fibroblasts in vitro. This virus was shown by molecular hybridization to be distantly related to MuLV and especially to certain strains of Asian mouse viruses.[100] GaLV has been isolated from several leukemic gibbons[31,51,52] (reviewed in Ref. 36). Interestingly, the nucleotide sequences and proteins of SiSV are closely related to each GaLV isolate.[31] It appears, then, that SiSV and the different GaLV isolates form members of a closely related group that may have originated in the past from an Asian mouse virus that entered at least two primate species by infection.[56,100] It is important to note that GaLV infection of gibbons can produce either acute lymphoblastic leukemia (ALL) or a disease mimicking human chronic myelogenous leukemia (CML). The latter is of particular interest in view of the lack of an animal model for CML and by the fact that this ape is a species closely related to man.

For over fifty years evidence for RNA tumor viruses in human disease has been sought, and within the past eight years data have slowly accumulated that indicate that information related to or derived from these viruses is present in the human population. Here we will but outline the evidence for the presence of RNA tumor virus information in human leukemia, since this subject has recently been extensively considered elsewhere.[32] The major emphasis of this review will be a critical examination of this evidence, which will be done by ex-

ploring the following questions: Why has infectious type-C virus not been frequently isolated from leukemic tissue? What role does the frequently detected intracytoplasmic particle play in human leukemia? What are the prospects for developing clinically useful oncornavirus markers in human leukemia? What are the prospects for specific antiviral therapy?

RNA TUMOR VIRUS INFORMATION IN HUMAN LEUKEMIA

The bulk of the evidence that has accumulated supporting the presence of RNA tumor virus components in man has been obtained from myelogenous leukemia (Table 2). The first attempts to demonstrate oncornaviruses in human leukemia were by electron microscopy. "Oncornaviral-like particles" were seen,[22] but the biochemical tools were not then available to follow up this observation.

Attempts to define oncornavirus components in human disease were markedly facilitated by a better understanding of these components in animal systems. Soon after the demonstration of an RNA-dependent DNA polymerase [reverse transcriptase (RT)] in murine[8] and avian[88] virions, this or a related DNA polymerase enzyme was sought and found in some human leukemic cells.[37] The major activity of this DNA polymerase was localized to a postmitochondrial particulate fraction.[2,27] The leukemic cell DNA polymerase resembling RT has been further characterized by several indirect and direct methods.[10,19,33,62,75,99] The cytoplasmic particles were shown to have some properties of known oncornaviruses (see below). These observations led to the development of the "simultaneous detection (SD) test" in Spiegelman's laboratory[10,82] (Fig. 1). This assay is an indirect sensitive test for RT and in Spiegelman's hands has almost been a universal indicator of leukemic disease.[82] The major advantage of this assay is its sensitivity, but correct interpretation requires extensive analysis of the products to show that a RNA-primed RNA-directed DNA polymerase is detected.[34,73] Also, the SD test does not verify the origin of the enzyme activity being detected, since the crude nature of the preparation precludes immunological analysis.

Direct isolation of RT from the postmitochondrial particulate fraction of leukemic tissue has been reported by several groups.[62,75,99] Once isolated, it is obviously desirable to distinguish biochemically this enzyme from the other cellular DNA polymerases and to attempt to define its origin. Stringent criteria for the demonstration of an intracellular human RT have been proposed.[102] These criteria have been established to assure proper biochemical classification

Table 2. Evidence for RNA Tumor Virus Information in Human Leukemia

Detection of viral components

  Intracytoplasmic particles with properties of known oncornaviruses (common)

  Reverse transcriptase related to the murine and certain nonhuman primate enzymes (occasional)

  Electron-microscopic demonstration (rare)

Demonstration of extracellular viral particles

  Noninfectious, nonbudding viruslike particles released in short-term tissue culture (common)

  Transforming, nonreplicating virus (rarely shown)

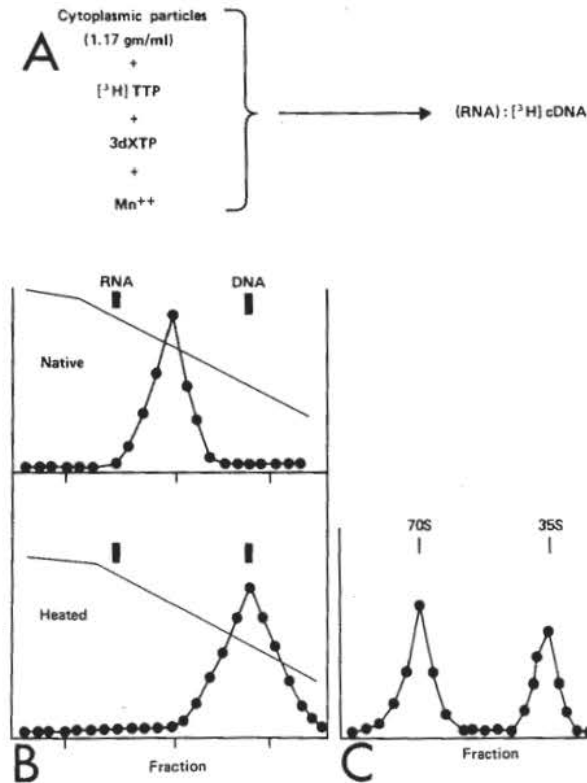 Replicating, budding virus (extremely rare reports)

Fig. 1. Simultaneous detection test. Small cytoplasmic particles are obtained, and in (A) endogenous reaction [^{3}H] complementary DNA (cDNA) is synthesized. (B) Cs_2SO_4 analysis indicates that [^{3}H] cDNA is hydrogen bonded to RNA (i.e., a hybrid and therefore suggesting reverse transcriptase activity). (C) Velocity gradient analysis indicates that some RNA sediments with S values of 35 and 70 (therefore a large viruslike RNA). Results then suggest both RT and viruslike RNA in one simultaneous assay.

of these enzymes. A study of the biochemical and immunologic relationships of human leukemic RT has shown several remarkable features.[27,28,62,92,102] Approximately 80% of the enzymes defined biochemically as RT are not immunologically detectably related to RT from the viruses tested. These include some primate and murine viruses. Of the remaining 20% with RT, viral relatedness has been shown immunologically, and the following features have been noted: these were invariable in cases of myeloblastic leukemia, and in each there was almost complete inhibition of the purified RT by antibodies to SiSV/GaLV RT. In some of these cases there was also partial inhibition by antibody to RT from $MuLV_R$. The development of an antibody to RT obtained from human tissue is an alternative approach to defining the immunologic relatedness of these enzymes. However, in only one instance has an antibody been reported to a human polymerase with properties of RT. This DNA polymerase was isolated from the spleen of a child with myelofibrosis.[15,83] Preliminary results show that this polymerase, like the enzyme found in some AML cells, is immunologically related to RT from SiSV.[83] Interestingly enough, this patient subsequently developed AML (Chandra P: personal communication).

Several important questions arise regarding the presence of RT in human leukemic cells. What is the nature of the enzyme activity being detected in the SD test? What is the origin of those RT detected in leukemic tissues that are biochemically an RT but to date are immunologically undefined? Is the RT found in human leukemic cells largely derived from exogenous viral informa-

tion or from the expression of endogenous type-C viral genes? Does the presence of viral-related RT imply etiologic involvement of an animal-related virus in human leukemia? Presently these questions cannot be answered, but recent findings with immunologic and nucleic acid probes may allow the development of some new approaches (see below).

Genomic information related to known RNA tumor viruses has been sought both in the cDNA synthesized endogenously by cytoplasmic particles found in fresh leukemic blood cells[29,61] and in the genome of leukemic blood cells.[6,11,35,101] Thus nucleotide sequences, the [^{3}H]cDNA probes prepared from the cytoplasmic particles, hybridize to 70S RNA from SiSV in about 50% of cases of myelogenous leukemia and in some of these cases to 70S RNA from MuLV$_R$. According to Spiegelman and Baxt "leukemic specific" sequences may be uniformly present in the [^{3}H]cDNA,[11,82] and in some cases may be related to MuLV$_R$.[9] However, there are great technical difficulties with these experiments, and the particle cDNA is limited in size and quantity.[29,40] A possible recent advance in this area has been an approach for direct isolation of the RNA that may be the template for the cDNA in these particles.[60] Characterization of the nucleotide sequences of this RNA should be of considerable interest.

Proviral information related to oncornaviruses has been reportedly found in DNA from only a few leukemic samples.[6,72,101] Some examples include the report of BaEV related sequences in seven samples,[100] and with a purified recycled MuLV$_R$ [^{3}H]cDNA probe nucleotide sequences distantly related to MuLV$_R$ were found in DNA from tissues of two of eight patients tested with myelogenous leukemia.[6] The latter observation indicates that a virus detectably related to but not the same as a mouse leukemia virus may have entered some leukemic cells. It is important to note that the positive results with MuLV$_R$ and BaEV cannot be attributed to the same or overlapping sequences; i.e., MuLV$_R$ and BaEV are not related RNA tumor viruses.

Recently, our laboratory has sought to identify unknown proviral sequences, that is, sequences not related to any known animal RNA tumor virus. This approach has the advantages of not having to screen every DNA for every known virus and of being able to pick up unknown proviral sequences. Briefly, the experiment depends on the fact that the proviral sequences of all known oncornaviruses have a G-C content of 50%, while main-band eukaryotic DNA has a G-C content of 40%. Using this information we have identified in leukemic cells an intracytoplasmic high molecular weight RNA that hybridizes to the 50% G-C region of human DNA.[60] This method is being explored further as a means to detect and characterize unique sequences in the leukemic cell genome. In view of the occasional detection of a DNA polymerase related to RT from SiSV and GaLV, the difficulty in proving that leukemic cells contain DNA nucleic acid sequences highly related to SiSV or GaLV will be discussed below.

Immunologic studies related to oncornaviruses and human leukemia are very recent and as yet unclear. The studies can be developed into three major areas: complement-mediated oncornavirus-lysing systems, direct detection of virus-related antigens, and antibodies to virus-related antigens.

A complement-mediated RNA tumor virus-lysing system has been reported

in the sera of primates, including humans, that is absent in some lower animals.[20,26] The studies clearly show that serum lytic activity does not completely protect against oncornavirus infection in a given species, but it may be important in limiting horizontal spread. More work needs to be done in this area to determine the role of this system in disease prevention.

The detection of virus-related antigens has given conflicting results.[58,59,78,84,85] The detection of p30 (the major structural protein—molecular weight 30,000 daltons—of most oncornaviruses) by radioimmunoassay,[78,85] $MuLV_R$ determinants on the leukemic cell surface,[58,84] and a GaLV-SiSV gp 69/71 (the major envelope protein of these viruses with a molecular weight of 69,000–71,000 daltons) antigen on the membranes of leukemic cells[59] gave variable results. Moreover, the results are difficult to interpret, since neither the antigens nor the antibodies are pure or chemically characterized.

The study of naturally occurring antibodies to oncornavirus components in man has also yielded conflicting results. In a recent report Aoki and co-workers found that human leukemia cells can be used to absorb selectively antibodies to envelop antigens of the BaEV group[5] and GaLV/SiSV group[4,5] and that some human sera contain antibodies that react with cells producing some of these viruses. Other groups have also reported positive results when human sera were tested for antibody to whole disrupted virus.[55,85] These reactions may have been to the viral envelope protein gp70. In fact, Snyder et al. were able to absorb specifically the positive human sera with partially purified SiSV gp 70.[85] However, negative results were obtained by a few groups in attempting to show antibodies in human sera that can precipitate purified gp70 or p30.[17,18,84] These conflicting results may be explained by artifacts of cross reactions (false positives) or, alternatively, may be due to loss of antigenicity of the viral probes during purification and/or iodination (false negatives).

It should be emphasized that even conclusive negative results for the detection of viral structural proteins or antibodies to them indicate only that it is unlikely that abundant replicating virus is present. They do not exclude the presence of a minimally replicating oncornavirus. There are now numerous examples of virus-infected and even virus-transformed cells where no viral structural proteins can be found in laboratory experiments. For instance, clones of rat cells have been obtained that, although transformed by SiSV, yield no SiSV proteins.[77] Moreover, we have examples in whole animals with naturally occurring leukemia where no virus has been isolated, proviral nucleic acid sequences can be difficult or impossible to detect, and no antibodies to viral structural proteins are found, yet the virus has infected these animals as evidenced by either antibody to RT or antibody to an antigen known to be specifically induced by the virus. Examples like this have been found in cattle (Burny A: personal communication) and cats (Jacquemin P, Wong-Staal F, Koshy R, et al: unpublished results).

Recently, antibodies reactive with RT purified from specific RNA tumor viruses have been identified on the surface of some human leukemic myelogenous blood and bone marrow cells not found in serum from the same patients (Jacquemin P, Saxinger W, Gallo R: manuscript in preparation). These antibodies show striking specificity, and have been identified only after ex-

tensive purification of Ig. The antibodies eluted from the membranes of AML cells in positive cases specifically inhibit RT from the GaLV-SiSV group, while antibodies eluted from the membranes of leukocytes from patients with CML in blast crisis specifically inhibit feline leukemia virus (FeLV). Prochownik and Kirsten found similar results with sera from two cases of AML, i.e., antibodies reactive with RT from SiSV.[70]

We believe that sufficient data from several groups have now accumulated to justify the conclusion that information derived from RNA tumor viruses is present in the human population. The major questions to ask now are: How did this information enter the human population? Is it important for leukemia or any disease? We can approach these issues by asking questions about specific findings, examining the difficulties with the available data with respect to classical mechanisms, and offering other mechanisms to explain these difficulties.

TYPE-C VIRUSES HAVE NOT BEEN FREQUENTLY ISOLATED FROM HUMAN LEUKEMIC TISSUES

An apparent major difficulty in linking type-C oncornaviruses with human disease is the rarity of isolating replicating biologically active virus from human tissue. This problem will be approached by first reviewing the conditions under which most animal isolates are obtained. Several important points emerge: Replicating virus can be isolated with high efficiency from several inbred strains of animals,[36,42,93] yet transformation without replication can be obtained by cloning.[69,77,79] Noteworthy is that in some of these nonproducing cases protein and nucleic acid viral components can be detected. Acquired oncornaviruses can be isolated from some wild-type animals, including birds, mice, cats, cattle, and nonhuman primates and endogenous viruses from many more species. The feline, cattle, and nonhuman primate leukemias illustrate several important points. For instance, even in frank epidemics infectious virus can be isolated only from about 50% of leukemic cats.[23] A recent study of the expression of RD-114 (an endogenous cat virus related to BaEV) and FeLV RNA and p30 illustrates the complicated situation that may exist regarding viral expression.[63] In this study it was found that RD-114 RNA and p30 were expressed in the lymphomas of both younger and older animals, whereas FeLV expression seemed to prevail among cats less than 5 yr old. Also, the level of expression ranged over 2 logs.

The nonhuman primate viruses BaEV, SiSV, and GaLV may be of special interest, since components related to these viruses have been reported in human leukemic tissues, yet an epidemiologic study using molecular, biologic, and immunologic approaches to detect components of these primate viruses has not been reported in non–virus-producing primates with spontaneous neoplasias. Of related interest is the situation in dog lymphomas and leukemias and similar diseases in other species, where to date no virus has been isolated. Is it that viruses are not important in leukemias and lymphomas of these species, or is it that the proper conditions have not yet been found to isolate virus? The question may be moot, but it is an important consideration in regard to the isolation of viruses from human tissues.

The nature of the oncornaviral isolates that have been reported deserves

comment. The reports can be divided into three major categories: (1) transient release of viral*like* particles; (2) partially characterized infectious or transforming virus; and (3) replicating infectious virus. Numerous reports of transient release of virallike particles have been made,[7,53,54,57,95] and reviewed.[32] These reports indicate that particles with some biochemical and biophysical properties of oncornaviruses can be released from some human cells. A large survey for these particles has not yet been reported, nor have extensive attempts been made to characterize them. Several reports suggest that following these particles or their components might be useful in monitoring residual leukemic diseases;[95,96] however, a longitudinal study has yet to be reported. The limitations in these experiments are the small quantity of particles and the inability to date to show biologic activity.

Several isolations of biologically infectious virus from leukemic tissues have been reported,[24,25,48,64,67] and several reports of a transforming virus from human tissue have been made.[24,65] Presently, the most characterized oncornaviruses reported as isolates from human tissues are HL-23V, HEL-12, SAK-21, and isolates from Gabelman and Kaplan et al. HL-23V has been isolated on multiple occasions from a middle-aged female with AML.[28] This isolate has been extensively analyzed, and the following points emerge: First, the virus has two distinct genetic components, one related to BaEV and one related to SiSV.[72,86] Second, SiSV and BaEV components related to those of the isolated virus have been detected in the patient's fresh tissue.[72] Third, the virus is more infectious for normal human bone marrow than SiSV itself (Ruscetti F, Gallo R: unpublished data). HEL-12, isolated by Panem and Kirsten, is released from late-passage human embryonic lung fibroblasts. It has been partially characterized, and it also has proteins and nucleic acids related to both SiSV and BaEV.[66,67] SAK-21 was isolated by Nooter and co-workers[64,65] from a child with ALL. It also resembles SiSV and may have a second component related to BaEV. Gabelman's isolate was obtained from the lung tissue of a patient with CLL and adenocarcinoma. Proteins and nucleic acids related to SiSV were identified,[24] but the virus was lost before full characterization was accomplished. The most recent virus isolate was obtained from a histiocytic lymphoma cell line by Kaplan et al.[48] This virus also shows some homology to the SiSV/GaLV group. These studies in toto suggest that type-C oncornavirus particles related to MuLV and to at least two nonhuman primate virus groups, SiSV-GaLV and BaEV, may sometimes enter man (for more complete review, see refs. 32 and 35).

The possibility that the detection of expression of information (as RNA or proteins) related to type-C viruses in some human leukemic cells is only a difference in gene expression between normal and leukemic cells is conceivable but unlikely, since sequences related to $MuLV_R$ or SiSV are not generally found in nucleic acids from normal tissues and therefore expression of information related to these viruses is not likely to be obtained only from endogenous sequences. However, the expression of murine sarcoma virus genes in uninfected rat cells subject to anerobic stress and other, earlier studies indicate that sarcoma genes in some cases represent recombinational events of virus and normal host cellular nucleotide sequences.[3]

The evidence to date then points strongly towards the presence of oncorna-virus components in human leukemia. The nature of the interaction, how this information entered man, and its role, if any, in producing the phenotypic change of malignant proliferation is elusive. We shall explore some of the possibilities in the following sections.

WHY ARE SiSV-RELATED PROVIRAL NUCLEIC ACID SEQUENCES RARELY FOUND IN LEUKEMIC TISSUES?

As mentioned earlier, the initial characterization of the RT isolated from some human leukemic cytoplasmic particles indicated that in some cases this polymerase was immunologically closely related to the RT of the SiSV-GaLV group and more distantly related to the RT of various murine leukemia viruses.[27,29,62,92] Prior to extraction and purification of this polymerase from the particles, it was shown that as a component of these particles this enzyme could catalyze endogenous synthesis of cDNA from an RNA template in the particle, a reaction analogous to that catalyzed by RT from isolated extracellu-lar animal RNA tumor viruses. This cDNA was analyzed and found to hybrid-ize significantly to 70S RNA from SiSV and to a lesser extent to 70S RNA from $MuLV_R$.[29] The identification of SiSV- and $MuLV_R$-related sequences in the cytoplasm of leukemic cells suggested then that proviral nucleotide sequences related to either the SiSV or the $MuLV_R$ genomes should be found in the leu-kemic tissue DNA. Despite extensive testing, highly related SiSV sequences were not initially found in human DNA from normal or leukemic tissue.[29]

If the integration of SiSV-related sequences is important for the phenotypic expression of leukemia, other mechanisms must be sought. We shall first con-sider the instance in which no sequences related to SiSV are detectable. The failure to detect highly related sequences to SiSV does not preclude their pres-ence. Several possibilities exist (Table 3). First, the SiSV could be completely integrated in only a rare cell. Conventional hybridization would not pick up these sequences. This possibility is given support by the demonstration by electron microscopy that probably only rare leukemic cells express intracyclo-plasmic viruslike particles.[22,97] Also, it has been clearly shown that from virus-producing cells some cells can be cloned that are transformed but that do not contain detectable proviral sequences,[69,77,79] yet in these same cells evidence of virus-related protein expression can be easily detected.[77,79] Presumably, if the

Table 3. Alternative Explanations for Failure to Detect SiSV Proviral DNA

Explanation	Cause of Phenotypic Expression of Leukemio	How to Show
SiSV integrated only in a rare cell	Rare cell produces abnormal regulatory factors	Hybridization in situ and detection of factors
"Hit and run," complete or partial SiSV integrated and then excised	Damage to critical regulatory genes	Chromosome mapping
"Double infection," SiSV integrated into tissue remote from its phenotypic effect	Abnormal factors or particles pro-duced by SiSV-infected cells that in turn affect appropriate hematopoietic cell	Detect abnormal factors or SiSV sequences consistently in a tissue type

rare cell producing virus is important in the phenotypic expression of leukemia, then the majority of cells are responding to a factor(s) secreted by these cells. To date no such factor(s) has been identified in leukemic cells, although leukemic cells grown in semisolid media may show aberrant growth characteristics.[41] It would be of interest to remove these cloned cells from semisolid media and examine them for viral expression, but to date this has not been reported.

An alternate approach is to look for proviral expression in single cells using the technique of hybridization in situ.[47] We are currently developing the system in situ simply for the detection of proviral information in single human leukemic cells. Of interest in this regard is a recent report with visna virus–infected tissues. Visna is a retrovirus, i.e., an RNA virus with RT that replicates through a DNA intermediate like all RNA tumor viruses but that produces neurologic disease, not neoplasia, in sheep. The report is concerned with the measurement of protein expression by immunofluoresence and proviral expression by hybridization in situ. The results indicate that 18% of the cells show evidence of protein expression, while less than 0.5% of cells contain detectable proviral sequences.[44]

A second possibility is that the complete SiSV could be integrated and then completely or partially excised. The integration and subsequent loss of sarcoma sequences has been described in known animal systems.[77] Peculiarly, what has not been adequately studied in animals naturally infected by viruses is the effect of the infection on chromosomes, especially in light of the experimental data showing an increase in the incidence of sister chromatid exchanges in mouse embryo fibroblasts infected with $MuLV_R$.[13] With the advent of sophisticated chromosome banding techniques, very specific chromosome lesions have now been found in some cases of AML.[74,103]

An important related question with regard to the loss of SiSV proviral sequences is whether or not the proviral sequences detected in leukemic tissue related to $MuLV_R$ sequences are also *distantly* related to SiSV. Sequences distantly related to SiSV have been detected in a large number of leukemic cells and tissues (Reitz M, Miller N, Gallo R: unpublished data). The experiments to determine the nature of these sequences have not yet been performed, but they may represent the same sequences that have been detected with nucleic acid probes (cDNA) prepared from $MuLV_R$ RNA by Aulakh and Gallo,[6] since murine viruses are distantly related to SiSV.[100] If so, this would argue that only a small number of specific nucleotides may be necessary for the phenotypic expression of leukemia. If a short transforming sequence could be identified, this sequence could be mapped and conceivably molecular manipulation entertained.

A third possibility is that the infecting virus is integrated in a tissue remote from the primary phenotypic expression. At least in animal systems this precedent has not been described. However, it is known that in some cases many tissues can be infected. No systematic study of SiSV-related sequences in tissue of patients with leukemia has yet been reported, but sequences *highly* related to SiSV have been detected in DNA purified from a *few* leukemic tissues. These tissues include the spleen of patient HL23, the patient from whom an infectious

type-C oncornavirus has been repeatedly isolated;[28] the spleen of one other patient with AML (Wong-Staal F, Reitz M, Gallo R: unpublished data); and the spleens of two other patients, one with osteosarcoma and the other with AML (Prochownik E, Kirsten W: personal communication). In none of these patients have nucleotide sequences highly related to SiSV been found in the *primary* leukemic cells. Significantly, sequences have also been detected in a few normal placenta-embryos, suggesting that the placenta may be a site of virus replication (Wong-Staal F, Reitz M, Gallo R: unpublished data). This finding is consistent with the isolation of infectious viruses highly related to SiSV from human embryo by Panem et al.[67] Finally, it is possible that small fragments of proviral sequences, rather than complete provirus, are integrated into the DNA of some human leukemic cells. This change could be very difficult to detect by conventional molecular hybridization techniques.

WHAT ROLE DOES THE INTRACYTOPLASMIC PARTICLE PLAY IN HUMAN LEUKEMIA?

As noted earlier, soon after RT was detected in human leukemic cells its major activity was localized to a postmitochondrial particulate fraction. This subcellular fraction was soon shown to contain particles with morphologic integrity in that they could be isopyknically fractionated and several of their characteristics determined. These cytoplasmic particles were found to have several properties similar to known oncornaviruses:

First, the particles themselves had several biophysical properties of oncornaviruses: (1) they fractionated isopyknically at a density of 1.15–1.20 g/ml in sucrose and upon treatment with nonionic detergent were converted to a density of 1.22–1.26 g/ml, a phenomen reminiscent of the liberation of inner cores by RNA tumor viruses after disruption,[29] and (2) they were 500 to 1000 Å in diameter as measured by molecular sieve exclusion chromatography (Saxinger W, Gallo R: unpublished observations). Initially, both these parameters were defined by following polymerase activity. More recently, we have labeled these particles directly in vitro with [3H]-uridine or [3H]-adenosine and have followed trichloroacetic acid–precipitable counts directly (Meyskens F, Gallo R, Saxinger W: unpublished data).

Second, the particles contained RT or RT-like activity and the other elements necessary to synthesize DNA.[10,29,61] An analysis of this reaction indicated that the synthesis of the DNA was RNA primed and RNA directed. A high molecular weight (HMW) RNA was present and hydrogen bonded to the synthesized DNA. This cDNA was analyzed in a few cases, and the nucleotide sequences shared significant homology with SiSV and to a lesser extent with MuLV.[29] In one case (fresh leukocytes of patient HL23), significant homology to the BaEV genome was also shown.[72]

Third, the particle contained RT that had been isolated and in some cases shown to be related to the RT of known oncornaviruses.

Fourth, the particle contained HMW RNA.[10,29,60] The presence of HMW RNA was indirectly shown by proving that the DNA synthesized in an endogenous reaction was hydrogen bonded to a molecule that sedimented at 70S

and was ribonuclease sensitive.[10,60] More recently, we have directly isolated and characterized this HMW RNA from the high-density leukemic cell particles.[60] The RNA had several properties of the RNA of known RNA tumor viruses: (1) the RNA was single stranded, as judged by nuclease, $CsSO_4$, and hydroxyapatite analysis; (2) the HMW RNA could be found native in a 70S to 50S form; (3) the HMW RNA could be denatured to 30–40S subunits; (4) the RNA had an associated poly(A) tract 200–300 nucleotides in length; and (5) the RNA hybridized to the 50% G-C region of human DNA, a region in which the RNA of known RNA tumor viruses hybridizes.

The available evidence then clearly indicates that some fresh human leukemic cells contain viruslike particles. In a few cases intracellular oncornaviruslike particles have been seen by electron microscopy.[22,97] However, the data suggest that these particles must be at a very low titer. To date similar particles have not been isolated from chronically infected cell lines (review, ref. 102); however, few attempts have been made to isolate these intracytoplasmic particles from fresh animal tissue.

Several ideas can be entertained to explain the presence of the particle: The presence of SiSV related sequences and an RT related to SiSV in the cytoplasmic particles in the absence of detectable SiSV proviral sequences might be explained by a "double infection" model, which proposes that particles are synthesized at a site distant to the site (leukocytes) of their phenotypic effects, requiring that the leukocytes destined to become leukemic engulf these particles. The particles then cause the observed phenotypic effects. Alternatively, the production of particles by the leukocytes may be incidental to a primary cell regulator defect; the particles would be a result rather than a cause of the observed phenotypic changes.

As noted above, the titer of intracytoplasmic particles is very low, at least as inferred from indirect electron microscopic measurements. A particularly relevant point here is that Fuscaldo et al. (Fuscaldo A, Fuscaldo K, Brodsky I: personal communication) have calculated that in polycythemia vera platelets, where similar if not identical particles have been discovered, the titer is less than 10^6 particles/g cells. Numerous investigators have attempted to propagate secondarily *released* particles (review, ref. 32), but these attempts have generally failed. This failure could be due to all particles being biologically inactive or because the appropriate bioassay systems have not been used. An alternative possibility is that there are two populations of particles in leukemic cells, a smaller population that is infectious and a larger population that is defective and interfering with infectivity of active particles. Defective interfering particles are seen in many different types of RNA and DNA viruses,[45,46] the best studied being the vesicular stomatitis virus (VSV) and influenza virus. At least in VSV the defective and infectious viruses can be simply separated by density gradient centrifugation.[46] Whether or not the same is true for leukemia cell particles has not been investigated.

The techniques to purify leukemia cell particles are presently available. Further studies of the biochemical, immunologic, and biologic nature of these particles will be of interest.

WHAT ARE THE PROSPECTS FOR DEVELOPING CLINICALLY USEFUL ONCORNAVIRUS MARKERS IN HUMAN LEUKEMIA? WHAT ARE THE PROSPECTS FOR SPECIFIC ANTIVIRAL THERAPY?

There are a few possibilities stemming from this work that may lead to useful markers for human leukemia. These include the detection of oncornaviruslike particles, reverse transcriptase, and the new finding briefly mentioned above—specific antibody to RT on the surface of specific types of leukemic cells. More distant possibilities include detection of HMW RNA viral sequences by hybridization in situ, leukemia-specific antigens, or other antibodies.

The presence of released viruslike particles in short-term tissue culture in many cases of leukemia tested has now been reported by many groups (review, ref. 32). Also, the presence of intracellular oncornaviruslike particles has been repeatedly shown.[10,28,48,95,96,98] We have recently been able to detect intracellular [³H]-uridine particles using as few as 10^4 leukemic cells (Meyskens F, Gallo R: unpublished data). To date, however, no serial studies of intracellular or extracellular particles in leukemia have been made.

Reverse transcriptase has been detected in leukemic cells with a high frequency using the SD test.[82] Only one group has examined this test in leukemic patients in remission.[95] This report indicates that the SD test is frequently abnormal in the normal-appearing peripheral blood leukocytes of patients in remission. It would be of interest to determine if this result has any prognostic value. The major disadvantages of this test are that it requires a relatively large number of cells and that it is indirect. However, more direct tests for RT are prohibitively complex for use in routine assays.

The detection of virus-related sequences by hybridization in situ in leukemic cells is a potentially very sensitive tool for detecting sequences in a single cell. The technique of hybridization in situ has been well worked out in some viral systems[47] but presents some difficulties with nucleic acid probes.

The presence of intracytoplasmic and especially extracellular HMW RNA might be developed into a sensitive indicator of residual disease. However, since the technical problems associated with the preservation of intracellular (and, to a lesser extent, extracellular) HMW RNA are formidable, it is unlikely that demonstration of HMW RNA could ever be used as a routine assay.

The status of leukemia-specific membrane antigens is too muddled to allow us to contemplate the development of specific probes at this point. However, as noted earlier, a recent development in our laboratory has been the detection of antibodies on the cell surface of myelogenous leukemia cells specific for certain viral RT (Jacquemin P, Saxinger W, Gallo R: unpublished data). These antibodies can be eluted and purified. The purified antibodies (IgG) in about 25% of patients with AML react chiefly with RT from SiSV. In CML in blast crisis most samples are positive (eight of nine cases), and the antibodies react chiefly with RT from FeLV. These results do not necessarily indicate that the antibodies are directed against the test viruses used, but they clearly suggest reactions with viruses at least related to the test viruses. The development of a secondary antibody may allow the development of specific and sensitive immunofluorescence assays.

Presently the prospects for specific antioncornaviral therapy are remote. The practical and conceptual difficulties in developing pharmacologic agents to RNA tumor viruses is great.[30,80,81] There are inhibitors of RT, but no absolutely specific inhibitor of RT has been found. Moreover, if one were available, it might be practically effective only in one instance, if relapse of leukemia during remission were secondary to infection of a new cell population. That reinfection might occur is suggested by some of the bone marrow transplantation data.[90] Chandra et al. have developed a relatively specific inhibitor of RT, i.e., one that inhibits RT at concentrations less than that required to inhibit any cellular DNA polymerase. This compound, a thiolated derivative of poly(C), has cytotoxic effects for some animal leukemias and has had reported beneficial effects in some end-stage leukemias of man.[16] Chandra et al. proposed that this compound might have efficacy as a selective cytotoxic compound not because it inhibits RT activity but because it may selectively bind to RT in leukemia cells, increasing uptake in these cells vis-a-vis normal cells. It was proposed that this in turn might lead to cell death by secondary effects on the cell.[16] Maintenance of the transformed state may or may not depend upon the continuing presence of RNA tumor virus integration and/or expression. If the transformed state is dependent on persistent viral integration only, then approaches would appear limited to genetic manipulation. Even if expression of oncornavirus components or the entire virus is needed to maintain the phenotypic result of leukemia, the processing of these molecules is presently not well enough understood to propose methods of inhibition that would not also significantly inhibit normal metabolism.

Animal models suggest that membrane antigens should be intensely investigated. For instance, the identification of a leukemia-specific membrane antigen (FOCMA) in feline leukemia is leading to the development of antiviral therapy and vaccines in cats.[23] The problem in human leukemia is that at least to date no absolutely leukemia-specific membrane antigen has been discovered.

FUTURE DIRECTIONS

The major directions of RNA tumor virus research in human leukemia will include at least the following areas: understanding the mechanism of proviral integration, delineation of the role of the intracytoplasmic viruslike particle, immunologic advances, investigation of preleukemic and other myeloproliferative disorders, and attempts to develop further more specific viral nucleic acid probes.

The presence of apparently acquired molecules related to SiSV, BaEV, and MuLV in human leukemia should be further established. Clearly, the mechanism of entry does not appear to follow the classical mechanism defined in chronically infected tissue culture cell lines.[93] The major question to ask now is whether or not these viruses are etiologically related to the final phenotypic expression of leukemia. Several questions need to be answered. What is the method of acquisition of foreign nucleotide sequences? Is acquisition of *any* foreign oncornaviral sequence sufficient for producing the leukemic state? Why are proviral sequences not detectable in most cases of leukemia? Is proviral information not found because we do not have the right probe, or are proviral

sequences lost after integration? Does proviral loss explain the inability to find highly related SiSV proviral sequences, or are the distantly related SiSV proviral sequences that we find at high frequency sufficient to cause leukemic transformation?

The presence of the intractyoplasmic viruslike particle in leukemia cells is a consistent finding that to date remains equally puzzling and intriguing. In chronically infected tissue culture systems no such particles have been identified, yet at least by the SD test the particle appears to be present frequently in a wide variety of primary animal and human mesenchymal malignancies. What is the role of this particle in human leukemia? Is the particle a partially assembled oncornavirus that is only a secondary manifestation of integration and unrelated per se to generation of the disease? Alternatively, it is an incoming particle released from some other tissues in the body, engulfed by early blood precursors, and somehow directly related to the generation of the leukemic phenotype? Investigation of these particles is still in a primitive phase. With the ability to obtain increasingly more pure populations of the relevant particle, biochemical, biologic, and immunologic characterization of it becomes possible. This aspect will be an emphasis of research in this area in the future.

Immunologic advances will depend on obtaining positive results in human leukemia with well-characterized and well-purified viral proteins. Possibly, a human oncornavirus membrane antigen analogous to FOCMA will be found. The detection of membrane-bound antibodies to RT in human leukemia is to us an interesting and exciting new result, and utilization of these antibodies as probes may allow the development of sensitive single-cell assays. Moreover, some antibodies appear to be specific to certain phases of specific leukemias, e.g., anti-FeLV RT specific to FeLV RT in CML blast crisis cells. This finding may be clinically useful in the detection of incipient blast crisis.

Recently, investigations with preleukemic conditions[96] and other hematopoietic neoplasms, including polycythemia vera,[98] multiple myeloma[76] (and Meyskens F: unpublished data), and lymphomas[19,48] indicate that components related to oncornavirus can also be detected in these disorders. Perhaps one of these neoplasms will have less restriction on expression of the complete virus or be more amenable to long-term manipulations in vitro.

Attempts to develop viral probes is significantly hampered by the fact that it is not yet certain on which viral probes efforts should be concentrated at this time. It seems reasonable to obtain purified viral proteins and their respective antibodies from SiSV, BaEV, and $MuLV_R$. However, even this goal is a formidable undertaking. An alternative and important approach is to assume that the intracytoplasmic particles from different cases of leukemia may be more related than presently known RNA tumor viruses. Purification and characterization of the proteins of the intracytoplasmic particles is an important goal that remains largely unexplored.

The field of RNA tumor viruses and human leukemia has progressed since the discovery in 1970 of RT in a few patients with leukemia. The evidence that has accumulated implicates oncornavirus components in select human cells. The data imply, in fact, that different classes of RNA tumor virus information may be associated with different leukemias. The emphasis of the future will be

to understand the relationship of this information to human disease and to generate reagents that will be useful clinically, either prognostically or therapeutically.

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REFERENCES

1. Aaronson SA, Tronick SR, Stephenson JR: Endogenous type-C RNA viruses of mammalian cells. Biochim Biophys Acta 458:323–354, 1976

2. Ackermann WW, Murphy WH, Miller BA, et al: RNA-dependent DNA synthesis in cell free preparations of human leukemic cells. Biochim Biophys Res Commun 42:723–729, 1971

3. Anderson GR, Matovcik LM: Expression of murine sarcoma virus genes in uninfected rat cells subject to anaerobic stress. Science 197:1371–1373, 1977

4. Aoki T, Luri M, Walling MJ, et al: Specificity of naturally occurring antibody in normal gibbon ape serum. Science 191:1180–1182, 1976

5. Aoki T, Walling MJ, Bushar GS, et al: Natural antibodies in sera from healthy humans to antigens on surfaces of type-C RNA viruses and cells from primates. Proc Natl Acad Sci USA 3:2491–2495, 1976

6. Aulakh GS, Gallo RC: Presence of Rauscher-leukemia virus-related sequences in DNA from some tissues from some hematopoietic neoplasias and their absence in DNA from other tissues. Proc Natl Acad Sci USA 74:353–357, 1977

7. Balabanova H, Kolter M, Becker Y: Transformation of cultured human embryonic fibroblasts by oncornavirus-like particles released from a human carcinoma cell line. Proc Natl Acad Sci USA 72:2794–2798, 1975

8. Baltimore D: RNA-dependent DNA polymerase in virions of RNA tumor viruses. Nature 226:1209–1211, 1970

9. Baxt WG: Sequences present in both leukemic cell nuclear DNA and Rauscher leukemia virus. Proc Natl Acad Sci USA 71:2853–2857, 1974

10. Baxt W, Hehlman R, Spiegelman S: Human leukemic cells contain reverse transcriptase associated with a high molecular weight virus-related RNA. Nature 240:72–75, 1975

11. Baxt WG, Spiegelman S: Nuclear DNA sequences present in human leukemic cells and absent in normal leukocytes. Proc Natl Acad Sci USA 69:3737–3741, 1972

12. Benveniste RE, Todaro GJ: Evolution of C-type viral genes: Inheritance of exogenously acquired viral genes. Nature 252:456–459, 1974

13. Brown RL, Crossen PE: Increased incidence of sister chromatid exchanges in Rauscher leukemia virus infected mouse embryo fibroblasts. Exp Cell Res 103:418–420, 1976

14. Callahan R, Lieber MM, Todaro GJ, et al: Bovine leukemia virus genes in the DNA of leukemic cattle. Science 192:1005–1007, 1976

15. Chandra P, Steel LK: Purification, biochemical characterisation and serological analysis of cellular deoxyribonucleic acid polymerases and a reverse transcriptase from the spleen of a pateint with myelofibrotic syndrome. Biochem J 167:513–524, 1977

16. Chandra P, Steel LK, Eberer V, et al: Chemical inhibitors of oncornaviral DNA polymerases: Biological implications and their mode of action. Pharmacol Ther (in press)

17. Charman HP, Kim U, White N, et al: Failure to detect in human sera antibodies cross-reactive with group-specific antigens of murine leukemia virus. J Natl Cancer Inst 52:1409–1413, 1974

18. Charman HP, Kim U, White M, et al: Natural and experimentally induced antibodies to defined mammalian type-C virus proteins in primates. J Natl Cancer Inst 55:1419–1424, 1975

19. Chezzi C, Dettori G, Manzari V, et al: Simultaneous detection of reverse transcriptase and high molecular weight RNA in tissues of patients with Hodgkin's disease and patients with leukemia. Proc Natl Acad Sci USA 73:4649–4652, 1976

20. Cooper N, Jensen FC, Welsh RM, et al: Lysis of RNA tumor viruses by human serum: Direct antibody independent triggering of the classical complement pathway. J Exp Med 144:970–984, 1976

21. Dameshek W, Gunz F: Leukemia. New York, Grune & Stratton, 1964

22. Dmochowcki L, Taylor AG, Grey CE:

Viruses and neoplasma (PPLO) in human leukemia. Cancer 18:1345–1368, 1965

23. Essex M: Horizontally and vertically transmitted oncornaviruses of cats. Adv Cancer Res 21:175–248, 1975

24. Gabelman N, Waxman S, Smith W, et al: Appearance of C-type virus-like particles after co-cultivation of a human tumor cell line with rat (XC) cells. Int J Cancer 16:355–369, 1975

25. Gallagher RE, Gallo RC: Type-C RNA tumor virus isolated from cultured human acute myelogenous leukemia cells. Science 187:350–353, 1975

26. Gallagher RE, Schrecker AW, Walter CA, et al: Oncornavirus lytic activity in the serum of gibbon apes. J Natl Cancer Inst (in press)

27. Gallagher RE, Todaro GJ, Smith RG: Relationship between RNA-directed DNA polymerase (reverse transcriptase) from human acute leukemic blood cells and primate type-C viruses. Proc Natl Acad Sci USA 71:1309–1313, 1974

28. Gallo RC: RNA tumor viruses and leukemia: Evaluation of present results supporting their presence in human leukemias, in Neth, Gallo, Mannweiler, et al. (eds): Modern Trends in Human Leukemia, vol 2. Munich, Lehmanns, 1976, p 431

29. Gallo RC, Gallagher RE, Miller N, et al: Relationship between components in primate RNA tumor viruses and in the cytoplasm of human leukemic cells: Implications to leukomogenesis. Cold Spring Harbor Symp Quant Biol 39:933–961, 1974

30. Gallo RC, Gallagher RE, Ting RC, et al: Interference with formation and expression of RNA tumor virus information: Rationale and experimental results. Cancer Chemother Rep 58:579–593, 1974

31. Gallo RC, Gallagher RE, Wong-Staal F, et al: Biochemical and biological studies on a gibbon ape (Hylobates lar) with lymphocytic leukemia: Presence and tissue distribution of type-C virus and viral components related to the GaLV-SiSV group. J Virol (in press)

32. Gallo RC, Meyskens FL: Evidence for the presence of RNA tumor virus components in human leukemia. Prog Exp Tumor Research (in press)

33. Gallo RC, Miller NR, Saxinger WC, et al: Primate RNA tumor virus-like DNA synthesized endogenously by RNA-dependent DNA polymerase in virus-like particles from fresh human acute leukemic blood cells. Proc Natl Acad Sci USA 70:3219–3224, 1973

34. Gallo RC, Reitz MS: Molecular probes for tumor viruses in human cancer. Int Rev Exp Pathol 16:1–58, 1976

35. Gallo RC, Saxinger WC, Gallagher RE, et al: Some ideas on the origin of leukemia in man and recent evidence for the presence of acquired and endogenous type-C viral related sequences, in Hiatt, Watson, Winston (eds): Cold Spring Harbor Conferences on Cell Proliferation: Origins of Cancer, vol 4. Cold Spring Harbor, Cold Spring Harbor Pr, 1976

36. Gallo RC, Todaro GJ: Oncogenic RNA viruses. Semin Oncol 3:8–95, 1976

37. Gallo RC, Yang SS, Ting RC: RNA-dependent DNA polymerase of human acute leukaemic cells. Nature 228:927–929, 1970

38. Gillespie D, Gallo RC: RNA processing and RNA tumor virus origin and evolution. Science 188:802–811, 1975

39. Gillespie D, Gallo RC: New concepts on human myelogenous leukemia based on studies of the simian RNA tumor virus family, in Clemmesen, Yohn (eds): Proceedings of the International Comparative Leukemia Lymphoma Meeting. Basel, Karger, 1976

40. Gillespie D, Saxinger WC, Gallo RC: Information transfer in cells infected by RNA tumor viruses and extension to human neoplasia, in Vohn K (ed): Progress in Nucleic Acid Research, vol 15. New York, Academic, 1969, p 1

41. Golde DW, Byers LA: Proliferation and maturation of human leukaemic cells in liquid culture. Bibl Haematol 40:243–250, 1975

42. Gross L: Oncogenic Viruses (ed 2). Oxford, Pergamon, 1975

43. Gunz FW: Genetics of human leukaemia. Ser Haematol 7:165–189, 1974

44. Haase AT, Stoving L, Narayan O, et al: Slow persistent infection caused by visna virus: Role of host restriction. Science 195:175–177, 1977

45. Huang AS, Baltimore O: Defective viral particles and viral disease processes. Nature 226:325–328, 1970

46. Huang AS, Greenawalt JW, Wagner RR: Defective T-particles of vesicular stomatitis virus. Virology 30:161–168, 1966

47. Jones KW, Murray K: A procedure for detection of heterologous DNA sequences in lambdoid phage by in situ hybridization. J Mol Biol 96:455–460, 1975

48. Kaplan HS, Goodenow RS, Epstein AL, et al: Isolation of a type-C RNA virus from an established human histiocytic lymphoma cell line. Proc Natl Acad Sci USA 74:2564–2568, 1977

49. Kawakami TG, Buckely PM: Antigenic studies in gibbon type-C viruses. Transplant Proc 6:193–196, 1974

50. Kawakami TG, Buckely PM, McDowell PS: Antibodies to simian C-type virus antigen in sera of gibbons (Hylobates sp.). Nature 246:105–107, 1973

51. Kawakami TG, Sun L, McDowell TS: Distribution and transmission of primate type C virus, in Bentzelzen P (ed): Advances in Comparative Leukemia Research 1977. Amsterdam, Elsevier North-Holland, 1978, pp 33–36

52. Kawakami T, Huff S, Buckley P, et al: C-type virus associated with gibbon lymphosarcoma. Nature 235:170–171, 1972

53. Klucis F, Jackson L, Parsons PG: Survey of human lymphoblastoid cell lines and primary cultures of normal and leukemic leukocytes for oncornavirus production. Int J Cancer 18:413–420, 1976

54. Kotler M, Weinberg E, Haskel O, et al: Particles released from arginine-deprived human leukaemic cells. Nature 244:197–199, 1973

55. Kurth R, Teich N, Weiss R, et al: Natural human antibodies reactive with primate type-C viral antigens. Proc Natl Acad Sci USA 74:1237–1241, 1977

56. Lieber MM, Sherr CJ, Todaro GJ, et al: Isolation from the Asian mouse Mus carole of an endogenous type-C virus related to infectious primate type-C viruses. Proc Natl Acad Sci USA 72:2315–2319, 1975

57. Mak TW, Kurtz S, Mancaster J, et al: Viral-related information in oncornavirus-like particles isolated from cultures of patients in relapse and remission. Proc Natl Acad Sci USA 72:623–627, 1975

58. Mann DL, Halterman R, Leventhal BG: Cross reactive antigens on human cells infected with Rauscher leukemia virus and on human leukemia cells. Proc Natl Acad Sci USA 70:495–499, 1973

59. Metzgar RS, Mohanakuma T, Bolognesi DP: Relationships between membrane antigens of human leukemic cells and oncogenic RNA virus structural components. J Exp Med 143:47–54, 1976

60. Meyskens FL, Gallo RC, Strayer DS, et al: Human leukemic leukocytes: Labeling of polyadenylated high molecular weight RNA in short-term tissue culture. (In preparation)

61. Miller NR, Saxinger WC, Reitz MS, et al: Systematics of RNA tumor viruses and virus-like particles of human origin. Proc Natl Acad Sci USA 71:3177–3181, 1974

62. Mondal H, Gallagher RE, Gallo RC: RNA-directed DNA polymerase from human leukemic blood cells and from primate type-C virus producing cells: High and low molecular weight forms with variant biochemical and immunological properties. Proc Natl Acad Sci USA 72:1194–1198, 1975

63. Neiman HL, Stephenson JR, Gardner MB, et al: RD-114 and feline leukaemia virus genome expression in natural lymphomas of domestic cats. Nature 266:357–360, 1977

64. Nooter K, Aaronson AM, Bentvelzen P, et al: Isolation of infectious C-type oncornavirus from human leukaemic bone marrow cells. Nature 256:595–597, 1975

65. Nooter K, Bentvelzen P, Zurcher C, et al: Detection of human C-type "helper" viruses in human leukemic bone marrow with murine sarcoma virus-transformed human and rat nonproducer cells. Int J Cancer 19:59–65, 1977

66. Panem S, Prochownk EV, Kirsten WH: Kinetics of type-C virion release from a human diploid fibroblast strain, in: Proceedings of the Seventh International Symposium on Comparative Leukemia Research. Basel, Karger, 1977

67. Panem S, Prochownik EV, Reale FR, et al: Isolation of C-type viruses from a normal human fibroblast strain. Science 189:297–299, 1975

68. Papas TS, Pry TW, Schafter MP, et al: Presence of DNA polymerase in lymphosarcoma in northern pike (Esox lucrius). Cancer Res 37:3214–3217, 1977

69. Peebles PT, Scolnick EM, Howk RS: Increased sarcoma virus RNA in cells transformed by leukemia viruses: Model for leukemogenesis. Science 192:1143–1145, 1976

70. Prochownik EV, Kirsten WH: Inhibition of reverse transcriptase of primate type-C viruses by 7S immunoglobulin from patients with leukaemia. Nature 260:64–67, 1976

71. Rasheed S, Toth E, Gardner MB: Characterization of purely esotrophic and amphlotropic naturally occurring wild mouse leukemia viruses. Intervirology 8:323–335, 1977

72. Reitz MS, Miller NR, Wong-Staal F, et al: Primate type-C virus nucleic acid sequences (woolly monkey and baboon types) in tissues from a patient with acute myelogenous leukemia and in viruses from cultured cells of the same patient. Proc Natl Acad Sci USA 73:2113–2117, 1976

73. Reitz MS, Smith RG, Roseberry EA: DNA-directed and RNA-primed DNA synthesis in microsomal and mitochondrial fractions of normal human lymphocytes. Biochem Biophys Res Commun 57:934–948, 1974

74. Rowley JD: Mammalian chromosomal abnormalities in hematologic disorders of man. Proc Natl Acad Sci USA 72:152–156, 1975

75. Sarngadharan MG, Sarin PS, Reitz MS, et al: Reverse transcriptase activity of human acute leukaemia cells: Purification of the enzyme, response to AMV 70S RNA, and characterisation of the DNA product. Nature 240:67–72, 1972

76. Sawada H, Tashima M, Nakamura T, et al: RNA-reverse transcriptase complex from cultured human myeloma-leukemia cells. Int J Cancer 20:15–20, 1977

77. Scolnick EM, Williams D, Ak JM, et al: Type-C particle-positive and type-C particle-negative rat cell lines: Characterization of the coding capacity of endogenous sarcoma virus-specific RNA. J Virol 20:570–582, 1976

78. Sherr C, Todaro G: Primate type-C virus antigens in cells from humans with acute leukemia. Science 187:855–857, 1974

79. Sliski AH, Essex M, Meyer C, et al: Feline oncornavirus-associated cell membrane antigen: Expression in transformed non-producer mink cells. Science 196:1336–1339, 1977

80. Smith RG, Gallo RC: Prospects for biologic and pharmacologic inhibition of ribonucleic acid tumor viruses. Biochem Pharmacol 25:491–495, 1976

81. Spiegelman S: Molecular evidence for viral agents in human cancer and its chemotherapeutic consequences. Cancer Chemother Rep 58:595–613, 1974

82. Spiegelman S: Molecular evidence for the association of RNA tumor viruses with human mesenchymal malignancies, in Neth, Gallo, Mannweiler, et al (eds): Modern Trends in Human Leukemia II. Munich, Lehmanns, 1976, p 391

83. Steel L, Laube H, Chandra P: Biochemical and serological characteristics of reverse transcriptase from human spleen in a case of myelofibrotic syndrome. Cancer Lett 2:291–298, 1977

84. Stephensen JR, Aaronson SA: Type-C RNA viruses and oncogenesis: Lack of evidence for the association of type-C viruses of the woolly monkey and gibbon apes with human neoplasia. Proc Natl Acad Sci USA 73:1725–1929, 1976

85. Synder HW, Pincus T, Fleissner E: Specificities of human immunoglobulins reactive with antigens in preparations of several mammalian type-C viruses. Virology 75:60–73, 1976

86. Teich NM, Weiss RA, Salahuddin SZ, et al: Infective transmission and characterisation of C-type virus related by cultured human myeloid leukaemia cells. Nature 256:551–555, 1975

87. Temin HM: The cellular and molecular biology of RNA tumor viruses, especially avian leukosis-sarcoma viruses and their relatives. Adv Cancer Res 19:47–104, 1974

88. Temin HM, Mizutani S: RNA-dependent polymerases in virions of Rous sarcoma virus. Nature 226:1211–1213, 1970

89. Theilen GH, Gould D, Fowler M, et al: C-type virus in tumor tissue of a woolly monkey (Lagothrix spp.) with fibrosarcoma. J Natl Cancer Inst 47:881–889, 1971

90. Thomas ED, Storb R, Clift RA: Bone-marrow transplantation. N Engl J Med 292: 895–902, 1975

91. Todaro G: Evidence for the interspecies transmission of tumor virus genes, in Gallo (ed): Recent Advances in Cancer Research: Cell Biology, Molecular Virology and Tumor Virology. Cleveland, CRC, 1977, p 51

92. Todaro G, Gallo RC: Immunological relationship of DNA polymerase from human acute leukaemia cells and primate and mouse leukaemia virus reverse transcriptase. Nature 244:206–209, 1973

93. Tooze J: The Molecular Biology of RNA Tumor Viruses. New York, Cold Spring Harbor Laboratory, 1974, p 618

94. Twardzik DR, Papas TS, Portugal FH: DNA polymerase in virions of a reptilian type-C virus. J Virol 13:166–170, 1974

95. Viola MV, Frazier M, Wiernik PH, et al: Reverse transcriptase in leukocytes of leukemia patients in remission. N Engl J Med 294:75–80, 1976

96. Vosika GJ, Kirvit W, Gerrand JM, et al: Oncornavirus-like particles from cultivated bone marrow cells preceding leukemia and malignant histiocytosis. Proc Natl Acad Sci USA 72:2804–2808, 1975

97. Warnaar SD, Teveloe J, Van Muijen G, et al: Biochemical and electron microscopical evidence for the presence of oncornavirus in spleen tissue from two patients with haematological malignancies. Mol Biol Rep 3:1–8, 1976

98. Weiman BJ, Kluge N, Dube SK, et al: Particle-associated RNA-dependent DNA polymerase and high-molecular-weight RNA in a human cell line derived from polycythemia vera bone marrow. J Natl Cancer Inst 55:537–541, 1975

99. Witkin SS, Ohno T, Spiegleman S:

Purification of RNA-instructed DNA polymerase from human leukemic spleens. Proc Natl Acad Sci USA 72:4133–4136, 1975

100. Wong-Staal F, Gillespie D, Gallo RC: Genetic relationship of a primate RNA tumor virus genome to genes in normal mice. Nature 256:670–672, 1975

101. Wong-Staal F, Gillespie D, Gallo RC: Proviral sequences of baboon endogenous type-C RNA virus in DNA of human leukaemic tissues. Nature 262:670–672, 1975

102. Wu AM, Gallo RC: Reverse transcriptase. CRC Crit Rev Biochem 3:289–347, 1975

103. Yamada K, Furasawa S: Preferential involvement of chromosome no. 8 and no. 21 in acute leukemia and preleukemia. Blood 47: 679–686, 1976