Cellular and Virological Studies Directed to the Pathogenesis of the Human Myelogenous Leukemias

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It is a particular honor for me to give the first Frederick Stohlman memorial lecture because of my personal respect and friendship with Fred. His courage, honesty, and stimulation of good science in the hematological field will be remembered by all who knew him. It is also very fitting to have these lectures in Wilsede where special friends were made at the first meetings and where association and friendship with Fred Stohlman became much closer for many of us.

Introduction

There have been enormous advances in the therapy of lymphocytic malignancies as exemplified by the treatment and apparent cures of some childhood acute lymphoblastic leukemias (see D. Pinkel elsewhere in this book). The myelogenous leukemias remain much more difficult to treat, and for this reason and because they also provide an interesting hematopoietic system for the study of differentiation our interests for the past several years has focused on the origin and pathogenesis of this disease group.

The origin of the myelogenous leukemias appears to involve proliferation of a stem cell with various degrees of commitment to differentiation and arrests in differentiation at various cellular levels. These diseases appear to be monoclonal (see Fialkow and also Rowley elsewhere in this book) when they are clinically manifest. However, it is not known if the initiation of the disease is multiclonal or monoclonal. Their appearance as monoclonal when presented to clinicians might be because of a select growth advantage of one clone as often seen in tissue culture. We do not yet know the mechanism(s) initiating the abnormality of growth characteristic of these diseases, but certain factors have clearly been shown in select cases to induce or play some role in leukemic development. Studies in mice, chickens, and humans implicate genetic factors which may be involved at multiple levels. Thus, some congenital diseases with chromosomal abnormalities and certain families have shown an unusual incidence of leukemia (see report by R. Miller elsewhere in this book). One family of unusual interest has recently been described by F. Gunz and his colleagues in Australia. Thirteen members of the family developed some form of myeloproliferative disease, clearly implicating genetic factor(s). Yet, some members developed the disease within a relatively short time of each other even

though the age of each varied [17]. This certainly suggests an environmental factor may have been operative as well as genetic factor(s). There was no known unusual exposure of any member of the family to chemicals or radiation.

What about environmental factors? Radiation can induce leukemias in animals, and it has been clearly associated with myelogenous leukemia in humans under unusual circumstances. Benzene also has been associated with leukemia in very rare instances, and some other chemicals with such rarity that their leukemogenic potential in man is undefined.

Epidemiological studies indicate that leukemia is not increased in people living at higher altitudes with greater exposure to radiation, nor is leukemia generally higher in industrial areas than in rural regions [24]. These observations together with the facts that 1. the incidence of childhood leukemia has apparently stayed about the same since industrialization and in fact has declined in recent years, 2. the incidence peaks in young children, 3. the incidence is greater in whites than in black people, and 4. association with chemicals and radiation is very rare (see R. Miller elsewhere in this book) all suggest to me that the leukemias may be predominantly biological diseases and that all biological factors must be thoroughly explored.

Retroviruses and Retrovirus Related Information: Reasons for Intensive Exploration of Human Tissues

We have felt that a search for retrovirus (RNA tumor virus, oncornavirus) information or related information in human cells was mandated by several considerations. 1. As discussed above epidemiological considerations are a stimulus for consideration of biological factors. Although these same broad studies also do not lend strong support to a virus causation of the disease in a conventional manner, I think they are quite consistent with a role for viral information if one considers: a) long latency, b) a second or third factor in addition to appropriate viral information may be a requisite, c) retroviruses can be vertically transmitted either in the germ line as an endogenous cellular element or by congenital infection (see R. Weiss elsewhere in this book). These factors would obscure epidemiological approaches. 2. Several retroviruses can produce leukemia in a variety of animals in laboratory experiments. 3. Retroviruses can sometimes transform cells and not be seen again as discrete virus particles in in vitro experiments (see P. Duesberg elsewhere in this book). Moreover, some data suggests this may also be true with some naturally occurring leukemias of animals. For instance, in a significant number of cats with leukemia (perhaps approaching 50%), feline leukemia virus (FeLV) has not been found (see O. Jarrett elsewhere in this book). In many of these cats antibodies to FeLV has also not been identified, and recent data from our laboratory in collaboration with W. Hardy and M. Essex indicate that FeLV proviral nucleic acid sequences may also not be readily detectable (Koshy, Wong-Staal, Gallo, Hardy, and Essex, in preparation). Yet there is evidence that FeLV may still cause the disease in

these so called "virus negative" cats. This is based on some epidemiological results (M. Essex, personal communication) and on the finding of feline oncornavirus membrane antigen (FOCMA) in the leukemic cells. This protein is believed to be specifically coded for or induced by FeLV and by feline sarcoma virus (FeSV) (see M. Essex elsewhere in this book). 4. Bone marrow transplants studies have indicated that in some exceptional cases normal bone marrow donor cells may be transformed to leukemic cells when given to a leukemic individual [43]. 5. We have recently been able to show that some of the primate type-C retroviruses can transform human B-lymphocytes and may also interfere with differentiation of myelopoietic cells. 6. The most compelling reason to think of retroviruses in the etiology of leukemias of humans is the results of animal models. It appears now that in every instance where we know the cause of *naturally* occurring leukemia in a *sizable* fraction of a leukemic animal population it involves a retrovirus. This now includes chickens (see R. Weiss and O. Jarrett, A. Burney elsewhere in this book), some wild type mice [16], cows (see O. Jarrett in this book), cats (see Jarrett and also Essex) and gibbon apes (see next section). 7. Since retroviruses can recombine with cellular genes and since some of them can affect cell differentiation (see later section of this report and also reports by M. Dexter, by N. Teich, by M. Moore, and by T. Graff in this book), it is possible and perhaps likely that sometimes these viruses contain cell derived genes involved with growth and/or differentiation. If so then it should be important to use retroviruses as probes in human leukemia to see if such genetic information is altered during leukemogenesis whether or not the disease is due to a virus. For this we would choose a primate retrovirus.

Primate Type-C Retroviruses

There were no isolates of any primate retrovirus before this decade. Now there are many, and they are from diverse species. I will focus on two groups since they were the earliest isolates and the only ones which have been shown to have pathological effects on cells. We have been particularly interested in the members of the infectious type-C virus group isolated once from a woolly monkey and called simian sarcoma virus (SiSV), simian sarcoma associated virus (SiSAV) complex and some isolated several times from gibbon apes and collectively called gibbon ape leukemia virus (GaLV). SiSV (SiSAV) and the various GaLV are very closely related viruses, and the evidence suggests their ancestral origin was probably a rodent virus which entered these primates by interspecies infection [27,47]. It is of particular interest that the virus entered two primates which are only distantly related. We have especially focused our attention on this virus group because the gibbon is the species closest to man for which a retrovirus has been isolated, because it is the species closest to man for which we have an animal model of leukemia and notably one which we know something of the etiology, and because we think viruses like this have been in humans. It

Table 1. Infectious primate type-C retroviruses: The Woolly Monkey (Simian) Sarcoma Virus (SSV-SSAV) – Gibbon ape Leukemia Virus (GaLV) Group[a]

Ancestral Origin	Vectors	Transmission	Biological Effects: Tumorigenicity in vivo natural	Biological Effects: Tumorigenicity in vivo experimental	Biological Effects: Transformation in vitro	Members	
Rodents	unknown (? man)	Horizontal to woolly monkey, gibbon apes, ? man. Vertical only by infection parent to progeny. Infections clearly evident gibbon to gibbon. Source of infection of one woolly monkey is unknown	Fibrosarcoma in a woolly monkey (SSV). CML and ALL by GaLV	Fibrosarcoma and fibromas in marmosets and cerebral tumors by SSV. CML and ALL by GaLV	SSV transforms fibroblasts. It's helper virus (SSAV) as well as GaLV can transform human blood B-lympho-blasts. (see text)	SSV (SSAV)	isolated once from a pet woolly monkey. Very related isolates from human cells reported by 5 laboratories
						$GaLV_{Thai}$	From leukemic animals in a colony in Thailand inoculated with human malaria blood
						$GaLV_{SF}$	From leukemic animals in San Franzisco Zoo
						$GaLV_{Br}$	From brains of 3 animals 2 of which were inoculated with extracts of human brains
						$GaLV_{H}$	From a spontaneous acute lymphoblastic leukemia of an animal free roaming with a group of gibbons on Hall's Island, Bermuda

[a] References to original reports (except for recent results) can be obtained from the review by Gallo and Todaro "Oncogenic RNA Viruses". In: Seminars in Oncology. Yarbro, J.W., Bornstein, R.S., Mastrangelo, M.J. (eds.), pp. 81–95. New York: Grune & Stratton, Inc. 1976

appears sufficiently clear that this virus entered gibbons directly or via an unknown vector from a rodent, and it is certain that the virus is transmitted by infection among gibbons in captivity. The immediate vector to the one woolly monkey is unknown. A summary of these viruses is given in Table 1.

Shortly after the isolation of viruses of the SiSV-GaLV group [9,21,22, 39,42,46], baboon endogenous type-C viruses (BaEV) were isolated from different tissues and different species of baboons [20,40]. These viruses exhibit all the characteristics of endogenous viruses, i.e., transmitted in the germ line of baboon cells, and had no demonstrable effect in vivo or in vitro on mammalian cells. Apparently BaEV entered an ancestor of domestic cats several millions of years ago, infecting the germ line, and now maintained as an endogenous virus of cats known as RD114 (reviewed by Todaro in reference [41]). There is also evidence from several laboratories that a related virus is sometimes identified in humans (see below).

Table 2. Endogenous Type-C Virus of Baboons (BaEV)[a]

Ancestral Origin	Transmission Vertical	Interspecies Infection	Biological Effects	Members
Babon	in germ line: baboon to baboon	1. to a feline ancestor of domestic cat; and now vertically transmitted in germ line of cat as a variant of BaEV known as RD114 2. ? to humans 3 reports of related virus from human tissue and several reports of related antigens and nucleic acid sequences (see text)	none reported to date –	Papio cynocephalus Papio anubis Papio papio Papio hamadryas

[a] References to original reports (except for recent results) can be obtained from either the text or the review by Gallo, R.C. and Todaro, G.J.: Oncogenic RNA Viruses. In: Seminars in Oncology. Yarbro, J.W., Bornstein, R.S., Mastrangelo, M.J. (eds.), pp. 81–95. New York: Grune & Stratton, Inc. 1976

Selected Results with Human Cells

Numerous reports suggest that some fresh uncultured human cells contain intracytoplasmic virus like particles (discussed in references [10] and [37]), but since these are not isolated as infectious virus and have not been shown to exhibit biological activity it is not certain thay they represent defective or abortive type-C viruses or are cellular artifacts. However, the presence of high molecular weight RNA with some sequence homology to RNA from

some viruses and, in some samples of human leukemic cells, a DNA polymerase with properties resembling reverse transcriptase (RT), associated with these "particles", emphasize their viral-like properties. In some cases these polymerases were shown to be immunologically specifically related to RT from SiSV-GaLV group (reviewed in references [10,11] and [12]), but in most cases immunological relatedness to primate viruses was not found and extensive comparisons (by immunological tests) to other animal viruses was usually not performed because of limited amount of enzyme. These results were a stimulus to consider the possibility that humans may harbor viruses related to this group.

Our objective was to purify sufficient enzyme to enable us to make peptide maps of the RT and compare this to RT from these viruses. This has not been achieved, and there has been little progress in our laboratory with this problem since the initial reports. On the other hand Chandra and colleagues [1,2] have reported the purification of this enzyme (related to SiSV-GaLV RT) from the spleen of a child with a preleukemic disease, myelofibrosis, which eventually turned into AML. Similar enzymes have been found in orbital chloromas from leukemic people living near Ankara, a disease appearing as a cluster (see R. Miller and also P. Chandra elsewhere in this book).

On rare occasions whole type-C virus has been reported isolated from human cells. These reports, reviewed in references [11] and [13], have come from our laboratory (HL23V), from Panem and Kirsten (HEL-12), from Nooter and Bentvelzen and their colleagues (SKA-21), from Gabelman and Waxman, and recently from H. Kaplan and colleagues. In each case the viruses are related to the SiSV-GaLV group, and at least in three cases (HL23V, SKA-21, and HEL-12), a second virus related to BaEV has been detected. This peculiar combination remains unexplained. Because of their similarity to existing primate viruses it is possible that these isolates are all laboratory contaminants. Regarding HL23V we offer the following results against contamination: 1. Reproducible isolation from separate clinical specimens; 2. previous evidence reported in references [30] and [7] for RT related to RT of SiSV-GaLV in the uncultured blood cells; 3. previous evidence for cytoplasmic RNA sequences related to SiSV and BaEV in the fresh uncultured blood cells of this patient [33]; 4. previous evidence for DNA proviral sequences related to BaEV [33,48], although SiSV proviral sequences could not be detected [33,48]. We did not subsequently find evidence for a humoral antibody response to either the SiSV or BaEV component of HL23V in the sera of this patient, but recently we have obtained confirmation of the presence of the BaEV provirus in the DNA of the uncultured blood cells from this patient. As described elsewhere in detail in this book [49], we have used the technique of DNA digestion with a restriction endonuclease followed by separation of DNA fragments by agarose gel electrophoresis, transfer of the DNA fragments to nitrocellulose filters by the Southern blotting technique, hybridization of I^{125}-labeled 35S viral RNA to this DNA, and examination for virus specific bands after development over X-ray films. This approach allows for more sensitive molec-

ular hybridization because much of the irrelevant DNA is excluded (the labeled viral RNA probe is in excess) and for qualitative assessment because the positive bands can be visualized and compared to bands of virus infected cells. As shown elsewhere in this book [49] DNA from fresh uncultured blood cells from patient HL-23 and from another AML patient labeled HL-49 contain several clearly visible bands after digestion with the endonuclease HIND III which hybridize to I^{125}-35S RNA from BaEV (M7). For control purposes DNA from normal human leukocytes and from human cells (A204) deliberately infected by BaEV are also shown. No viral specific fragments with BaEV as a probe were found in the normal cell DNA. The only band seen is from ribosomal DNA which was detected with labeled rRNA which was used as a control because sometimes rRNA can contaminate viral RNA. Multiple viral specific bands are, or course, found in the positive control, BaEV infected A204 cells. It is interesting that some of the proviral bands found in the DNA from the leukemic cells of patients HL-23 and HL-49 are not found in the BaEV infected A204 cell positive control. This suggests that the integration sites may be different or that the viral fragments detected in HL-23 and HL-49 are related but not identical to the M7 strain of BaEV. Despite the evidence for some cytoplasmic RNA sequences related to SiSV (SiSAV) in HL23 fresh blood cells [33] no novel proviral bands of SiSV (SiSAV) were found. M. Reitz in our laboratory had previously shown that DNA from the kidney and spleen of patient HL-23 contained more SiSV (SiSAV) hybridizable sequences than did DNA from normal cells or DNA from the leukemic blood cells of patient HL-23. It is possible that only a small percent of the cells contained the SiSV (SiSAV) proviral sequences, and failure to detect them is due to insufficient sensitivity. Such cells may have been concentrated in the kidney and spleen. Unfortunately, DNA or tissue from these organs is no longer available so they cannot be examined by the restriction endonuclease blotting technique. In summary, we have evidence now that the BaEV component of HL23V was present in the *primary uncultured blood cells* of patient HL-23, but inconclusive data for the SiSV (SiSAV) component.

The important question regarding these or similar viruses is to ask whether they could be important causes of human leukemia. In an effort to obtain a preliminary answer to this question we surveyed DNA from many human tissues for the presence of integrated novel viral sequences by the standard techniques of molecular hybridization. We used molecular probes (both labeled RNA and labeled cDNA) from many animal viruses, including SiSV and BaEV. We did not find significant differences between normal and leukemic cells with probes from the majority of viruses, and the level of hybridization were insignificant. An example of this is the negative data obtained with a rat type-C endogenous virus and with GaLV illustrated in Fig. 1 (parts B and C). The data is a summary of results of many samples presented as a distribution frequency, i.e., it represents the percent of human DNA samples (ordinate) which hybridize to a certain maximum extent (abscissa). Note that the vast majority of cases (normal and leukemic hybridize very little to rat virus nucleic acid probes and the distri-

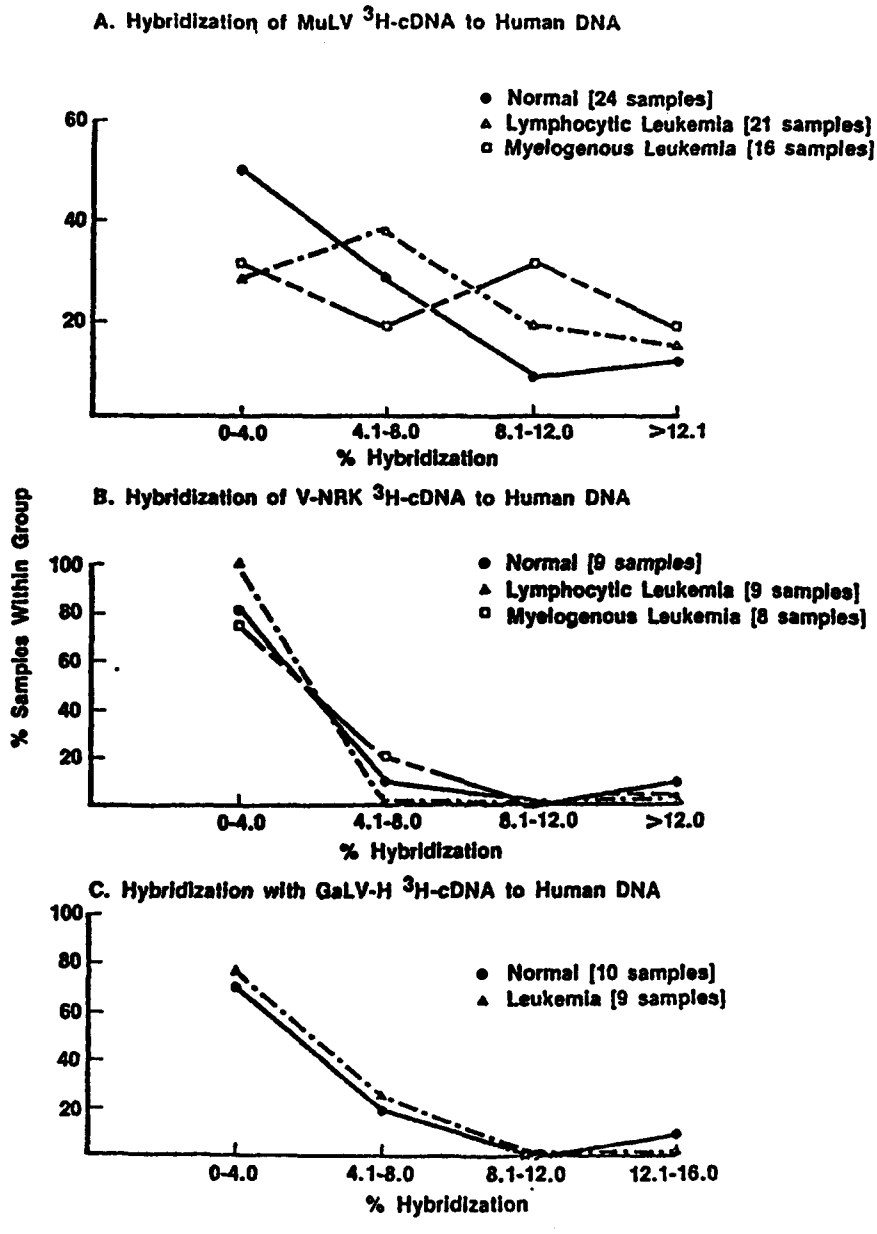

Fig. 1. Survey of human DNA from normal blood and from people with various types of leukemia for murine leukemia virus (MuLV) (Rauscher strain) related sequences (panel A), rat endogenous virus (V-NRK) related sequences (panel B), and gibbon ape leukemia virus (GaLV) related sequences (panel C).

The probes are ^{3}H-cDNA prepared from endogenous reverse transcriptase reactions of the respective viruses. Data are given as the frequency and extent of hybridization found in the various DNA samples. As shown all samples hybridized less than 8% of cDNA probes from V-NRK and GaLV, and there are no differences between normal and leukemic samples. These hybridization results are not significantly above background levels. Therefore, within the limits of sensitivity of the assay such sequences could not be detected in humans. In contrast, some human DNA samples did hybridize some of the MuLV probe to levels which appear to be significant. These results do not discriminate between MuLV sequences themselves or sequences related to MuLV. This survey was carried out by N. Miller and M. Reitz in our group.

bution of samples is virtually identical with DNA from normal and leukemic cells. In contrast, using BaEV probes we find a small number of leukemic DNA samples which hybridize significantly more of the probe (data not shown). We have not conducted an extensive survey for BaEV related sequences using the more sensitive approach of restriction endonuclease-Southern blotting, but we have applied it to a few very select cases and verified the higher hybridization obtained in the molecular hybridization survey. These results were mentioned above and presented with AML

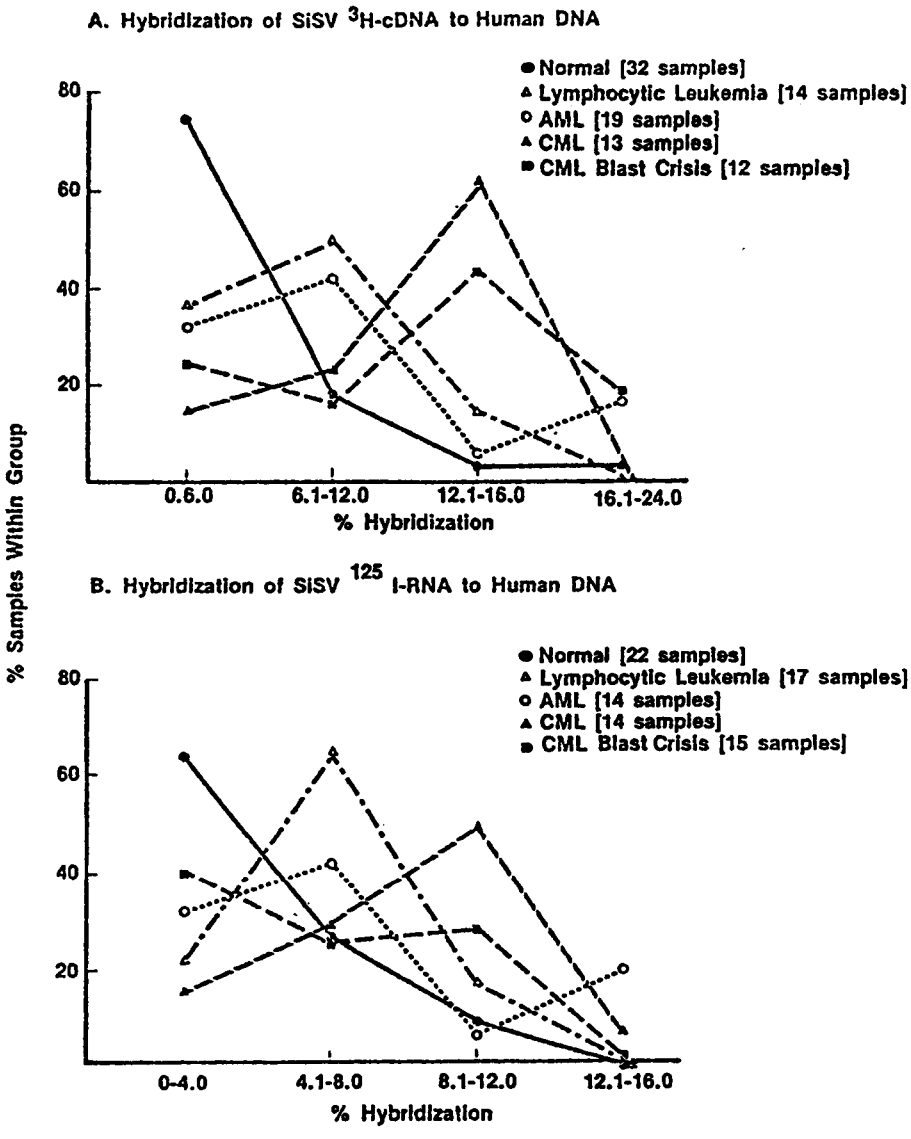

Fig. 2. Survey of human DNA from normal blood and from people with various types of leukemia for simian sarcoma virus (woolly monkey) (SiSV) related sequences.
In the upper panel (A) the probe used was ^{3}H-cDNA prepared from endogenous reverse transcriptase reactions. In the lower panel (B) the probe was I^{125} labeled 70S RNA. In both cases the vast majority of DNA samples from normal tissues hybridized less than 8% and all but a few less than 16%. In contrast, a significant percent of leukemic samples hybridized more than 12% and several more than 16%. Again, these results do not discriminate between SiSV sequences and SiSV related sequences. This survey was carried out by M. Reitz and N. Miller in our group.

patients HL-49 and HL-23 elsewhere in this book [49]. If the survey results are a true indication of the presence of these sequences then we can safely rule out BaEV or a closely related virus as commonly associated with human leukemia and therefore as a *common* cause of human leukemia. However, it may be important to keep in mind that the results could be underestimations, e.g., if only fragments of provirus are present and/or only a small number of cells in a population contain viral sequences, the sequences might easily be undetected, but possibly sufficient for leukemic transformation.

We also surveyed DNA purified from many human tissues for SiSV related sequences, and as illustrated in Fig. 2 several leukemic DNA samples hybridized more nucleic acid probes of SiSV (grown in marmoset cells) than did DNA from normal tissues. This is more clearly evident when one compares the distribution of hybridization of viral probes to various human DNA samples between rat virus (Fig. 1) and SiSV (Fig. 2). However, one DNA sample from a normal tissue (one of six human placentas tested) also showed unusually high hybridization to SiSV probes, so clearly higher hybridization is not unique to certain leukemic cells. To verify these higher hybridization results and to obtain greater sensitivity, some of these samples were also analyzed by restriction endonuclease digestion–Southern blotting, and hybridization of the specific fragments to SiSV 35S RNA. As described elsewhere in this book [49], the positive results with the one placenta derived from a normal person (termed NP3) obtained in the standard molecular hybridization survey was confirmed as was one leukemic sample. In these cases it is difficult to escape the conclusion of infection by an SiSV related virus. The presence in placenta is particularly interesting since the finding suggests the possibility of congenital virus transmission, a mechanism apparently involved in natural virus transmission seen in chickens [44], cats [19], cattle [26], and gibbon apes [23]. The data are also consistent with the isolation of an SiSV related virus from a human embryo by Panem and Kirsten [32].

As noted earlier SiSV and GaLV are not endogenous to primates but present in them only after infection. Although their recent history (? vectors) is unknown their ancestral origin is believed to be rodents. An unexpected observation, that came out of the search of human DNA for inserted viral sequences related to SiSV and GaLV was the finding of an SiSV related sequence in *all human DNA*. This was detected after digestion of the DNA with BAM I, with HIND III, and with Xpa restriction endonucleases (see paper by F. Wong-Staal et al. elsewhere in this book). This sequence was specific to this virus group since no endogenous viral sequence (i.e. sequences in DNA from normal uninfected tissues from various cell samples) were detected with probes from other viruses. We found this sequence using either SiSV (SiSAV) or SiSAV, and the results were obtained with viruses grown in non-human cells. Therefore, the results cannot be due to a trivial explanation such as presence of human cellular sequences with the viral probe. Moreover, it appears that this is not an evolutionarily conserved sequence since it was not found in the DNA we

have tested from other normal uninfected primates. We, therefore, tentatively interpret these results to indicate that this group of viruses has been in the human population or an ancestor of humans.

The Possibility of an Immune Response to Type-C Viruses in Humans

One of the most important questions relevant to type-C viruses and humans is whether a serological response against them is detectable. Results presented previously [25,38] and elsewhere in this book by R. Kurth and also by H. Snyder and by N. Hogg and their colleagues deal with this complex and as yet unsettled issue. We recently explored a different approach. Stimulated by the findings of I. Witz and his colleagues [45], P. Jacquemin, C. Saxinger, and I examined human blood cells for surface immunoglobulins. Both IgG and IgM were found, and appear to be chiefly associated with non-lymphocytic cells, a finding in agreement with Metzgar et al. [29] and Cotropia et al. [5]. We discovered that among the IgG were some which react with high specificity and at low concentrations with purified reverse transcriptases (RT) from select mammalian leukemia type-C viruses. In AML we find the IgG generally reacts with RT from SiSV, and to our surprise the reaction can be specific enough to distinguish this RT from the RT of the other members of the SiSV-GaLV group. In normal people (bone marrow) we find about 20% positive for IgG chiefly reactive with RT from one of the GaLV isolates, namely $GaLV_{SF}$. In CML, we find the remission and chronic phase patients to be like normal (negative or reactive with RT from $GaLV_{SF}$), while in almost every CML in blast crisis the IgG is chiefly reactive with RT from FeLV and rodent type-C viruses [18,36]. Unlike the sporadic detection of apparent inserted viral nucleic acid sequences the finding of IgG with specific RT enzymatic neutralizing activity is common. Because of this and because we have not as yet developed an assay independent of RT activity neutralization,[1] it is premature to conclude that this IgG represents an immune response to a viral protein. It is possible that the IgG is directed against determinants of a protein coincidentally very close to the amino acid sequences of the catalytic sites of certain RTs. The answer to this question should come from the isolation and characterization of the putative cell surface antigen.

Growth and Differentiation of Human Leukemic Myeloid Cells and Effects of Primate Type-C Viruses

We have reported that in the presence of conditioned media (CM) from certain human embryo cell strains, human myelogenous leukemic cells can often be induced to grow in liquid suspension tissue culture [8, 14]. In many instances the cells terminally differentiate [14, 15]. Sometimes they retain marker chromosomes, and this observation combined with the fact that this CM generally does not induce growth of normal myeloid cells led us to

conclude that the maturation arrest of many human myelogenous leukemias is not always irreversible, in agreement with L. Sachs [35] and M. Moore [31] and their colleagues. The differences in response of normal myeloid cells and leukemic cells also suggested to us that membrane receptors for regulation may be modified in leukemia. An interesting by product of this research was the establishment of the first human cell line with distinct myeloid characteristics [3]. This line, called HL-60, was shown to consist mostly of promyelocytes [31] which can be terminally differentiated when DMSO or other compounds active in the Friend murine erythroleukemia system are added to the culture [3].

Effect of Primate Type-C Viruses on Normal Fresh Human Blood and Bone Marrow Cells

To our knowledge there are surprisingly no reported studies of the effects of primate type-C viruses on fresh human blood cells. We have recently completed a preliminary study of the effects of various type-C viruses, including the primate viruses described here on human blood and bone marrow cells. We find that SiSV (SiSV), SiSAV alone, GaLV, HL23V (SiSV component), but not BaEV induce growth (Fig. 3) of B-lymphocytes (Fig. 4). In about one third of cases studied, the cells become immortalized, and despite apparent normal karyotype, they are tumorigenic in nude mice (Fig. 5). These cells are Ig and EBNA positive and do not have myeloid or T-cell characteristics [28]. The observation is different from that of "spontaneous" transformation of EBV positive human B-lymphocytes because the induction of growth is faster, the frequency of establishment of cell lines in our hands much greater, and the cells are tumorigenic despite apparent normal chromosomal makeup. The addition of BaEV or FeLV (despite some virus replication in a few cases) did not produce this effect. Finally, recent results indicate that the same phenomenon can be induced with EBV negative cord blood but at a lower frequency. We conclude that by indirect or direct means and by mechanisms not yet understood, some primate type-C viruses can be involved in the in vitro transformation of human B-lymphocytes.

Working Hypothesis

A currently interpretable and simple model for leukemogenesis is that the leukemogenic event, whatever the cause, leads to auto-production of a growth promoting molecule which prevent binding of normal regulatory molecules. This model is similar to one proposed by Todaro and colleagues in the genesis of sarcomas [6]. The alternative is that receptors for regulators are themselves modified. These ideas should become testable in the very near future.

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[1] Since this talk precipitation of RT has been obtained with these IgG.

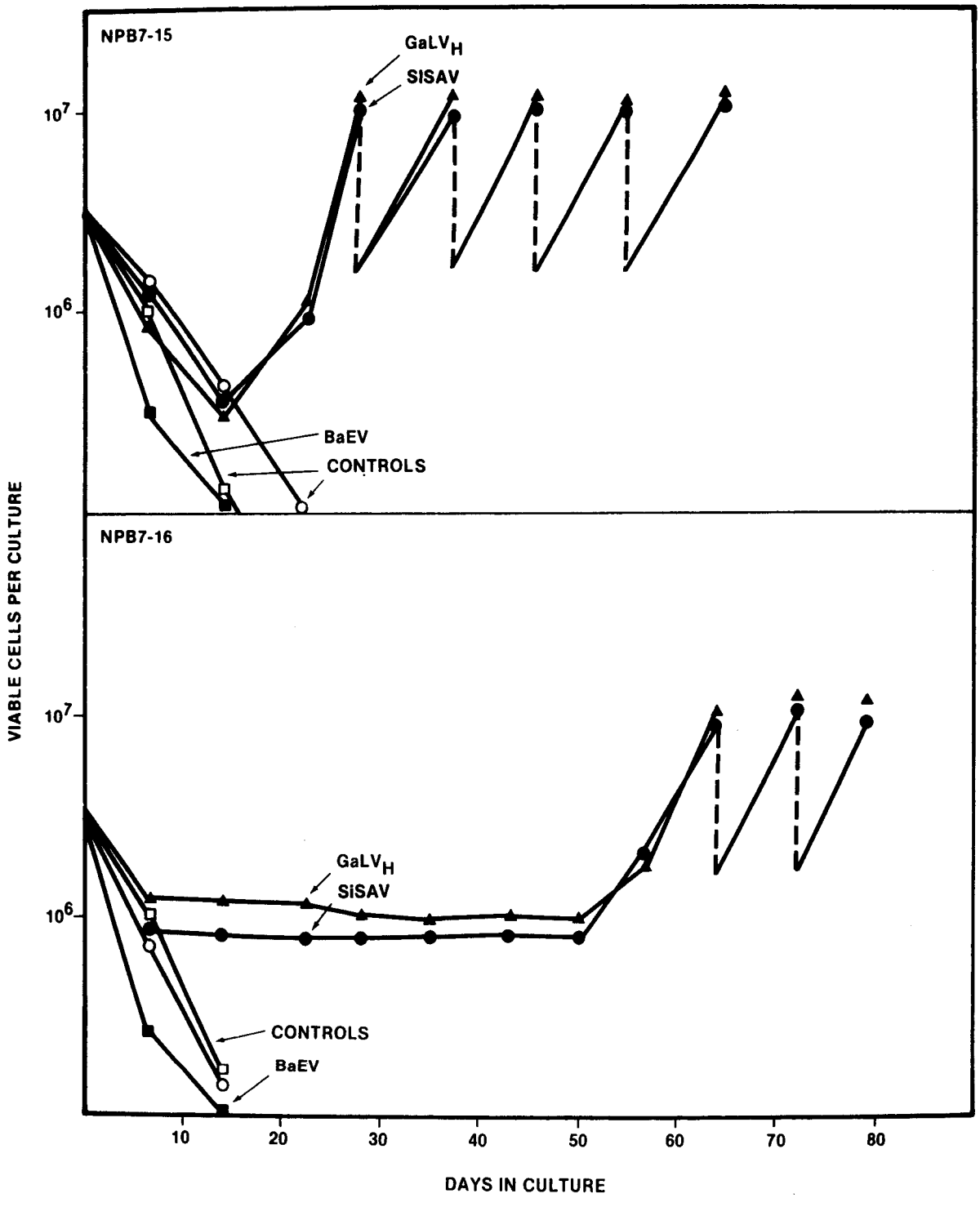

Fig. 3. Induction of growth of B-lymphocytes from normal human blood by primate type-C retroviruses ($GaLV_H$ and SiSAV).

Human blood leukocytes were cultured with or without the Hall's Island strain of GaLV or with the simian sarcoma associated virus (helper virus) (SiSAV). In some cases (e.g., sample NPB 7–15 shown in the figure) induction of growth was rapid. In other cases (e.g., sample NPB 7–16 shown in the figure lower panel) growth induction occurred after 50 days. Spontaneous growth (no virus) occurred in only 1 to 2% of samples but in more than 40% of cells treated with virus if a co-cultivation method was used (P. Markham, F. Ruscetti, Z. Salahuddin, R. Gallagher, and R. Gallo, in press).

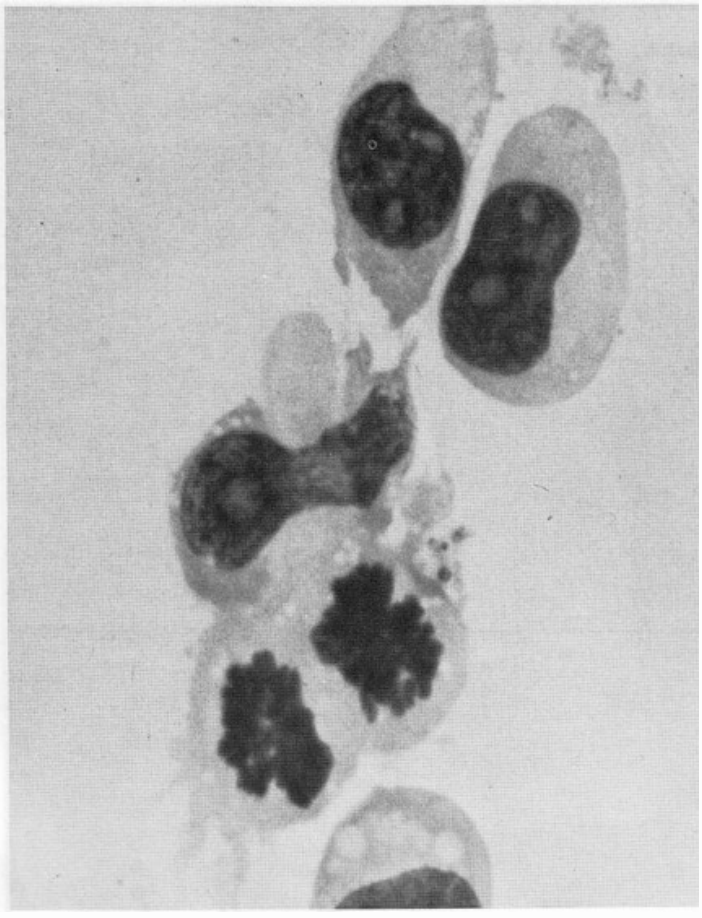

Fig. 4. Morphology of "immortalized" cell line induced from normal human blood after infection by Ganot lV_H.
Cells are lymphoblasts which are IgG and EBV positive
(Magnification 1000×).

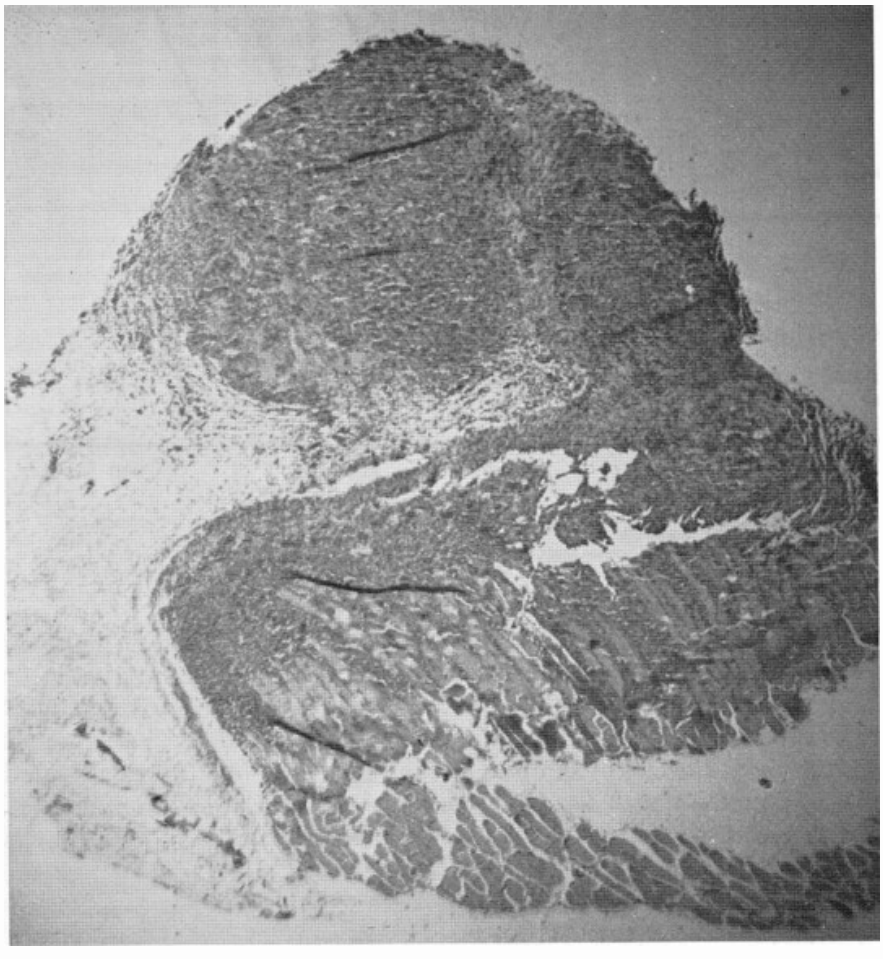

Fig. 5

Summary and Conclusion

1. Work over the past years and especially results of the past few years indicate that type-C viral or viral related genetic information exists in humans.
2. We do not know how this information entered humans or whether it causes disease, but it is of interest that the probes from the viruses used to detect this information are from the very same viruses which we find can affect growth and differentiation of some human hematopoietic cells.
3. The status of actual virus isolates from humans, though encouraging because of similarities of isolates from five different laboratories, remain very perplexing and so far have not been especially informative to human leukemogenesis.
4. In the near future we hope to clone in bacteria the viral related sequences detected in human DNA in order to more precisely determine their chemical and biological properties. The HL-60 system may also afford an opportunity to purify receptors for CSF. When CSF and other, perhaps more important, regulatory factors are purified, we would like to determine if they bind differently to leukemic and normal cells.

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Fig. 5. B-Cell lymphoma of nude mouse induced by injection of mouse with cells induced to grow by $GaLV_H$.
Cells are human as determined by karyotype analysis. (From: P. Markham, F. Ruscetti, Z. Salahuddin, R. Gallagher, and R. Gallo, in press).

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