Retroviruses as Etiologic Agents of Some Animal and Human Leukemias and Lymphomas and as Tools for Elucidating the Molecular Mechanism of Leukemogenesis

By Robert C. Gallo and Flossie Wong-Staal

It has long been known that retroviruses (RNA tumor viruses, type-C viruses, oncornaviruses, leukemia viruses) can induce a variety of neoplasias (leukemias and lymphomas, sarcomas, and occasionally carcinomas) in animals, and they have been identified as the prime factor in the pathogenesis of many naturally occurring leukemias and lymphomas in several animal species. Although there are recent reviews on retroviruses, they focus chiefly on the molecular biology of viral replication and in vitro cellular transformation. In our view, several advances in retroviral oncology have merged within the past few years, resulting in a heightened appreciation of the role of retroviruses in naturally occurring leukemias and a rapid expansion in our understanding, although still fragmentary, of the mechanisms by which they transform cells. Simultaneously, the search for a possible viral etiology of some human leukemias and lymphomas has led to the isolation of a novel retrovirus, HTLV, from cultured human neoplastic T cells. Recent epidemiologic and biochemical evidence has associated HTLV with a specific type of adult T-cell leukemia and lymphoma. The purposes of this review are: (1) to summarize briefly the role of retroviruses in naturally occurring leukemias and lymphomas of animals, drawing specifically on features that are instructive in considering a viral etiology of certain human leukemias and lymphomas; (2) to describe our current concepts of the molecular mechanisms of retroviral transformation and how they may be applicable to human leukemias; and (3) to summarize the recent results dealing with the isolation of HTLV and its characterization as a possible etiologic agent of certain T-cell leukemias and lymphomas in man.

Naturally Occurring Leukemias of Animals Caused by Retroviruses

Since retroviruses have been identified as etiologic agents of naturally occurring leukemias and lymphomas in some animals, they are often called leukemia viruses. Leukemia in domestic poultry and cattle was suspected to be transmissible because of disease clustering decades before virus isolation was achieved. In addition, retroviruses have also been associated with spontaneous leukemias of cats, wild mice, and gibbons in captivity. Most of the diseases are lymphoid malignancies (Table 1), but some strains of gibbon ape leukemia virus produce chronic myelogenous leukemia (CML) extremely similar to CML in humans, and some feline leukemia virus strains may produce AML. In all of these diseases it is now clear that the virus is an acquired infectious agent (exogenous) rather than transmitted in the germ line (endogenous) (Table 1). Two animal systems (feline and bovine leukemia) deserve special mention since they illustrate some relevant points in considering the etiology of a naturally occurring leukemia.

Feline Leukemia

Following Ludwik Gross's now classical work on the viral etiology of leukemia in some inbred strains of
of either FeLV or FeSV. Although exogenous FeLV is detectable virus or viral components. These virus-virus (FeSV), although it is not coded by the genomes seems to be induced specifically in FeLV-induced our consideration of "virus-negative" human leukemias of these virus-positive tumors, express FOCMA, and are negative tumors are clinically indistinguishable from 30%-40% of leukemic cats have no readily detectable in most spontaneous leukemias and leukemic cells or cells transformed by feline sarcoma virus (FeSV), although it is not coded by the genomes of either FeLV or FeSV. Although exogenous FeLV is readily detectable in most spontaneous leukemias and lymphomas of cats, 30%-40% of leukemic cats have no detectable virus or viral components. These virus-negative tumors are clinically indistinguishable from the virus-positive tumors, express FOCMA, and are derived from cats with a history of exposure to FeLV. Despite these similarities there is no evidence for integration of exogenous FeLV sequences in the virus-negative tumors. If FeLV is indeed etiologically associated with "virus-negative" tumors, then novel mechanisms have to be invoked to explain the role of FeLV such as: (1) a "hit and run" mechanism whereby FeLV transiently infects the cell, turns on some cellular genes and induces transformation, but the provirus is not maintained; (2) infection of cells that do not themselves become transformed but as a result of the infection produce an abnormal amount of a regulatory protein. Thus, the tumor cells themselves may have no detectable viral information, and the infected (regulator) cells may be too rare to detect. Obviously, if either of these mechanisms did occur, it could be important in our consideration of "virus-negative" human leukemias.

**Table 1. Retrovirus-Associated Naturally Occurring Leukemias in Animals**

<table>
<thead>
<tr>
<th>Species</th>
<th>Etiologic Agent</th>
<th>Disease</th>
<th>Predominant Target Cell</th>
<th>Major Known Routes of Transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken</td>
<td>Avian Leukosis Virus</td>
<td>Leukemia</td>
<td>B lymphocyte</td>
<td>Congenital (egg)</td>
</tr>
<tr>
<td>Wild mice</td>
<td>Amphotropic</td>
<td>Lymphoma</td>
<td>Nonthymic B cell</td>
<td>Congenital (milk)</td>
</tr>
<tr>
<td>(Lake Casitas)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat</td>
<td>FeLV</td>
<td>Leukemia, lymphoma</td>
<td>T lymphocyte, B lymphocyte, Erythroid</td>
<td>Horizontal (saliva)</td>
</tr>
<tr>
<td>Cattle</td>
<td>BLV</td>
<td>Leukemia, lymphoma</td>
<td>B lymphocyte (and/or mixed)</td>
<td>Horizontal (insect vectors; and unknown route)</td>
</tr>
<tr>
<td>Gibbon ape</td>
<td>GalV</td>
<td>Leukemia, lymphoma</td>
<td>Lymphoblast, myeloblast</td>
<td>Horizontal (saliva; urine; feces)</td>
</tr>
</tbody>
</table>

**Bovine Leukemia**

Bovine leukemia virus (BLV) is the causative agent of two related lymphoproliferative diseases in cattle: the enzootic forms of lymphoid leukemia or lymphosarcoma and persistent lymphocytosis. The latter is a benign polyclonal lymphoid proliferation that may develop into the monoclonal lymphosarcoma. An interesting feature of BLV is the lack of detectable homology of its genome with other retrovirus genomes or with sequences of cell DNA from any species tested. Furthermore, all BLV structural proteins, including the major core protein, called p24, are serologically unrelated to other retroviral proteins, even though the corresponding core protein of other viruses contain antigenic determinants well conserved among mammalian retroviruses. Another unusual aspect of bovine leukemia caused by BLV is that the infected lymphocytes of leukemic cattle do not produce BLV in vivo. Only after culture did the infected cells produce virus. Furthermore, viral RNA transcripts and viral proteins are not even detected in the primary tumor tissues. Nonetheless, there is evidence for horizontal transmission of BLV. Lymphosarcoma and persistent lymphocytosis can spread from herds with leukemia clusters to leukemia-free herds. Because virus is not detected unless the leukemic cells are grown in vitro and because leukemic lymphocytes can be transmitted by insects and by other means, it has been suggested that the infected lymphocytes themselves are transmitted.

There are several implications of these observations with BLV to human disease. (1) Prior to the isolation of BLV, biochemical or immunologic evidence of a virus could not be found using previously isolated retroviruses as source of probes since BLV is unrelated to other retroviruses. By analogy, failure to detect viral "footprints" in human leukemias with probes made from animal viruses does not preclude nonproductive infection of the leukemic cells by a novel virus. (2) The
lack of BLV expression in primary tumors but positive expression after growing infected cells in vitro emphasizes the importance of growing the correct human cells in vitro for virus isolation. (3) The complete lack of nucleotide sequences in the DNA of uninfected cattle homologous to BLV makes it unlikely that the mechanism of BLV-induced leukemogenesis involves the formation of recombinant viral envelope genes as suggested for viral leukemias of inbred mice, and therefore, this is not likely to be a universal mechanism of leukemogenesis. (4) If BLV is transmitted via whole lymphocytes, then transmission must involve intimate contact or vector(s), as suggested by Ferrer and co-workers. (5) Since infection does not always lead to leukemia, other additional factors must be invoked. One likely factor is age at time of infection. Gross demonstrated that newborn and young animals are more susceptible to development of leukemia than adult animals, but in some instances, long latent periods are observed so that the disease does not become manifest until the animals become adults. As we shall illustrate later, all these findings have bearings on our results with a human retrovirus, HTLV, and some forms of human T-cell leukemias.

HOW DO RETROVIRUSES CAUSE LEUKEMIAS AND LYMPHOMAS?

Viruses Without Transforming (onc) Genes (Chronic Leukemia Viruses)

The pathogenic retroviruses commonly associated with naturally occurring leukemias in animals are chronic leukemia viruses. Examples include the avian leukemia viruses, wild type mouse leukemia viruses, feline leukemia viruses, bovine leukemia viruses, and gibbon ape leukemia viruses. Such viruses induce a wide spectrum of diseases in animals after long latency periods; they are nondefective, i.e., they do not require a helper virus for replication. The structures of their RNA genome and integrated proviral DNA are depicted in Fig. 1 (also see ref. 24 for review). The RNA genome contains a linear arrangement of three genes: gag (for group specific antigens), coding for a precursor protein that is cleaved into four virion core proteins; pol, the viral RNA-directed DNA polymerase (reverse transcriptase); and env, the virion envelope glycoproteins. These viruses do not contain genes that code for proteins specific for cellular transformation (onc genes). In addition to the structural genes, there are stretches of noncoding sequences including a short stretch of nucleotides that are repeated at both termini (designated r) and unique sequences near the 5' (U5) and 3' (U3) termini. Retroviruses replicate through a DNA intermediate, which integrates into the host chromosome. Immediately to the right of U5 is the binding site of a tRNA molecule that serves as a primer for the synthesis of the DNA strand. The integrated viral DNA has the same gene order as the viral genome, except that the U3 and U5 sequences are also duplicated at the ends (Fig. 1). Thus, the U3, r, and U5 sequences constitute a terminally repetitive unit, referred to as the long terminal repeat (LTR) unit. Within the U3 region of the LTR is the binding site for RNA polymerase (promotor site). This may be important for the altered expression of cellular genes, which are near the viral integration site (see below). There appears to be a large number of sites on the host DNA where the provirus can integrate into, and in nontumor cells the integration sites are multiple in a given cell population. However, the tumor cells are clonal populations and all cells of a given tumor have viruses integrated at the same sites. Since these viruses do not contain onc genes and generally have not been shown to transform cells in vitro, the mechanism by which they induce tumors in vivo is not yet clear, but some possibilities are considered later.

Viruses Containing "onc" Genes

Another type of retrovirus, known as the acutely transforming retroviruses, has been isolated both from naturally occurring tumors and from animals with tumors induced in the laboratory. However, isolates in nature are relatively uncommon compared to the "chronic" leukemia viruses. The genomes of these viruses carry transformation-specific genes (onc genes) coding for part or all of a protein required for the initiation and maintenance of the transformed phenotype, but the acquisition of this gene is generally associated with the loss of at least portions of some replicative gene(s), leading to a defect in replica-
tion of the virus. Consequently, they need nondefective helper viruses for replication. A notable exception is the Rous sarcoma virus of chickens, which carried both an onc gene and a full complement of replicative genes. The acutely transforming retroviruses can induce tumors rapidly in animals and can transform cells in culture. Viruses of this class include all the sarcoma virus isolates and all of the "acute" leukemia viruses (Table 2).

Many of the onc gene products still lack functional definition. The best characterized one is that of Rous sarcoma virus, a 60K phosphoprotein called pp60src. This protein was first identified in lysates from RSV-transformed cells and from in vitro translation products of src mRNA by immunoprecipitation with antisera obtained from rabbits bearing sarcomas induced by RSV.30,31 Since phosphorylation is a common cellular device to regulate protein activity, it is of interest to find that pp60src is not only a substrate for phosphorylation but is itself a protein kinase. Subsequently, pp60src was found to contain phosphotyrosine,32 an unusual phosphoamino acid that is also present in the middle T antigen of polyoma virus.33 Furthermore, the kinase activity associated with pp60src also catalyzes the transfer of phosphate groups to tyrosine residues of other proteins.34 The products of several other (but not all) viral onc genes have associated tyrosine-specific protein kinase activity as well (see Table 2). In most cases, the presence of the kinase activity of these proteins has been shown to be necessary for transformation. However, the actual mechanism by which cell transformation is induced and the in vivo substrates of the various viral protein kinases are still far from clear.

Furthermore, some viral onc gene products lack protein kinase activity35 and do not contain phosphotyrosine,36 suggesting that there may be different mechanisms by which this class of retroviruses transform cells. The lack of high titer antibody against these transforming proteins has often limited their characterization. However, with the advent of DNA cloning and sequencing technology, it is possible to predict the primary structure of proteins coded by specific genes and to raise antibody against defined synthetic polypeptides from different regions of the genes.36 Results with such approaches should be forthcoming.

**CELLULAR "ONC" GENES: TARGETS FOR A COMMON PATHWAY OF LEUKEMOGENESIS?**

The transforming (onc) genes of retroviruses seem to have been acquired by some recombination events. Data from numerous laboratories have shown that viral onc genes originated from normal genes of vertebrate cells (termed generically c-onc without implying that these normal cellular genes are themselves transforming). Although the extent of conservation of these genes varies from one to another, it is in general greater than the majority of cellular genes.37 Genetic loci homologous to most if not all onc genes have been detected in DNA from chicken to man.38-41 Although the high degree of conservation of cellular onc genes sustains the premise that these genes are important in normal cell growth, there is only limited documentation for expression of these genes in normal cells. RNA transcripts of four cellular onc genes (c-src, c-myc, c-erb, and c-myb) (see Table 2 for nomenclature) have been detected in tissues of several avian species42,43 (T. Gonda and D. Sheiness, personal communication), but attempts to detect transcripts of some other onc genes (c-mos and c-fes) have been unsuccessful.44 Where

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**Table 2. Some Properties of Acutely Transforming Retroviruses**

<table>
<thead>
<tr>
<th>Species of Origin</th>
<th>Nomenclature of v-onc*</th>
<th>Prototype Virus Strain</th>
<th>Genomic Structure</th>
<th>Protein Product</th>
<th>Kinase Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avian</td>
<td>src</td>
<td>Rous sarcoma virus (RSV)</td>
<td>5' gag-pol-env-src 3'</td>
<td>pp60 src</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>myb</td>
<td>Avian myeloblastosis virus (AMV)</td>
<td>5' gag-pol-myb 3'</td>
<td>?</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>E26</td>
<td>Avian myeloblastosis virus (MC29)</td>
<td>5' gag-myc-env 3'</td>
<td>p130 gag-myc</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>erb-A</td>
<td>Avian erythroblastosis virus (AEV)</td>
<td>5' gag-erbA-erbB 3'</td>
<td>p75 gag-erb-A</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>erb-B</td>
<td></td>
<td></td>
<td>p44 erb-B</td>
<td>-</td>
</tr>
<tr>
<td>Rodents</td>
<td>abl</td>
<td>Abelson murine leukemia virus</td>
<td>5' gag-abl 3'</td>
<td>140 gag-eps</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>mos</td>
<td>Moloney sarcoma virus</td>
<td>5' gag-mos 3'</td>
<td>80-90 gag-mos</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>ras</td>
<td>Kirsten murine sarcoma virus</td>
<td>5' rat-ras 3'</td>
<td>21 ras</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Feline</td>
<td>Snyder-Thelen strain feline sarcoma virus</td>
<td>5' gag-fes 3'</td>
<td>85 gag-fes</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>fms</td>
<td>McDonough strain of feline sarcoma virus</td>
<td>5' gag-fms 3'</td>
<td>180 gag-fms</td>
<td>+</td>
</tr>
<tr>
<td>Primate</td>
<td>sis</td>
<td>Simian sarcoma virus</td>
<td>5' gag-sis 3'</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>

*Taken from ref. 85.
examined, the detectable RNA transcripts have been found to be associated with polyribosomes, suggesting that they would be translated into proteins.

The protein products of three normal cellular onc genes have been identified. Two of these, pp60 c-src and p21 c-ras, are similar in size to their respective viral (Rous sarcoma virus and Harvey murine sarcoma virus) onc proteins. The third is p150 c-abl, a 150,000-dalton protein which, unlike the corresponding product (p120) of Abelson mouse leukemia virus, lacks gag-related elements. Although it is known that the viral and cellular forms of pp60 src are similar antigenically, structurally, and functionally, the level of the c-onc proteins generally is too low to allow further characterization.

If v-onc and c-onc genes code for functionally similar proteins, why do viral onc genes induce transformation? There are two ideas to consider: (1) the dosage of the onc gene product may be abnormally high in viral-transformed cells and/or (2) v-onc and c-onc genes have subtle qualitative differences. Several observations suggest that it is the dosage and not the quality of the onc gene product that is critical in the transformation event. The most powerful argument for this is the successful transformation of cells by a molecular clone of a c-onc (mouse c-mos) linked to portions of a murine leukemia virus genome containing promoter sequences (specifically, the LTR sequences, see Fig. 1). This result implies that modification of the protein-coding sequences of c-onc is not necessary for transformation, but rather the regulation of transcription of this cellular gene is the determining factor. This may be the mechanism by which some chronic leukemia viruses cause leukemias and lymphomas as well. In B-cell lymphomas of chickens induced by two distinct retroviruses, namely, avian leukosis virus and avian syncytia virus, the DNA forms (proviruses) are integrated near a particular cellular onc gene, c-myc, and cause expression of this gene to be greatly enhanced. Less frequently, avian leukemia virus also induces erythroblastomas, and in those tumors the provirus is integrated next to the cellular onc gene known as c-erb (the gene corresponding to the onc gene of avian erythroblastosis virus), and the transcriptional activity of that gene is enhanced (H. J. Kung, personal communication). It is of considerable interest that the proviruses in these tumors are usually defective. Some have been pared down to little more than a single LTR unit, which contains the important controlling sequences. In these tumors, the proviruses would be detected only with probes highly enriched in LTR sequences. It appears then that although the chronic leukemia viruses do not carry onc genes as an integral part of their genomes, they may transform cells by activating cellular onc genes. Since a virus integrates at multiple sites initially after infection, the proper alignment of a viral LTR with a cellular onc gene may be a matter of chance. Once a productive arrangement occurs, a sequence of events including activation of a gene to produce an abnormally high level of protein and cell proliferation may follow, leading finally to tumor production. This is an attractive model since it explains the long latency requirement, the often wide spectrum of pathogenicity associated with the chronic leukemia viruses, and the monoclonality of tumors caused by these viruses. Furthermore, activation of cellular onc genes potentially provides a common mechanism of tumorigenesis by the acute and chronic leukemia viruses as well as by nonviral agents. One can conceive of mutations in the regulatory elements for c-onc induced by chemicals, radiation, or other factors. However, it is premature to think that this model is generally applicable. There is as yet no evidence for activation of cellular onc genes in nonviral-induced tumors in animal systems, although much more work is needed in this regard. C-onc may only be a subset of a number of cellular genes that could play a role in tumorigenesis.

HUMAN onc GENE ANALOGUES AND THEIR EXPRESSION

Since most c-onc's have been shown to be phylogenetically conserved, it is of obvious interest to study the homologous genes in man and to determine their role in normal or neoplastic cell processes. Two of these human genes (c-sis and c-fes) have been cloned in our laboratory. Both genes are homologous to the entire v-onc, but the homologous sequences are interrupted. Therefore, like many eukaryotic cellular genes, c-sis and c-fes contain intervening nucleotide sequences (introns). The availability of other cloned human onc genes will be forthcoming and may shed light on the general organization and transforming potential of these genes.

A wide spectrum of human cells of neoplastic and normal origin have been examined for active transcription of six c-oncs: c-myc, c-myb, c-abl, c-Ha-ras, c-sis, and c-fes by our laboratory in collaboration with S. Aaronson and his colleagues, and in some instances M. Baluda and T. Papas and coworkers. The types of cells we have used as our source of mRNA included fresh peripheral blood cells from normal and leukemic patients and various neoplastic hematopoietic and solid tumor cell lines. Radiolabeled viral onc gene probes were hybridized to these RNAs using the technique of RNA gel blotting. The results with hematopoietic cells are summarized in Table 3. The abl and Ha-ras genes are expressed at low levels as multiple RNA
transcripts in all hematopoietic and solid tumor cells examined. The myc gene is also transcribed in all human cells examined but as a single 2.7 Kb transcript. These included normal peripheral blood lymphocytes prior to or after stimulation with phytohemagglutinin (PHA) and normal fibroblasts. The only exception is HL6O cells induced to differentiate into more mature metamyelocytes and granulocytes with DMSO or retinoic acid where myc transcription is turned off. The almost universal expression of abl, Ha-ras, and myc genes suggests that these genes play a role in basic cellular functions. It is interesting to note that the viral counterparts of these genes are able to induce neoplasias of hematopoietic and nonhematopoietic cells. All three viruses, MC29, Abelson-MuLV, and HaMSV, transforms fibroblasts in vitro. In addition, MC29 (v-myc) induces myelocytomatosis and less frequently carcinomas and sarcomas in chickens, and transforms macrophage-like cells in vitro. A-MuLV (v-abl) causes lymphosarcoma in mice and the transformed cell is a pre-B-cell. HaMSV (v-Ha-ras) induces erythroleukemias as well as fibrosarcomas in mice.60

The myb gene is expressed only in certain hematopoietic cells, including the early precursor cells of lymphoid, myeloid, and erythroid lineages, but there is little or no expression in B cells or in mature T or myeloid cells. Like myc, myb is transcribed in undifferentiated HL60 cells, but not in HL60 cells induced to differentiate with either DMSO or retinoic acid. It is not transcribed in nonhematopoietic cells. Thus, this gene may be involved in hematopoietic cell differentiation. The sis gene is not transcribed in the vast majority of hematopoietic cells, normal fibroblasts, melanoma and carcinoma cell lines, but is frequently transcribed in sarcoma and glioblastoma cell lines. So far, we have not detected transcripts of fes in any human cells.

Although AMV induces myelogenous leukemias in chickens and cells transformed in vivo or in vitro are myeloblasts, we did not find inordinately high levels of c-myb transcripts in various human myeloid leukemic cells. In contrast, immature T-cell leukemias express higher levels of c-myb.61 In the absence of control studies with normal myeloblasts or pre-T-cells, we cannot conclude whether the given levels of myb expression are linked to the leukemic state of the cells examined. We also failed to detect high levels of myc expression in human B-cell lymphomas analogous to the high levels expressed in ALV-induced lymphomas of chickens. The highest level of expression of c-myc was detected in HL60, a promyelocytic leukemia cell line. The enhanced level of expression in this cell is apparently due to amplification of the functional c-myc gene. In summary, our results do not implicate enhanced expressions of these 'onc' genes in most spontaneous human leukemias. However, the possible
roles of \( c-amv \) and \( c-myc \) in immature T-cell leukemias and promyelocytic leukemias, respectively, are worth further investigation. In addition, it is important to establish the “basal” levels of onc gene expression in normal immature cells of comparable stages of differentiation.

HUMAN T-CELL LEUKEMIA VIRUS (HTLV): ISOLATION, CHARACTERIZATION, AND EVIDENCE FOR AN ETIOLOGIC ASSOCIATION WITH SOME ADULT T-CELL LEUKEMIAS AND LYMPHOMAS

As discussed above, retroviruses are useful tools for defining the nature of genetic information which leads to neoplastic transformation and studies using them as such have been directly applied to human neoplasias. In addition, since many naturally occurring animal leukemias and lymphomas are caused by infectious type-C retroviruses, it has been of considerable interest to look for the presence of this kind of virus in comparable human diseases. Most studies prior to 1970 centered on electron microscopic examination of primary tumor cells and did not result in any clear-cut positive results. The discovery in 1970 of the viral-specific DNA polymerase (reverse transcriptase) by Temin\(^2\) and Baltimore\(^3\) opened up two new approaches for biochemically probing human cells for retroviruses: (1) a sensitive enzyme assay that exploits the novel feature of this enzyme to distinguish it from cellular DNA polymerases; (2) synthesis of radiolabeled DNA complementary to viral RNA (cDNA) to determine if nucleotide sequences homologous to a given animal retrovirus are present in DNA or RNA of human cells. These approaches could be used in the absence of release of detectable virus particles. Concomitantly, advances in the immunochemistry of viral structural proteins\(^4\) made it possible to explore cells for viral-related antigens. Extensive experiments of this kind were carried out in many laboratories, including our own and particularly that of S. Spiegelman and coworkers. Some of these results, especially studies of identical twins by Spiegelman and colleagues,\(^5\) indicated that new retrovirus-related nucleotide sequences are present in the DNA of some leukemic patients and not in the DNA of a normal but otherwise identical twin. These results implied that the leukemic patient was infected, presumably by a retrovirus.\(^6\) In our experience, however, this kind of experiment yielded scanty findings that did not merge into an interpretable pattern.\(^7\) In fact, extensive studies carried out in our laboratory in the last decade suggest to us that some forms of human leukemia are not clearly associated with retroviruses. However, their presence in other human leukemias and lymphomas has now been established. Recent results strongly implicate an etiologic association between a newly discovered human type-C retrovirus with certain types of T-cell leukemias and lymphomas. The virus, known as human T-cell leukemia-lymphoma virus or HTLV, has some analogous features to BLV in its biology, some of its biochemistry and some of its history. As shown for BLV-induced bovine lymphomas, culture of the appropriate tumor cells in vitro is sometimes requisite for virus identification and isolation. Since T-cell leukemias are often caused by retroviruses in animals, the discovery of the factor that supports long-term growth of T lymphocytes\(^8\) availed us the opportunity to routinely examine human T-cell tumors for these viruses. These efforts led to the isolation of HTLV.

Growth of Human T Cell and Isolation of HTLV

The proliferation of mature T cells normally involves at least three major steps\(^9\): (1) activation of a subset of T cells by an antigen or lectin to synthesize receptors for TCGF; (2) interaction of the antigen or lectin with an adherent cell and a different subset of T cells to produce TCGF; and (3) interaction of the activated (receptor-positive) T cells with TCGF leading to T-cell proliferation. However, a significant percentage of all the neoplastic mature T cells examined in our laboratory possess TCGF receptors without prior lectin or antigen activation. Apart from the intrinsic interest of this observation, a practical outcome is that some neoplastic T cells can be selectively grown free of normal T cells with purified TCGF.\(^6\) Utilizing this approach, some human neoplastic mature T-cell lines have been established and some have subsequently become independent of exogenous TCGF apparently by producing their own TCGF.\(^7\) A number of these cultured neoplastic T-cell lines release type-C virus (HTLV).\(^8\) The first virus, HTLV strain CR, was obtained from a lymph-node-derived T-cell line from a patient (C.R.) with a aggressive variant of cutaneous T-cell lymphoma.\(^9\) Additional isolates were obtained from separate blood samples of the same patient and a second virus-inducing cell line, CTCL-3, was established.\(^7\) HTLV, strain MB, was also isolated from cultured T cells of a patient (M.B.) with cutaneous T-cell leukemia (Sézary's syndrome).\(^2\) Subsequently, HTLV has also been isolated and/or identified in cultured T cells from many patients from around the world. These include patients with diseases called acute lymphosarcoma T-cell leukemia, a T-cell variant of Hairy cell leukemia, Japanese adult T-cell leukemia, primary tumor cells from peripheral T-cell lymphoma, and additional patients with Sézary leukemia (Gallo et al. and Popovic et al., submitted). A
recent report from Japan suggests that a retrovirus associated with Japanese adult T-cell leukemia is able to induce permanent growth of T cells from normal human cord blood independent of TCGF. We have found by nucleic acid hybridization and protein immunochemistry that HTLV is indeed present in Japanese adult T-cell leukemia cells (M. Popovic et al., submitted). Its effect on cord blood T cells either suggests that HTLV contains a transforming gene or represents an unusual observation of high frequency in vitro transformation of hematopoietic cells by a chronic leukemia virus.

Characterization of HTLV

Like all retroviruses, HTLV contain reverse transcriptase and an RNA genome. The two characterized HTLV strains (HTLV\textsubscript{CB} and HTLV\textsubscript{MB}) are highly related if not identical to each other, but unrelated to the known animal retroviruses. The analyses have included nucleic acid hybridization\textsuperscript{74} and immunologic studies of the viral reverse transcriptases\textsuperscript{75} and core proteins.\textsuperscript{76} Recently, the major core protein, p24, of HTLV was purified to homogeneity\textsuperscript{76} and subjected to amino acid sequence analysis.\textsuperscript{77} The results showed overall similarities but distinct features of this protein compared to other retroviral core proteins. It shares the common NH\textsubscript{2}-terminal proline, COOH-terminal leucine, and certain internal “stretches” of amino acids of all mammalian type-C retroviral core proteins. Alignment of the first 25 amino acid residues of HTLV p24 with those of BLV p24 and FeLV p27 showed a distant evolutionary relationship of HTLV to BLV, but not to FeLV.

HTLV is an exogenous human virus, since HTLV sequences are not present in the DNA of normal human donors.\textsuperscript{74} The opportunity to find out if HTLV was transmitted in the germ line or acquired by postzygotic infection in patient C.R. was provided when his B cells were grown in cultures parallel to his neoplastic T cells (established independently in T. Waldman's laboratory by Drs. Takashi Uchiyama and Samuel Broder). HTLV sequences were detected only in DNA of the neoplastic T-cell lines but not in that of normal T cells and B cells of the same patient.\textsuperscript{78} Thus, HTLV was not transmitted in the germ line of patient C.R. Rather, it was acquired by infection by an as yet unknown mode of transmission.

In Vitro Transmission of HTLV

Transmission of HTLV to cultured cells was monitored using a competitive radioimmune assay for HTLV p24 and an indirect immunofluorescence assay with a monoclonal antibody to HTLV p19, a protein shown to be expressed only in cells known to produce HTLV.\textsuperscript{79} Initial attempts to infect many types of cultured cells from different species were unsuccessful. This result, coupled with the apparently very low level of distribution of this virus among contacts of infected people (see next section), suggests that HTLV is poorly infectious, perhaps requiring an intermediary vector or close contact for transmission. However, in vitro transmission of HTLV was successful with peripheral blood T cells from a few relatives of patients with an HTLV-associated T cell neoplasia\textsuperscript{78} and more recently, to normal T cells from cord blood (M. Popovic et al., unpublished results). These results clearly show that HTLV has biologic function and suggest that it is tropic for T cells.

Specific Antibodies to HTLV in Human Sera

A sero-epidemiologic survey of patients with T-cell lymphomas and leukemias was conducted using three assays: (1) a solid-phase radioimmunoassay using disrupted HTLV, (2) a radioimmunoprecipitation assay using purified p24 labeled by iodination, and (3) indirect immunofluorescence against virus-infected cells. In an initial survey of patients and normal donors from the United States and Europe, one patient with cutaneous T-cell lymphoma (patient C.R.) and two with cutaneous T-cell leukemia showed high titer natural antibody against HTLV. Serum samples from other forms of leukemia, including T-CLL, childhood leukemias, Hodgkin’s disease, all forms of myeloid leukemia, B-cell lymphomas, and several other T-cell malignancies, including many more U.S. cases with cutaneous T-cell lymphomas, were negative. Of hundreds of samples from normal U.S. and European donors, only two sera reacted strongly in the immunoprecipitation test. Both are close relatives of patients positive for HTLV, namely the wife of C.R. and sister of M.B. The positive results were the first evidence for a specific immune response to a type-C retrovirus in humans,\textsuperscript{80,81} and they were clearly specific for a subtype of T-cell malignancies and for people who were close contacts and/or in identical environment as the HTLV-positive cases. Yet they were disappointingly few in number when the survey was based on random sampling.

Clusters of Japanese adult T-cell leukemia (ATL), a subtype of T-cell malignancy, occur with high frequency on the islands of Kyushu and Shikoku in Southwestern Japan. In collaboration with Y. Ito, M. Maeda, Y. Nakao, and T. Aoki, sera of Japanese patients with ATL and other hematopoietic malignancies and normal donors from endemic and nonendemic areas\textsuperscript{83,84} were examined for anti-HTLV antibodies in our laboratory. In contrast to the U.S. and European patients, a high proportion of the Japanese patients...
with T-cell leukemias contain natural antibodies to HTLV that score positive in all three assays. Furthermore, in a survey of normal relatives of ATL patients in the endemic area, 16 of 182 scored positive. A few patients with non-T-cell leukemias also scored positive. Thus, it is clear that HTLV is associated with sporadic cases of T-cell leukemias and lymphomas where such cases occur sporadically. In contrast, in Japan where one form of T-cell disease (ATL) is characterized by rapidly growing pleomorphic lymphocytes, splenomegaly, and hypercalcemia, the disease can be endemic and HTLV is a common infection. Such relatively frequent findings of HTLV-associated T-cell leukaemia are, however, not limited to Japan. Recent results in collaboration with William Blattner of the NCI Epidemiology Branch and Daniel Catovsky, David Galton, and Mel Greaves and their colleagues in London indicate that a similar situation exists in the West Indies (Blattner et al., submitted). These and more recent studies (Table 4) indicate that there will be “pockets” of HTLV leukemia-lymphoma in other parts of the world.

A Hypothesis for Interaction of HTLV and TCGF in the Pathogenesis of Mature T-Cell Leukemias-Lymphomas

Although the molecular structure of the HTLV genome has not been elucidated, it probably resembles other nondefective leukemogenic retroviruses such as MuLV, BLV, FeLV, ALV, and GaLV. Assuming it does not contain an onc gene, we would anticipate that its effect will be an indirect one involving the induction of cellular gene(s) involved in T-cell proliferation. Two such candidates are the genes coding for TCGF or its receptor protein. We observed that HTLV-producing cells are unusual in that they produce and respond to their own TCGF. One possible sequence of events following HTLV infection may be as follows (Fig. 2). The HTLV envelope protein interacts with the population of T cells, normally designed to make TCGF receptors, mimicking an antigen stimulation of blastogenesis. These cells then synthesize receptors for TCGF. Simultaneously, the HTLV provirus, complete or incomplete, may integrate “upstream” from the TCGF gene, and its promotor (LTR) could activate this gene, leading to TCGF release. This release of TCGF by a cell bearing a TCGF receptor may result in increased proliferation. Alternatively, HTLV may simply induce the presence and maintenance of TCGF receptors (perhaps in an increased number), and this could conceivably be the only mechanism needed to maintain abnormal growth. We have recently identified a gene that is actively transcribed in all HTLV-infected cells (V. Manzari et al., unpublished). This same gene is also actively transcribed in activated T cells and T cells constitutively producing TCGF, suggesting that it plays a role in T-cell proliferation involving TCGF. The exact nature of the product of this gene and whether its transcription is enhanced by the HTLV provirus are problems currently under investigation.

CONCLUSION

Retroviruses cause neoplasia by: (1) transducing into infected cells onc genes incorporated into the viral genomes from cellular genes during previous rounds of infection, as in the case of the acute leukemia viruses; (2) activating resident cellular onc genes by proximal integration of the proviruses, as has been documented for the ALV-induced B-cell lymphomas and erythro-

Table 4. Distribution of Antibody to HTLV by Clinical and Geographical Status

<table>
<thead>
<tr>
<th>Sera</th>
<th>Origin</th>
<th>Antibody to HTLV*</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Normal&quot; donors</td>
<td>USA, Europe</td>
<td>0/181</td>
</tr>
<tr>
<td>Cutaneous T-cell lymphoma-leukemia†</td>
<td>USA</td>
<td>2/245</td>
</tr>
<tr>
<td>Healthy relatives of HTLV positive patients‡</td>
<td>USA</td>
<td>2/18</td>
</tr>
<tr>
<td>Normal donors</td>
<td>Japan (nonendemic area)</td>
<td>0/47</td>
</tr>
<tr>
<td>Normal donors</td>
<td>Japan (endemic area)</td>
<td>21/182</td>
</tr>
<tr>
<td>Adult T-cell leukemia</td>
<td>Japan</td>
<td>23/26</td>
</tr>
<tr>
<td>Other T-cell lymphomas</td>
<td>Japan</td>
<td>6/12</td>
</tr>
<tr>
<td>Acute lymphocytic leukemia</td>
<td>Japan</td>
<td>4/20</td>
</tr>
<tr>
<td>Other leukemias</td>
<td>Japan</td>
<td>5/49</td>
</tr>
<tr>
<td>Normal donors</td>
<td>Caribbean (West Indies population)§</td>
<td>12/337</td>
</tr>
<tr>
<td>T-cell lymphosarcoma</td>
<td>Caribbean (West Indies population)</td>
<td>4/4</td>
</tr>
</tbody>
</table>

*HTLV-specific antibodies were assayed as described elsewhere.†Sézary's syndrome or mycosis fungoides. Two positives include one of each disease. Patient M.B. with Sézary's syndrome was born in the Caribbean but moved to the U.S.
‡Positive sera were from the wife of patient C.R. and the daughter of patient M.B., who was also born in the Caribbean.
§Data taken from Blattner et al., in preparation. More than 50% of the positive samples were taken.
Human type-C retroviruses (HTLV) have recently been isolated from T cells of patients with leukemia-lymphomas of mature lymphocytes. Sero-epidemiologic studies indicate a strong association of HTLV with certain subtypes of T-cell neoplasia, particularly Japanese adult T-cell leukemia and similar sporadic and endemic diseases in other parts of the world. The origin and present reservoir of HTLV, the molecular mechanism of its action on T cells, and ramifications for containment of the virus are all areas of future research. Furthermore, the findings with HTLV may stimulate more careful research into the possible viral etiology of other human cancers.

**SUMMARY**

Retroviruses are etiologic agents of naturally occurring leukemias and lymphomas in several animal species (chickens, wild mice, cats, rats, cows, and gibbon apes). One class of viruses (the acutely transforming viruses) carry transformation-specific genes derived from highly conserved normal cellular genes that may be important in basic cellular functions and/or differentiation. The other class of viruses lack definable onc genes, do not transform cells in vitro, and require long latency periods for disease induction. One mechanism by which retroviruses that lack onc genes induce leukemias may be activation of resident cellular onc genes. It has been speculated that this may be a common mechanism of tumorigenesis regardless of etiology. However, studies on the expression of onc genes in human tumors are so far inconclusive.

Added to the list of retroviruses that are associated with leukemias is a human retrovirus called HTLV, repeatedly isolated from T-cell lymphoma and leukemia cells that had been established in culture with T-cell growth factor. HTLV is an exogenous type-C
virus distinguished from other mammalian retroviruses by assays of nucleic acid homology and immunologic assays of viral proteins. The amino acid sequence of the purified core antigen (p24) revealed a distant but significant homology to bovine leukemia virus p24. Transmission of the virus has been successful with T lymphocytes from relatives of HTLV-positive T-cell leukemia patients (i.e., antigen or antibody-positive patients) and more recently to normal human cord blood T lymphocytes. These data suggest a T-cell tropism for HTLV and a possible genetic factor for susceptibility to HTLV infection.

A wide survey of human sera for antibodies to HTLV was conducted. Among those positive for antibodies to p24 and p19 were almost all serum samples from Japanese patients with adult T-cell leukemia, a disease that is endemic in South-Western Japan. A similar phenomenon has also been found in other parts of the world, especially in the West Indian black population. These results indicate that HTLV is much more prevalent in certain geographical regions than in others. The biochemical characterization of HTLV and sero-epidemiologic studies demonstrate that HTLV is a unique retrovirus and suggest that it may be etiologically involved with certain forms of adult T-cell leukemia and lymphoma. Many more epidemiologic surveys for HTLV and studies of its molecular mechanism of influencing T-cell proliferation are needed.

ACKNOWLEDGMENT

We are grateful to our many colleagues who contributed to various aspects of the work described here, to Drs. E. Gelmann, R. Dalla Favera, M. Robert-Guroff, V. Manzari and G. Franchini for critical review of the manuscript, and to A. Mazzuca for expert secretarial assistance.

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